Pakistan Journal of Biological Sciences 14 (24): 1090-1099, 2011
ISSN 1028-8880 / DOI: 10.3923/pjbs.2011.1090.1099

Biological Study of *Plutella xylostella* (L.) (Lep: Plutellidae) and It's Solitary Endoparasitoid, *Cotesia vestalis* (Haliday) (Hym. Braconidae) under Laboratory Conditions

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Abstract: *Plutella xylostella* (L.) (Lep: Plutellidae), is a destructive pest of brassicaceous crops in the world. *Cotesia vestalis* (Haliday) is one of most important biological control agents of *P. xylostella* in the world and Iran. Both of *P. xylostella* and *C. vestalis* biology were carried out in laboratory condition. Results showed that development time of immature stages of *P. xylostella* including egg, Instar I, Instar II, Instar III, Instar IV, prepupa, pupa were 2.39±0.17, 2.18±0.17, 2.06±0.28, 2.14±0.14,2.54±0.12, 0.40±0.12 and 4.23±0.23 days, respectively. Longevity of female and male were 28.26±0.05 and 30.22±0.05 days. By dissecting the parasitized larvae, the egg incubation period of *C. vestalis* was recorded 1.73±0.06 days. In long-term oviposition trials, females laid eggs on *P. xylostella* larvae for up to 10 days. Larval development of the parasitoid in host only required 6.47 days: the first instar larva required 3.25±0.047 days; the second instar larva needed 2.78±0.1 days and the third instar larvae exited the host and pupated in, 0.4±0.07 days. Prepupal and pupal period of wasp were 1.9±.0.06 and 2.13±0.09 day, respectively. Unmated female and male longevity of wasp were 16.83±0.37, 16.25±0.17 and sex ratio is male-biased. When a mixed group and isolated of instars were presented for parasitoid, the 2nd and 3rd instar larvae were so preferred and the 4th instar was less attractive for selection. In choice experiment, the percentage parasitism of 2nd, 3rd and 4th instars was 78.58, 69.94 and 4.36%, respectively. The rapid oviposition rate, short life duration and high percentage parasitism increases parasitoid potential for suppression of host population. Present results suggest that *C. vestalis* has considerable potential as a biological control agent for *P. xylostella*.

Key words: *Plutella xylostella*, *Cotesia vestalis*, biological parameters, host stage preference

INTRODUCTION

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is one of the most destructive cosmopolitan insect pest of cruciferous plants in many parts of the world (Talekar and Shelton, 1993). Sometimes due to outbreak of *P. xylostella* in Southeast Asia, damage to cabbage plants has reached 90% (Verkerk and Wright, 1996). In many countries, *P. xylostella* has developed multiple and cross-resistance to a wide range of conventional organic insecticides and *Bacillus thuringiensis* (Bt) products (Tabashnik *et al*., 1990; Tabashnik, 1994; Sarfraz and Keddie, 2005; Zhao *et al*., 2006; Raymond *et al*., 2007; Gassmann *et al*., 2009; Nehare *et al*., 2010; Santos *et al*., 2011). Insecticide resistance development of DBM due to the high frequency of insecticides application led to use alternative strategies and control methods such as biological control agent's particularly hymenopterous parasitoids (Talekar and Shelton 1993; Sarfraz and Keddie, 2005). The braconid wasp, *Cotesia vestalis* (=*plutellae*) (Haliday) (Hymenoptera: Braconidae) is the most widely distributed koinobiotic solitary larval endoparasitoid of *P. xylostella* and could parasitize all instars, but preferred to parasitize instarIII (Talekar and Yang, 1991). In Iran, It was first recorded by Karimpour *et al*. (2005) from Orumieh in west Azarbayejan Province and now it was the dominant parasitoid of *P. xylostella* in most province of Iran with high parasitism. Therefore, this parasitoid has high potential as biological control agents of *P. xylostella*. Undoubtedly, Successful in integrated pest management programs is more dependent on investigation and knowledge about biology of the pest, natural enemies and its interactions with host plants (Sequiera and Dixon,

