

Orijinal araştırma (Original article)

Cold storage possibilities of a larval parasitoid, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae)¹

Larva parazitoiti *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae)'in soğuk koşullarda depolanması

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Summary

The effects of cold storage on the biology of the larval parasitoid *Venturia canescens* were tested. Storage studies were conducted in two stages, pre-adult and adult stage of the larval parasitoid, *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae). Pre-adult stage of parasitoid was stored in last larval stage of the hosts, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) and *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) at 5, 10, 15°C for 1, 3, 5, 7, and 15 days. Similarly adult parasitoids were stored the same temperatures for honey feeding and non-feeding condition. Storage temperatures, Storage period and host effects on *V. canescens* development were evaluated. The parasitoid did not develop at 5°C for 5, 7 and 15 day storage period on both hosts. Similarly parasitoid did not develop at 10°C for 15 day storage period on the host *P. interpunctella*. Decreasing of temperature and increasing of storage time resulted in increasing in the parasitoid development time, but reducing emergence rate. This reduction of emergence rate was higher in *P. interpunctella*. Longevity and adult dry mass were less affected by low temperature in both hosts., the most suitable temperature for feeding and non-feeding condition on both hosts was found to be at 10°C for the adult. The results suggest that *E. kuehniella* could be more suitable host for *V. canescens*.

Key words: *Venturia canescens*; *Ephestia kuehniella*; *Plodia interpunctella*; parasitoid, Cold storage

Özet

Bu çalışmada parazitoit *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae)'in biyolojisi üzerine soğuk depolamanın etkileri çalışılmıştır. Depolama çalışmaları larva parazitoiti *V. canescens*'in ergin öncesi ve ergin döneminde gerçekleştirilmiştir. Parazitoitin ergin öncesi dönemi 5, 10, 15°C sıcaklıklarda ve 1, 3, 5, 7, 15 gün konukçuları *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) ve *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae)'nin son larva dönemi içinde depolanmıştır. Benzer olarak ergin parazitoitler aynı sıcaklık derecelerinde besinli ve besinsiz koşullarda depolanmıştır. Çalışma sonucunda depolama sıcaklığı, depolama süresi ve konukçu farklılığının *V. canescens*'in gelişimini etkilediği belirlenmiştir. Parazitoitin iki konukçuda da 5°C'de 5, 7 ve 15 gün depolama sürelerinde gelişim göstermediği belirlenmiştir. Benzer olarak parazitoitin konukçu *P. interpunctella* üzerinde 10°C' de 15 gün depolanmasıyla da gelişim göstermediği bulunmuştur. Depolama sıcaklığının düşmesi ve depolama süresinin artması parazitoitin gelişme süresini artırırken, çıkış oranını azaltmıştır. Çıkış oranındaki azalma konukçu *P. interpunctella* üzerinde daha fazladır. Yaşam süresi ve ergin ağırlığı iki konukçuda da düşük sıcaklıktan daha az etkilenmiştir. Ergin parazitoitler için hem besinli hem de besinsiz ortamda ve heriki konukçuda en uygun sıcaklık derecesinin 10°C olduğu belirlenmiştir. Sonuçlar, depolama çalışmalarında *V. canescens* için *E. kuehniella*'nin daha uygun bir konukçu olduğunu göstermektedir.

Anahtar sözcükler: *Venturia canescens*; *Ephestia kuehniella*; *Plodia interpunctella*; parazitoit, soğuk depolama

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Introduction

In recent years, biological control is more ordinarily utilized, especially in ecologically based integrated pest management. Parasitoids are extensively used in biological control programs. Mass rearing of parasitoids has been regarded a necessity for biological control against pests, particularly biological control applications supported on inundative releases (van Lenteren & Tommasini, 2002).

The main difficulty of the prosperous execution of mass releases is the capability to rear high numbers of parasitoids when requirement is maximum and cost of producing natural enemies in high numbers for augmentative release at the suitable time (Glenister & Hoffmann, 1998). Pesticides have a long shelf life, however many beneficial insects used in biological control have a comparatively short longevity, so that beneficial insects should be reared for a short time before they are utilized. The improvement of an effective method of storage can decrease the cost of biological control by spreading the long term (McDonald & Kok, 1990; Venkatesan et al., 2000).