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1996; Awmack and Leather, 2002). Beckage and Gelman (2004) reported that parasitoids have evolved with mechanisms to maneuver host physiology and biochemistry to create a conditions increasing own and offspring fitness. For improving their fitness, parasitoids have shown preference for specific larval stages, because there are differences in host developmental stage quality (McGregor, 1996; Li *et al*., 2006). With increasing age of the host, they increase their physical and immune defense mechanisms, so these phenomena are not benefit for parasitoid (Li *et al*., 2006). Thereafter, it is important to determine the host stage most effectively parasitized by *C. vestalis*. for management of *P. xylostella* by releasing parasitoid at the most effective time. Detailed biology of DBM presented by Marsh (1917); Harcourt (1957); Talekar *et al*. (1985) but all of them carried out in the field condition. The braconid wasp, *C. vestalis* has been preliminary studied by Chiu and Chien (1972) and Yu *et al*. (2008) presented details on the developmental biology and morphology of *C. vestalis* but results were only about immature stages. Therefore, the first objective of present work was to evaluate the life cycle duration of *P. xylostella* and *C. vestalis* on Chinese cabbage for improving mass rearing and second specific objectives were to determine the effects of host stage on parasitoid preference and parasitism.

MATERIALS AND METHODS

Host plant: The Chinese cabbage (*Brassica pekinensis* cv. Spring Smile) was grown in plastic pots (10×10×10 cm) under greenhouse condition (25±5°C, 65±10% r.h. and L16:D8 h).This plant were used to rear *P. xylostella*.

Insects culturing procedure: Both of *P. xylostella* larvae and the *C. vestalis* specimens (originally collected from parasitized *P. xylostella* larvae) were collected from the *Brassica* fields in Karaj (Alborz province, Iran) and brought to the laboratory during 2010 growth seasons. The stock culture of DBM was maintained on 8-week-old Chinese cabbage in screened hyaline cages (40×40×40 cm) under standard constant environment (25±1°C, 65±5% r.h. and a photoperiod of L16:D8 h; (Karimzadeh *et al*., 2004). Rearing of *C. vestalis* was performed according to Talekar *et al*. (1997) with slight modification. A small cotton-wool wick soaked in 10% honey solution was placed in each oviposition cage as a source of carbohydrate for adults of DBM and wasps.

Biology of *Plutella xylostella* (L.): For obtain the same synchronized eggs of DBM, a potted Chinese cabbage (8-week-old) were placed inside oviposition cages containing 50 pairs of newly emerged *P. xylostella* (female 1: male 1) for one hour. The eggs laid on the leaves were then used for the experiments. By using a stereomicroscope (Olympus, SZ11), about 100 eggs transferred on fresh cabbage leaf discs (3 cm diameter) within Petri dishes (5.5 cm diameter) and the edge of each Petri dishes was covered with a layer of Parafilm® (Laboratory Film, Chicago, IL). Finally, 64 fertile egg (as 64 replicate) selected. New and fresh leaf discs were replaced every 8 h. The immature stages were checked every 4 h under stereomicroscope and developmental time of each stage was recorded until become adult and all of them died. Starting of each larval instar was recorded when molting or exuvia that observed under stereomicroscope. In addition to, other parameters including size of all stages, width of larval head capsule, total ovipositional periods, sex ratio (female number/total adult number) adult longevity and fecundity (eggs per female) were investigated in this experiment. All experiments were replicated four times in a growth chamber under controlled conditions as noted previously.

Biology of *Cotesia vestalis* (Haliday): The larva of DBM were reared on the Chinese cabbage as described above and exposed to *C. vestalis* to obtain a cohort group of parasitoid eggs in host larva. The early 3rd instar of host (n = 500 larvae) parasitized by mated 3-days-old female of *C. plutella* (1 Wasp: 2 Host ratios) for 1 h. Every 8 h, 20 parasitized larvae were selected and dissected (by entomology needle No. 00) under a stereomicroscope (Olympus, SZ11). For this purpose, at first parasitized larva were placed in a phosphate-buffered saline (pH 7.4), that dropped on microscope slides, then larval body pulled until it was ruptured. Finally, the DBM larvae abdomen was gently pressed with needle until parasitoid (egg or larva) flowed out in the body fluid. Thereafter, according to Lim (1982), duration of life cycle was recorded from egg to adult based on immature stage morphological characters. If superparasitism was occurred the larvae removed. In each dissecting time, eggs and larval stages were photographed using a stereomicroscope (Olympus, SV6) and inverted Zeiss phase contrast microscope (Oberkochen, Germany) equipped with on DinoCapture 2.0 software (AnMo Electronics Corporation).