Cold storage has pointed to be an important method for increasing the beneficial insects shelf-life. Storage at low temperature permits synchronized field releases of beneficial insects during the appropriate stage of a suitable host insect (McDonald & Kok, 1990; Venkatesan et al., 2000). Storage at low temperature can allow a more cost-effective insect production (Glenister & Hoffmann, 1998). It may also preserve natural enemies when not instantly needed. Commercial producers may supply exceptionally large numbers during peak demand periods (Pitcher et al., 2002).

Low-temperature storage of the beneficial insect is commonly applied. Permissiveness to low temperature is a very flexible trait determined by a broad range of factors (biotic and abiotic) experienced before, during, or after cold exposure (Colinet & Boivin, 2011). Various factors may affect the cold storage studies. Storage period, this is one of the most important factors for this research. For this reason, cold storage studies are divided into two parts such as short term storage and long term storage. Leopold (1998) defined short-term storage as less than 1 month and anything over that as long-term storage. In this study, short-term storage was used for *V. canescens*.

Solitary, koinobiont larval parasitoid *Venturia canescens* (Gravenhorst) (Hymenoptera: Ichneumonidae), is an endoparasitoid of several lepidopterous. Its host spectrum contains many moth species including *Ephestia cautella* Wk, *Ephestia elutella* Hübn, *Ephestia kuehniella* Zell, *Nemapogon granella* Linn, *Pyralis farinalis* Linn, *Aphomia sociella* Linn, *Esperia sulphurella* Fabr, *Peralipsa gularis* Zell, *Plodia interpunctella* Hübn, *Prays citri* Mill, *Homeosoma neubella* Hübn, *Vitula edmanse* Pack, *Clepsis peritana* Clem, *Galleria mellonella* Linn, *Achroia grisella* Fabr, *Corcyra cephalonica* Staint, *Ephestia figuliella* Greg, *Paramleyosis transitella* Wk, *Pexicopia malvella* Hübn, *Phthoraea operculella* Zell. (Frilli, 1965; Salt, 1975, 1976). Both arrhenotokous and thelytokous reproduction can be seen in this parasitoid (Beukeboom et al., 1999). Schneider et al. (2002) reported that arrhenotokous and thelytokous parasitoids have different geographical distribution. Particularly; in arrhenotokous parasitoids, there are different ecological requirements. Thelytokous parasitoids is spreading to larger areas. This situation is explained by two reasons. First, most of these studies made use of a few commonly split laboratory strains. The second reason is that several of the hosts of *Venturia* are stored product pests and the strains were generally gotten from bakeries or granaries (Press et al., 1982; Cline et al., 1983; Driessen et al., 1995; Harvey & Vet, 1997; Bonsall & Hassell, 1998). These places supply a relatively stable environment and several pyralid hosts (Freeman, 1980; Goater, 1986; Harvey & Thomson, 1995). Under such conditions arrhenotokous populations are likely to be quickly surmount by thelytokous ones. Regular shipments of flour between mills and bakeries can expand a thelytokous strain quickly over a large area once it has occurred. So bakeries may supply as source populations from which thelytokous parasitoids can get in the environment (Schneider et al. 2002). These parasitoids have a wide tolerance about changing in abiotic factors and differences in host distribution and specificity. Additionally, thelytoky strain of this parasitoid may have different biology (Beling, 1932; Gade & Parker, 1997; Jokela et al., 1997; Vrijenhoek, 1999; Beukeboom & Pijnacker, 2000; Barke et al., 2005).

Thelytokous parasitoid *Venturia canescens* is occurred in different storages of Turkey. In this study the parasitoids, obtained from grain storages of Ankara province were used. There are many studies on the biology and effectiveness of *V. canescens*. (Kansu & Uğur, 1985; Özkan & Gürkan, 2002; Özkan et al., 2003, Özkan, 2004; Gökçek, 2005; Boz, 2006; Ozkan, 2007; Sahin & Ozkan, 2007; Boz & Gülel, 2012). However, there is no record on the storage of the thelytoky strain of this parasitoid in Turkey.. The aim of this study is to find out the suitable cold storage conditions for mass rearing of *V. canescens*.