Host age preference in non-choice tests: In the non-choice experiment, to determine the larval stages of *P. xylostella* that were most completely parasitized by *C. vestalis,* 3-day-old mated female wasps were exposed to host larvae in Petri dish (diameter, 5 cm), for 2 h containing 10% honey water on lid of dish. Each Petri dish

contained a female parasitoid and 10 host larvae of a particular stage. Experiments were replicated 10 times. After parasitism, the host larvae were separated and placed individually on fresh cabbage leaf discs (5 cm diameter) within Petri dishes (7.5 cm diameter). Larvae checked daily until they had pupated, died, or produced parasitoid cocoons.

Host age preference in choice tests: In this experiment to choose the instar of *P. xylostella* preferred by *C. vestalis* for parasitization, 3-day-old mated female parasitoids were exposed to host larvae in the hyaline Plexy glass (height 4 cm; diameter, 12 cm). Ten replicates were performed. Each hyaline Plexy glass contained three parasitoids and 30 larvae. After a 2 h exposure period, larval instars were separated and placed fresh cabbage leaf discs (5 cm diameter) within Petri dishes (7.5 cm diameter). Exposed host larvae were checked daily as described in the non-choice tests.

Parasitoid oviposition dynamic: Ten parasitoid females obtained treatment were caged individually in oviposition unit (1 female: 1 male) and provided daily with smears of honey. Each oviposition unit was 40 early-3rd instar *P. xylostella* larvae for an exposure period of 6 h. Male parasitoids were replaced in the event of death. Host larvae previously exposed to wasp females were dissected within 24 h after exposure to determine if parasitism had occurred and number of parasitoid eggs or larvae checked. Female lifespan was recorded too.

Statistical analysis: Data were transformed and analyzed using PROC General Linear Model. Fisher's Least-Significant Difference (F-LSD) test was applied for mean comparisons among treatments. The Two-sample t-test was used for paired comparisons between treatments. Computations for this experiment were done using the statistical software package SYSTAT version 12.02 (SYSTAT, 2007).

RESULT

Life cycle of *Plutella xylostella* (L.): Life cycle of DBM consists of egg, four instars larvae, prepupa and adult. Diagnosis of these stages is important for biological purpose. The some morphological features of all stages are given below:

Egg and larvae: Eggs are oval and protuberant in shape and lubricant pale to strong yellow in color. Hatching occurred in 2.39±0.17 days in laboratory conditions

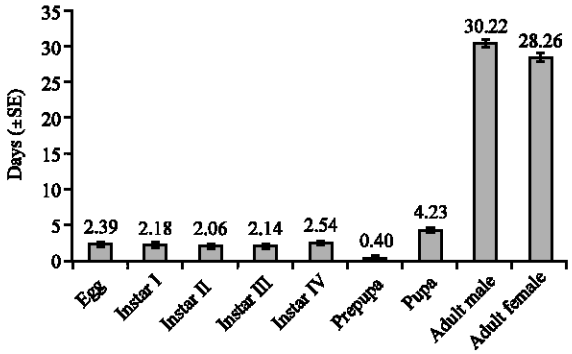

Fig. 1: The duration of immature and adult development (days; Mean±SD) of *P. xylostella*

(Fig. 1). Of 323 eggs oviposited by each female on Chinese cabbage, 219 were laid on lower leaf surfaces, 104 on upper leaf surfaces (Table 2). First instars start feeding immediately. They are leaf miners and feed in the spongy mesophyll tissue of leaves. First instar development was completed about 2.18±0.17 days (Fig. 1). Hence, other larval fed on lower leaf surface and often fed all tissue at end of larva period. Anal legs of larva are distinctive and V-shape. Larva is pale yellow in color at early instars and gradually become pale green to dark in other instars. The developmental time of 2nd–instar, 3rd-instar and 4th-instar were lasted about 2.06±0.28, 2.14±0.14 and 2.54±0.12, respectively (Fig. 1). Data analysis have shown body length ($F_{7, 232}$=1729, $p<0.001$) and weight ($F_{8, 261}$=1409, $p<0.001$) of immature stages are significantly different (Table 1). There are no differences between late 1st-instar and early 2nd-instar in both length and weight, so apparent recognition between these two instars is very difficult and distinguishes is occurred based on head capsule width (Table 1). In other instars with increases age of larvae the body length and weight increases (Table 1). The changes in the head capsule width (HCW) of all instar were measured and result showed significantly differences ($F_{3, 116}$=17700, $p<0.001$) (Table 1). This character would be useful for diagnosis and separate larva stage from each other.