Materials and methods

Insect culture

Both hosts *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) and parasitoid *Venturia canescens* Grav. (Hymenoptera: Ichneumonidae) were obtained from the laboratory at Ankara University, Faculty of Agriculture, Department Plant Protection. The host *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) was reared at $25 \pm 1^\circ\text{C}$, 60-70% R.H., 16 h light and 8 h dark condition. Culturing was undertaken using clear plastic containers (27 x 37 x 7 cm) on a 2:1 mixture of wheat flour and rough wheat bran containing approximately 400 g food, which was sterilized at 60°C for 3 days, and 5.000 host eggs. *E. kuehniella* eggs were homogeneously dispersed in this food (Bulut & Kılınçer, 1987).

The host *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae) was reared in laboratory condition ($25 \pm 1^\circ\text{C}$, 60–70% R.H., 16 h light and 8 h dark). In the rearing of *P. interpunctella*, wheat bran, corn flour, dry yeast, honey, milk powder, and glycerin were mixed in a ratio of 2:1:0.25:0.50:0.25:0.25, respectively. Before using the mixed preparation, it was kept in an incubator. Sterilized plastic breeding containers with 15 x 20 x 7.5 cm in size were filled with 300 g of sterilized food and over this nearly $400 \geq 1$ -day-old *P. interpunctella* eggs were homogeneously dispersed using a soft tip brush (Ozkan, 2006).

The parasitoid *V. canescens* was cultured on mature larvae of *E. kuehniella* in plastic containers under laboratory condition ($25 \pm 1^\circ\text{C}$, 60–70% R.H., 16 h light and 8 h dark). In order to rear the parasitoid, 10 (4- to 5-day-old) adult parasitoids which had been daily fed with pure honey were transferred into the container including approximately 250 g sterilized food and approximately 300 twenty nine day-old mature larvae. After 24 h parasitization, parasitoids were removed from the container in order to prevent probable superparasitism (Ozkan, 1999).

Cold storage treatments

Cold storage treatments were carried out for pre-adult and adult stage of *V. canescens*. For pre-adult stage, fifth instars of *E. kuehniella* and *P. interpunctella* were singly parasitized by *V. canescens*. Following a successful oviposition, the parasitoid preens and transfers a new egg to the tip of her ovipositor via a characteristic flexing motion of the abdomen (the 'cocking' motion described by Rogers, 1972). Singly parasitized larvae were transferred to plastic boxes containing enough fresh food. The parasitized larvae were fed with food for two hours.

Treatments consisted of combinations of three temperature levels (5, 10, 15 °C) with five cold storage times (1, 3, 5, 7, and 15 days). There was also a control group (without cold storage). The control group was kept at standard rearing conditions ($25 \pm 1^\circ\text{C}$, 60–70% R.H., 16 h light and 8 h dark). Once the storage period was over, each treated group was transferred to the laboratory at standard condition. The effect of cold storage on the quality of the parasitoid was evaluated by measuring the following parameters in each replicate: development time, emergence ratio, adult longevity and adult dry mass. In order to determine parasitoid dry mass (mg), they were frozen upon emergence and later oven-dried for 5 days at 60°C (Harvey et al., 1993).

To evaluate the effect of cold treatments on the adult parasitoid, the parasitized larvae were incubated at the rearing condition until adult emergence. The newly (0-6h) emerged *V. canescens* adults were put into glass tube (1.5 X 11 cm) individually. The glass tube containing adult parasitoid was stored in cold incubator at 5, 10 and 15°C . The control group was kept at standard rearing conditions ($25 \pm 1^\circ\text{C}$,

60–70% R.H., 16 h light and 8 h dark). Parasitoid adults were stored at two different conditions (with food and without food). With food condition, parasitoids were fed on diluted honey solution (%10) at two day interval during storage.

Statistical analysis

Development time, emergence ratio, adult longevity and adult dry mass data were analyzed with one-way analyses of variance (ANOVA). Means were separated by using Duncan's Multiple Range Test and t-test was used for comparing two means. Percentage data were arcsine transformed before analysis. All statistical analyses were carried out using MINITAB computer software Release 14 (McKenzie & Goldman 2005). MSTAT C was used for variance between treatment means. All the analyses were carried out at the 5% significance level.

Results

Cold storage of pre-adult stage

The effects of cold storage on pre-adult stage are presented in Table 1. Emergence of *V. canescens* occurred in all treatments, except for those parasitized *E. kuehniella* larvae stored 5, 7, 15 days at 5°C. The development time (df=2, F= 49.12, P=0.000), longevity (df=2, F= 0.79, P=0.458) and dry mass (df=2, F=1.71, P=0.186) of parasitoids did not affect by the storage 1 day at 5°C. However, development time of the parasitoid was significantly affected by the storage 3 day at 5°C. Storage of parasitized larvae for 1 and 3 days at 5 °C resulted in significantly lower adult emergence rates than that of the control (df=2, F=14.45, P=0.005).

Development time of the parasitoid significantly increased with extending the storage period at 10°C (df=5, F=712.01, P=0.000) while longevity of parasitoid was not affected by 1-, 3-, 5-, 7-, and 15-day of storage at this temperature (df=5, F=1.44, P=0.210). There was significant difference in dry mass of parasitoid only between 7-day cold storage at 10°C and control group (df=5, F= 3.00, P=0.012). Emergence ratio of parasitoid in all storage periods decreased compared to control (df=5, F=5.99, P=0.005). Development time of parasitoid varied significantly among storage period at 15°C, except for 1 day (df=5, F= 655.34, P=0.000). Longevity of adult parasitoid was positively affected after 1-, 3-, 5-, 7-, and 15- day storage at 15°C (df=5, F=17.20, P=0.000). There was no significant differences in dry mass between 1-, 3-, 5-, 7- and 15-day storage at 15°C and control group (df=5, F=3.73, P=0.003). Similarly, there was no significant differences in emergence ratio between control group and 1-, 3-, 5- and 7-day storage at 15°C, except for 15-day storage (df=5, F=3.21, P=0.04).

As in the host *E. kuehniella*, there was no parasitoid emergence from parasitized *P. interpunctella* larvae after 5-, 7-, 15- day storage at 5°C. Development time of parasitoid was significantly affected after storage for 1 and 3 days at 5°C (df=2, F= 65.71, P=0.000). Similarly, longevity, dry mass and emergence ratio of parasitoid were significantly affected by 1- and 3- day storage at 5°C (df=2, F=18.83, P=0.000; df=2, F= 68.84, P=0.000; df=2, F= 18.99, P=0.003). There was no parasitoid emergence from parasitized *P. interpunctella* larvae after 15- day storage at 10°C. Development time of parasitoid was significantly decreased with increasing storage periods at 10 °C (df=4, F=88.54, P=0.000). Longevity of parasitoid was significantly reduced after 5-, 7- day storage at 10°C (df=4, F= 35.34, P=0.000). Significant differences in dry mass and emergence ratio of F1 progeny were found between storage treatments and the control group (df=4, F= 21.88, P=0.000; df=4, F= 25.51, P=0.000). Development time of the parasitoid was significantly affected by 3-, 5-, 7- and 15- day storage at 15 °C (df=5, F= 357.90, P=0.000). Longevity and dry mass of parasitoid were significantly reduced after 5-, 7- and 15- day storage at 15°C (df=5, F= 15.77, P=0.000; df=5, F= 9.40, P=0.000). Emergence ratio of parasitoid in all storage periods, except for 1- and 3-day storage decreased compared to control (df=5, F=5.80, P=0.000).

Table 1. Development parameters of F1 progeny *Venturia canescens* after stored at different temperatures and storage periods in host of *E. kuehniella* and *P. interpunctella*