Prepupa and pupa: There are two inactive, non feeding stages called prepupa and pupa. The duration of prepupa was lasted 0.42±0.018 days (Fig. 1). Then this quiescent prepupa molt in its cocoons and larval skin remains attached to the posterior end of the pupa (successive observations). Development of pupal period was completed 3.82±0.06 days. In normal pupa the length and weight were 5.06±0.027 mm and 5.29±0.092 mg, respectively (Table 1).

Table 1: Estimating of body length (mm; Mean±SE.) and weight (mg; Mean±SE) of *P. xylostella* (n = 100) feed on Chinese cabbage

	L^1_1		L_2		L_3	
Parameter	Early	Late	Early	Late	Early	Late
Length	0.88±0.04a[3]	1.78±0.038a	1.8±0.045a	2.82±0.051bc	2.83±0.048bc	4.99±0.083e
Weight	0.064±0.004a[3]	0.177±0.005b	0.209±0.009b	0.588±0.016c	0.629±0.022c	1.802±0.044d
HCW[4]	-	0.157±0.01a[3]	-	0.245±0.01b	-	0.361±0.01c

	L_4			Adult wing span[2]	
Parameter	Early	Late	Pupa	Female	Male
Length	5.0±0.067d	9.0±0.01f	5.06±0.027	15.03±0.04a	13.67±0.17b
Weight	2.297±0.096d	7.40±0.188e	5.29±0.92	-	-
HCW[4]	-	0.598±0.002d	-	-	-

[1]L_1, L_2, L_3 and L_4 denote the 1st, 2nd, 3rd and 4th instar moth larvae, respectively. [2]In adult wing span:means marked with the same small letter within the same row are not significantly different (t-test, $p<0.05$). [3]Means marked with the same small letter within a same row (L_1 untile L_4) are not significantly different ($p<0.05$; Fisher-LSD Test). [4]Head capsule width

Table 2: Ovipositional period, adult longevity and fecundity (eggs per female) of *P. xylostella on* Chinless cabbage

Total ovipositional period (day's± SE)			Adult longevity(day's± SE)		
Pre-oviposition	Oviposition	Post-oviposition	Female	Male	Fecundity
0.37±0.01	7.20±0.13	20.7±0.5	28.26±0.50a*	30.22±0.50b*	323.45±6.03

*Means marked with the same small letter within the same row are not significantly different (t-test, $p<0.05$)

Adult performance: Wingspan of male and females were different each other ($t = 8.45$, $df = 31$, $p<0.001$; Table 1) and it was larger in females than males. There was no differences between both sex length ($t = -1.51$, $df = 58$, $p>0.05$; Table 1). The longevity of females was significantly reduced than males ($t = -2.79$, $df = 62$, $p<0.05$; Table 2). Mean longevity of males and females are 30.22±0.50 and 28.26±0.50, respectively. Oviposition mainly was occurred in darkness. Preoviposition, Ovipositon and post ovipositional periods for female lasted 0.37, 7.20, 20.7 days. The sex ratio is 0.48 (female per total emerged adult). The mean number of total eggs oviposited by a *P. xylostella* female in its oviposition period (about 10 days) was 323±6.03. Ovipositional peak in the first 24 to 48 h had taken place and it was reduced gradually till reached to zero after 10 days of adult emergence (Fig. 2).