Temperature (°C)	Storage period (day)	Development time (day) (Mean±SE)		Longevity (day) (Mean±SE)		Dry mass (mg) (Mean±SE)		Emergence ratio (%)	
		<i>E. kuehniella</i>	<i>P. interpunctella</i>	<i>E. kuehniella</i>	<i>P. interpunctella</i>	<i>E. kuehniella</i>	<i>P. interpunctella</i>	<i>E. kuehniella</i>	<i>P. interpunctella</i>
5	1	24.45 ± 0.155 B n = 55	24.33 ± 0.314 B n = 36	12.09 ± 1.20 A n = 53	7.77 ± 0.615 AB n = 36	2.0 ± 0.102 A n = 43	0.95 ± 0.040 B n = 35	61.00 B	52.33 B
	3	27.08 ± 0.679 A n = 12	27.45 ± 0.366 A n = 11	9.16 ± 0.386 A n = 12	6.00 ± 1.17 B n = 11	1.65 ± 0.096 A n = 8	0.82 ± 0.049 B n = 12	13.33 C	16.00 C
	5	-	-	-	-	-	-	-	-
	7	-	-	-	-	-	-	-	-
	15	-	-	-	-	-	-	-	-
Control	1	23.47 ± 0.111 B n = 73	21.28 ± 0.262 C n = 50	12.06 ± 0.876 A n = 73	12.04 ± 0.558 A n = 50	1.93 ± 0.047 A n = 57	1.49 ± 0.036 A n = 50	81.33 A	72.33 A
	3	26.71 ± 0.195 C n = 57	23.15 ± 0.190 C n = 26	11.65 ± 1.13 A n = 57	10.19 ± 0.304 A n = 26	2.17 ± 0.048 AB n = 52	1.10 ± 0.043 B n = 26	66.66 AB	37.66 B
	5	26.79 ± 0.198 C n = 49	24.80 ± 0.264 B n = 21	11.83 ± 1.07 A n = 48	9.90 ± 0.497 A n = 21	2.05 ± 0.069 AB n = 32	1.04 ± 0.055 B n = 19	54.33 B	30.33 BC
	7	28.90 ± 0.228 B n = 42	25.20 ± 0.368 B n = 15	10.61 ± 1.12 A n = 41	3.73 ± 0.228 B n = 15	2.15 ± 0.093 AB n = 30	1.06 ± 0.056 B n = 15	46.66 B	21.66 BC
	15	29.46 ± 0.195 B n = 52	30.66 ± 0.373 A n = 9	14.78 ± 1.28 A n = 51	3.55 ± 0.338 B n = 9	2.21 ± 0.082 A n = 40	1.08 ± 0.061 B n = 9	53.00 B	13.00 C
Control	1	38.46 ± 0.213 A n = 41	21.28 ± 0.262 D n = 50	12.58 ± 1.39 A n = 41	12.04 ± 0.558 A n = 50	1.98 ± 0.090 AB n = 31	1.49 ± 0.036 A n = 50	44.33 B	-
	3	23.47 ± 0.111 D n = 73	21.69 ± 0.369 E n = 42	12.06 ± 0.876 A n = 73	12.04 ± 0.558 A n = 50	1.93 ± 0.047 B n = 57	1.49 ± 0.036 A n = 50	81.33 A	72.33 A
	5	22.94 ± 0.149 E n = 72	22.66 ± 0.227 D n = 39	19.56 ± 0.806 A n = 71	12.04 ± 0.388 A n = 42	2.10 ± 0.065 AB n = 66	1.38 ± 0.044 AB n = 36	73.66 AB	61.00 AB
	7	25.04 ± 0.108 D n = 75	25.35 ± 0.079 C n = 69	19.64 ± 0.895 A n = 71	11.12 ± 0.375 A n = 39	2.14 ± 0.071 A n = 61	1.36 ± 0.095 AB n = 26	74.33 AB	58.00 AB
	15	32.73 ± 0.127 A n = 68	28.06 ± 0.148 B n = 68	12.14 ± 0.971 B n = 68	9.18 ± 0.297 B n = 37	2.18 ± 0.089 A n = 58	1.20 ± 0.036 BC n = 36	70.33 AB	54.00 B
Control	1	30.02 ± 0.264 C n = 68	34.41 ± 0.221 A n = 48	12.53 ± 0.860 B n = 65	8.31 ± 0.457 B n = 32	1.90 ± 0.062 AB n = 50	1.22 ± 0.052 BC n = 28	75.66 A	46.00 B
	15	23.47 ± 0.111 E n = 73	21.28 ± 0.262 E n = 50	12.06 ± 0.876 B n = 73	12.04 ± 0.558 A n = 50	1.84 ± 0.097 B n = 33	1.07 ± 0.045 C n = 29	53.33 B	45.00 B
								81.33 A	72.33 A

*: Means followed by same letter within columns are not significantly different.

-: Parasitoid eclosion was not seemed.

Cold storage of adult stage

Temperature during storage significantly affected the longevity of *V. canescens* reared on *E. kuehniella* and *P. interpunctella* with food and without food condition (df=3, F =23.46, P=0.000; df=3, F=233.42, P=0.000; df=3, F =19.71, P=0.000; df=3, F=191.99, P=0.000, respectively) (Table 2 and Table 3). The longevity of *V. canescens* significantly decreased in food condition at 5 °C. However, parasitoid lived longer compared to control group in without food condition at 5 °C. The highest mean longevity was obtained for *V. canescens* stored at 10 °C on both hosts. Mean longevity of parasitoids stored at 10 and 15°C with food condition was longer than that without food condition on *E. kuehniella* and *P. interpunctella* (df=47, T=5.46, P=0.000; df=58, T =9.49, P=0.000; df=57, T =2.84, P=0.006; df=58, T=6.90, P=0.000, respectively) except for 5 °C (df=58, T=0.31, P=0.759; df=58, T=1.84, P=0.071, respectively). Similar result was obtained for control group on *E. kuehniella* and *P. interpunctella* (df=59, T=9.7, P=0.000; df=58, T=14.95, P=0.000, respectively) (Table 2; Table 3).

Table 2. Longevity of *Venturia canescens* stored at different temperature in the host *Ephestia kuehniella*

Temperature (°C)	Longevity (Mean±SE)	
	With food	Without food
5	8.067 ± 0.745 Da n=30	7.833 ± 0.136 Ba n=30
10	22.263 ± 1.914 Aa n=19	13.400 ± 0.466 Ab n=30
15	18.600 ± 1.168 Ba n=30	6.433 ± 0.422 Bb n=30
Control	12.880 ± 1.447 Ca n =25	1.722 ± 0.162 Cb n =36

*:Means followed by same letter with lower-case letters in the rows and capital letters in the columns do not differ statistically

Table 3. Longevity of *Venturia canescens* stored at different temperature in the host *Plodia interpunctella*

Temperature (°C)	Longevity (Mean±SE)	
	With food	Without food
5	7.767 ± 0.354 Ca n=30	6.866 ± 0.338 Ba n=30
10	15.103 ± 0.904 Aa n=29	12.300 ± 0.421 Ab n=30
15	11.467 ± 0.731 Ba n=30	6.000 ± 0.303 Bb n=30
Control	13.833 ± 0.803 ABa n=30	1.733 ± 0.095 Cb n=30

*: Means followed by same letter with lower-case letters in the rows and capital letters in the columns do not differ statistically

Discussion

In this study the larval parasitoid *V. canescens* was stored in the short term. Cold storage tolerance of *V. canescens* showed differences depending on temperature, storage period and host. Bigler (1994) reported that cold storage resistant/tolerance of insects may be influenced by a range of internal such as mass and body reserves, life-history strategy, nutrition, mode of reproduction, age/stage, dormancy status, gender and external factors like temperature, duration of exposure, acclimatization, developmental temperature, constant or fluctuating cold exposure, combined cold exposure, humidity, photoperiod, chemicals, oxygen concentration, handling. Cold storage of *V. canescens* at 5 °C, for more than 3 days on both hosts, had detrimental effects on development. Parasitoid completed their development at other

tested temperatures, except for at except for 15 days at 10 °C in the host *P. interpunctella*. Colinet & Boivin (2011) reported that for the purpose of augment shelf-life of beneficial insects benefit from the cold storage, such as temperature ranging from 0 to 15°C. But these moderately low temperatures have a detrimental effect on lifespan of beneficials (Leopold et al., 1998; van Lenteren & Tommasini, 2002). The lower the storage temperature resulted the higher the mortality in many studies. (Ballal et al., 1989; Venkatesan et al., 2000 Rundle et al., 2004, Lopez & Botto, 2005; Bernardo et al., 2008).