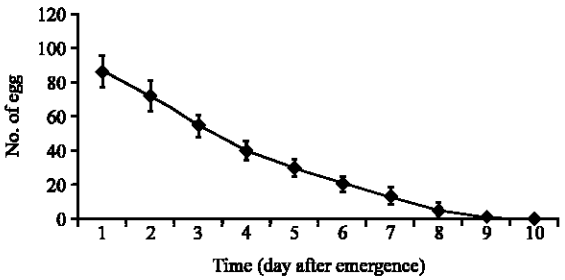

Fig. 2: Number of eggs (Mean±SE) laid by *P. xylostella* over 10-days period

Parasitoid biology, host age preference and oviposition dynamics: Life stages of the *C. vestalis* include egg, three larval instars, pupa and the adult. The egg and two larval stages were found on the hosts. Characteristics seen under microscopic examination included.

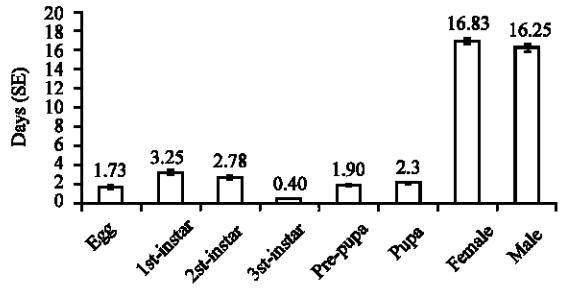

Fig. 3: Mean developmental time (days±SD) of *C. vestalis* on parasitized larvae of *P. xylostella*

Eggs: Spindle shape, slightly curved and hyaline colorless. It has a short and thin pedicle with 17.72±0.18 μm length at the end 24 h after parasitism. Eggs have distinct 3-layered membrane. The external membrane was named; serosal membrane that produce teratocyte cells. Gut was visible in the embryo 24 h after wasp oviposition. The segmented larvae and big head capsule was visible in the egg nearly 36 h after parasitism. The incubation period of egg was 1.73±0.06 days (Fig. 3).

First instar: 1st -nstar were Caudate-mandibulate form. It has a large sclerotized head capsule with sickle and sclerotized mandibles which were unfolded and closed freely. Moreover mouthparts were included Labium and Maxilla. Caudal horn was measured 66.40±0.51 μm in length. The number of body segments wasn't countable at first day of first instar, because of serosal membrane

(external membrane embryo) and another membrane (Amnion) were still attached to the embryo and had dissociated, respectively. The first instar larvae have 13 segments (3 thoracic segments and 10 abdominal segments after the first day. At early first instar larva head capsule was too wide than the body, But gradually it became older; this width ratio was reduce. The size of cauda was more reduced that develops in to the transparent part namely anal vesicle at connecting to anal segment at the end of first instar close to molting. The first instar was completed about 3.25 ± 0.05 day (Fig. 3). Our result was according to description of Lim (1982) and supported it.

Second instar: The 2nd-instar larvae were named vesiculate form (Lim, 1982) because of horn tail at end of larval body in first instar replaced by vesiculate structure (anal vesicle) which attached to the midgut with a visible constriction. In early 2nd-instar, the ratio of body width to anal vesicle was 1.226±0.09 mm and in late 2nd-instar this ratio was 1.78± 0.09 millimeters. The sclerotized head was existing in early 2nd-instar. The mandibles were absent and mouthparts were included: Labrum and a pair of maxillae. The body was transparent at early instar but it became cream color with light green gut at middle course (seven days after parasitism) and the tracheal system was visible. When the larva became older sclerotized head and body were disappeared and became more Hymenopteriform. After One day, the body color white greenish with dark green gut. At the end of second instar and close to molting the body color became light to dark yellow and anal vesicle was smallest at size. The second instar duration was completed 2.78±0.10 days (Fig. 3).

Third instar: The 3rd-instar larva was named hymenopteriform according to Lim (1982) description, because they were tapered anteriorly with distinct segmentation and there were not anal vesicle and mouthpart. Second instar of parasitoid chewed DBM larval cuticle (4th instar larvae) and made an emergence hole on the lateral side of abdomen segments , thereafter molted to 3th instar during emergence from the host larva and spin a cocoon immediately beside the host. The body color of parasitoid larvae was yellowish green. While spinning cocoon, the color of larvae change to yellowish white. The parasitized larvae were alive with no feeding and low movement 2-2.5 days, approximately.

Cocoon: The cocoon was oval and its color was opaque white with a light green tint and was inclined to pale cream at end. The spinning of cocoon was lasted 0.158±0.0008 day. The cocoon dimension was 3.87±0.032 mm in length and 1.74±0.016 mm in most width.

Prepupa: From the cocoon formation time to shedding of the exuvium of 3rd instar was considered as prepupa. The prepupa has 13 segments and its body length was 3.25±0.024 mm. The body color was cream in head and thorax and white pus in abdomen at early and then whole of body became yellow approximately 4 h after pupation. The meconium (undigested food) was visible from through cuticle that filled gut. The *C. vestalis* meconium was black and moved to posterior end of cocoon, 0.32±0.015 days after pupation that it is obvious from out of cocoon by naked eye. The prepupa had two russet compound eyes were obvious approximately 2 days after pupation. The prepupa stage lasted 1.9±0.06 days (Fig. 3).

Pupation: The exuvium of 3rd instar attached to the posterior end of abdomen at end of cocoon. It was completely hard and visible unlike the exuvium of two previous instars that those were seen difficulty. Two compound eyes became dark jujube color and three dorsal ocelli became fawn antenna, wings and legs were formed at second day after cocoon formation. Then appear blackening spot in the body from head and thorax at 3rd day after spinning of cocoon and were continued to whole of body became black gently. About 4.03±0.11 day after cocoon formation, the adult wasp was emerged (Fig. 3).

Adults: The adult was black with transparent yellow in semi primary of abdominal segments at whose tergum pleura and sternum. The antenna was black and had 16 segments with uniform slender shape. The body length (head to end of abdomen) of females was more than that of males. It was 2.7±0.08 mm for females and 2.5±0.04 mm for males. Wing span of females and males were 5.8±0.12 mm and 5.76±0.09 mm, respectively. Unmated female and male longevity of wasp were 16.83±0.37, 16.25±0.17 and sex ratio is male-biased (Fig. 3).

Host age preference in choice and non-choice tests: In both choice and non-choice experiment, second and third instars of DBM a significantly higher percentage parasitism than 4th instars (Table 3). This resulted in a significantly higher preference of *C. vestalis* to 2nd and 3rd-instar. In choice test, *C. vestalis* preferred significantly 2nd-instars of *P. xylostella* than two others. The percentage parasitism of 2nd, 3rd and 4th instars was 78.58, 69.94 and 4.36%, respectively (Table 3). Percentage of mortality and adult emergence was not significantly affected at both experiments.

Parasitoid oviposition dynamic: In the experiment *C. vestalis* endoparasitoid laid significantly more eggs on the first day than on any of the other nine days that

Table 3: Influence of larval instars on percentage (Mean±SE) *P. xylostella* larvae parasitized by *C. vestalis* within a 2-h oviposition period in non-choice and choice tests

	Non-choice test exp.[1]			Choice test exp.[2]		
Host instar	Parasitism (%)	Mortality (%)	Adult emergence	Parasitism (%)	Mortality (%)	Adult emergence
L_2[3]	73.55±3.81 a[4]	8±2.9 a	94.9±3.43a	78.58±3.27a	9±2.77a	95.5±3.14a
L_3	62.52±3.27 a	10±2.58 a	95.7±2.31a	69.94±2.8 b	5±2.23a	96.8±3.10a
L_4	24.8±3.14 b	6±5.7 a	97.1±1.13a	4.36±1.79 c	4±2.21a	95.2±2.13a
F	54.97	.540	12.70	170.1	1.99	55.6
d.f[5]	2, 27	2, 27	2, 27	2,27	2, 27	2. 27
P	<01	0.589ns	<001	<001	0.318ns	<001

[1]Larval instars separated (L_2,L_3 and L_4) during parasitism period. [2]Larval instars mixed (L_2,L_3 and L_4) during parasitism period. [3] L_2, L_3 and L_4 denote the 2nd, 3rd and 4th instar moth larvae, respectively. [4]Means followed by the same letter within columns are not significantly different ($p<0.05$; Fisher-LSD Test). [5]The numbers given show the degrees of freedom of treatment and error, respectively

Table 4: Number of eggs (mean±SE) laid by *Cotesia vestalis* over 10-days period

Ovipositional time (day)	1	2	3	4	5	6	7	8	9	10
Eggs	13±0.96a	12.1±0.64a	10.2±0.57b	7.3±0.72c	7.2±0.51c	4.1±0.57d	2.3±0.62e	1±0.37f	0.3±0.15g	0.1±0.1h

Means marked with the same small letter within a same row are not significantly different ($p<0.05$; Fisher-LSD test)

followed ($F_{9,57}$ = 95.6, $p<0.001$; Table 4). Total egg production per female (Mean±SE) was about 58.2±1.34. Females oviposited for up 10 days; over 50% of the eggs were laid within first 4 days.