At all tested temperatures, 1, 3, 5, 7, and 15 days of storage periods were found to be extremely important for development of *V. canescens*. Similarly, Colinet & Boivin (2011) showed that storage period was also grave component of cold storage studies. Temperature and storage period are thought together two concepts for cold storage. For this reason dose of cold exposure can be explained by a combination of storage period and temperature (Kostal et al. 2004, 2006). With the increasing the dose of cold exposure, negative effect of cold storage enhances on natural enemies. Several studies on cold storage at different exposure times reported that survival rates of the parasitoids were reduced with exposure time (Okine et al., 1996; Langer and Hance, 2000; Pitcher et al., 2002; Colinet et al., 2006, Foerster and Doetzer, 2006; Ayvaz et al., 2008; Abd El-Gawad et al., 2010). Similarly in this study, development time and emergence ratio of *V. canescens* were significantly affected by low temperature and exposure time in both hosts. Development time increased significantly with increasing exposure time within the range of 1-15 days. There was no *V. canescens* development after 5-, 7- and 15-day storage at 5°C in both hosts and 15-day storage at 10°C in host *P. interpunctella*. Mortality of the parasitoids can be attributed to the ultimate cost of prolonged cold exposure. In addition to chilling injuries, parasitoids stored as immature may not have sufficient energy resources to complete their development and/or to emerge (Colinet & Boivin 2011). Many studies reported that mortality generally increases with increasing cold storage period in both solitary and gregarious parasitoids (Langer & Hance, 2000; Colinet et al., 2006, 2007b; Foerster & Doetzer, 2006; Colinet & Hance, 2010).

Nutritional resource was also important factor for biological properties of progeny in cold storage. The host represents the nutritional resource in the parasitoid (Coudron et al., 2007). In this study, cold storage of *V. canescens* was carried out on two different hosts. The host differences affected development time, longevity, progeny size/weight and emergence ratio of *V. canescens*. But longevity and weight of *V. canescens* were slightly affected by cold storage in the host *E. kuehniella*. Statistical analysis showed no significant difference in the weight of parasitoid but larger parasitoids were obtained compared to control. However, weight of *V. canescens* decreased in the host *P. interpunctella*. As describe above, this situation depends on dietary source of immature stage of parasitoid.

Liu et al., 2007 reported that variation in nutritional resource can influence low temperature tolerance by regulating low-molecular-weight sugars and polyols levels (e.g. trehalose and glycogen), body size and lipids levels. Atkinson (1994) and Angilletta (2009) reported that insect body size increased as temperature decreases and this situation was also applied for insect parasitoid (Bazzocchi, 2003; Colinet et al., 2007a).

Cold storage studies can be divided into two main parts in parasitoid; immature stage and adult stage. In this study, cold storage of adult *V. canescens* was also tested. Storage temperature had a significant influence on the longevity of *V. canescens* in both condition and host. The highest mean longevity was obtained for *V. canescens* stored at 10 °C. If adults were fed with honey and water before, during and after storage, tolerance to low temperature significantly increased. (Shalaby & Rabasse, 1979; Ganteaume et al., 1995; Riddick, 2001; Uçkan & Ergin, 2003).

Many studies conducted on the storage of beneficial insects indicated that emergence, lifespan and/or reproduction of cold-stored pest and beneficial insects were negatively affected (Leopold, 1998). For instance, storage of *Trissolcus basalus* (Wollaston) and *Telenomus podisi* Ashmead (Hymenoptera:

Scelionidae) pupae at 12°C and 15°C for 4-7 moths showed different results. There was no emergence of adults at 12°C (Foerster et al., 2004). Gautam (1986) showed that there was no significant difference in the development of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) stored at 10°C for 7 days within host eggs of *Spodoptera litura* (F.) (Lepidoptera: Noctuidae) when compared to control. In other study reported by Liu & Tian (1987), *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) within the mummified aleyrodid host was stored at temperatures ranging from 3-12°C and the ratio of adult development was positively correlated with storage temperature and negatively correlated with the exposure time. When this species was stored at <12°C for 20 days, there was no significant difference in the development of the parasitoid when compared to control group. The tiny parasitoid wasps, *Trichogramma* spp. were stored at 10°C for 30 days on the host *Ephestia kuehniella* and no adverse effect was observed on the development of parasitoid (Vigil, 1971).

All these studies confirm that storage conditions of parasitoid can vary with the species, stage, temperature and exposure time. Low temperature storage is important scientific method in mass-rearing of biological control agents. It supports flexibility and efficiency in mass production and allows to synchronize releases with appropriate host presence (Leopold, 1998). Based on our laboratory experiments, storage of *V. canescens* will permit researchers to profit flexibility and admit of them to provide a completely biological control agent on request. The affectivity of any biological control agent utilized for insect control purpose depends on being extricated at the suitable time. We believe that the result of this study is going to provide fine data to help in the mass-rearing and timely release of *V. canescens*.

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