DISCUSSION

Higher oviposition preference of *P. xylostella* was occurred on lower surfaces of Chine's cabbage. This result is similar to results of Charleston and Kfir (2000), Andrahennadi and Gillott (1998) and Satpathy *et al.* (2010) but in other previous researches, oviposition preference of *P. xylostella* was higher on upper surface of tested crussifera leaves (Harcourt, 1957; Talekar and Shelton, 1993). In crussifera, the Glossy and waxy state of leaf surfaces caused the egg deposition by females was affected (Uematsu and Sakanoshita, 1989). Total Developmental time (egg to pupa) of *P. xylostella* on *B. pekinensis* cv. Spring smile was very close to result of Fathi *et al.* (2011) and Soufbaf *et al.* (2010) on cauliflower cultivar. The pupal period of DBM was shorter than (Karimzadeh and Wright, 2008) that they used *B. pekinensis* cv. Tip Top as host plant. Shorter development times indicate greater suitability of a host plant (Awmack and Leather, 2002; Talekar and Shelton, 1993). In this study, adult longevity of *P. xylostella* was significantly higher than other investigations such as of Fathi *et al.* (2011), (6.67±0.60 days) and Soufbaf *et al.* (2010), (6.29±0.41 days) on cauliflower cultivar. These differences might be referred to low fitness of DBM produced offspring on cauliflower cultivars. Another reason is referred to the quality and quantity of adults feeding, in our experiment, adult fed with 10% honey solution but in another experiment when we remove the honey solution as main food of adult they could not survive only 2-4 days and died (unpublished data). Winkler *et al.* (2005) have shown long time survival of adult completely dependent to sources of carbohydrate-rich food as main source of energy for longevity, fecundity and mobility. Male longevity was significantly higher than female longevity that it was in consistent with finding of Abdel-Razek (2003), Gholizadeh *et al.* (2009) and was opposite with results of Fathi *et al.* (2011) and Soufbaf *et al.* (2010). All of these differences among our result and research mentioned above are probably due to difference in cultivar of host plant and geographic populations of DBM that used in their experiment. *C. vestalis* larval development was rapid, requiring only 6.47 days to complete all three instar at 25±1°C. The morphology of first instar was very different from that of the other two instar. Similar result have been reported in *C. plutellae* (Lim, 1982; Chiu and Chien, 1972; Yu *et al.*, 2008) and other solitary larval endoparasitoid braconid (Hegazi and Fuhrer, 1985; Grossniklaus-Burgin *et al.*, 1994; Luo *et al.*, 2007). According to endocrine pathways, parasitoids regulators induced developmental interruption in the hosts. Some of these regulators consist of polydnaviruses (PDV), venoms, teratocytes (Dahlman and Vinson, 1993; Beckage, 1998; Rana *et al.*, 2002). For *C. vestalis*, we were able to confirm the presence of teratocytes several hours after egg hatching of *C. vestalis* that these giant cells envelop early first instar larva and then distributed in the host hemocell. However, we didn't study the egg layers of *C. vestalis* by electron microscope but one day later after hatching body segmentation is visible. Therefore, we concluded that the serosal membrane (which occurs below the chorion) began to dissociate to from teratocytes several hours after hatching

and then amnion layer (which occurs inside the chorion) is dissociated one day after hatching. The results of Yu *et al.* (2008), were supported our finding in this subject. Hagen (1964) said that the exact number of parasitic Hymenoptera instars is very crucial to determine. Like, Lim (1982) and Chiu and Chien (1972), we found that the *C. vestalis* has three larval instars. We couldn't observe the exuviae of *C. vestalis* larval instars in the host hemolymph therefore we determined times of molting based on Lim (1982) description for larval recognition. Quicke (1997) reported the some of braconidae family endoparasitoids have been shown to have anal vesicles in two first instars. In braconid, *C. vestalis* larvae, there is not anal vesicle in first instar, so we could be used anal vesicle for separating the first and second instars by using light microscopy. The anal vesicle has two basic functions including, secretory and uptake of nutrients from the host's hemolymph (Edson and Vinson, 1977; Kaeslin *et al.*, 2006). The gut development in Hymenoptera, including in parasitoid wasps is variable. In most Apocrita gut remain imperfect before pupation (Quicke, 1997). In the Apocrita species, merging of the mid and hindgut usually occurs at the end of the final instar, providing excretion of undigested material and nitrogenous waste in the form of meconia (Yu *et al*., 2008). Our observation confirmed the result of Yu *et al.* (2008) about excreting the meconia at the prepupal stage of *C. vestalis,* so in pupal stage gut is complete and voids the meconium in adult eclosion. Many hymenoptera parasitoids have the capacity to determine host nutrient quality during the oviposition period and will often accept or reject hosts on this foundation (Michael and Pech-Louis, 1995). The host nutrition quality may be altered in various hosts or different host instars (Harvey and Strand, 2002). Monnerat *et al.* (2002), tested 2nd to 4th instars and demonstrated that 2nd-3rd instars of *P. xylostella* were suitable for parasitization by *Diadegma* sp. In this study when a mixed group of instars was presented, the 2nd instar larvae was so preferred and the 4th instar was less suitable and less attractive for parasitoid. Probably, required time for parasitoid development, better immunological defense in larger hosts and ability of last instar to fight-off an approaching parasitoid, caused lower preference of *C. vestalis* to 4th instar than others. When given no choice and only a 4th instar larva was presented the percentage parasitism is higher than 4th instar in comparison with choice test. This result is consistent with other Monnerat *et al.* (2002), however in our experiment larval mortality in all tested instars did not increased at both mixed and isolated group. We concluded that some parameters including the size, age and mobility of *P. xylostella* larvae had significant influence on oviposition by *C. vestalis* and the parasitization rate decreased as the host size increased. This finding according to result of Liu *et al.* (2004) on *Heliothis armigera-Microplitis mediator* system. Another reason for decrease of parasitism of 4th instar *P. xylostella* by *C. vestalis* supports the approach that the host's immune system was strong enough by the 4th instar to interrupt development of the parasitoid. Webb *et al.* (2001) reported host's defense system which increases in performance with the age of the host. Therefore, according to this finding, it is acceptable to use 2nd and 3rd instars to mass rearing of *C. vestalis*, so these caused to optimize the percentage parasitism, parasitoid development time and survival. Gauld and Hanson (1995) reported that many endoparasitoids are pro-ovigenic which has been shown to be case for *Microplitis bicoloatus* as well Luo *et al.* (2005). In present study, *C. vestalis* females oviposited on the 3rd larvae of *P. xylostella* for up 10-days, but a high proportion of eggs were laid on the first three days. This rapid oviposition rate increases the potential of parasitoid for biological suppression of DBM populations because the likelihood of mortality for the parasites due to exposure to detrimental environmental factors or generalist predators increases with time. Muniappan *et al.* (2004) were observed the gregarious larval parasitoid, *Euplectrus maternus* (Eulophidae), to gently lay eggs on the second larvae of fruit-piercing moth, *Eudocima fullonia*, for up 30 days and the large number of eggs were laid during the first week after exposure. In the parasitoids mass rearing process, high reproductive capacity and shorter oviposition period are important factor for biological control program. In conclusion, the development of *C. vestalis* is in many respects similar to other braconids. By understanding the development of the *P. xylostella* and *C. vestalis*, we have produced a solid foundation for further studies on laboratory mass-rearing, ecological and physiological investigations on *P. xylostella* and *C. vestalis*. The rapid oviposition rate of parasitoid increases the potential for biological suppression of host. Present results suggest that *C. vestalis* has considerable potential as a biological control agent for *P. xylostella*.

ACKNOWLEDGMENTS

This publication is a part of the first author's Ph.D. thesis funded by the Department of Plant Protection of Tehran University that which we thank for financial support.

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