

## Effects of Abiotic Factors and Host Preference on the Biology of the Oriental Cabbage Webworm, *Hellula undalis* Fabricius (Lepidoptera: Pyralidae)

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### ABSTRACT

Because of the importance of *Hellula undalis* (Lepidoptera: Pyralidae) as a serious pest of crucifers in Iran and inadequate information, this study was conducted to investigate its biology under laboratory and greenhouse conditions on different host plants during the years 2007-2008. The result showed that the maximum percentage of larval mortality and survival were observed at  $15\pm 0.5^{\circ}\text{C}$  and  $25\pm 0.5^{\circ}\text{C}$ , respectively, at each given temperature, different relative humidity produced a significant effect on the mortality while the influence of same relative humidity at different temperatures was not significant. At  $20\pm 0.5^{\circ}\text{C}$ , pupae were heavier than  $25\pm 0.5^{\circ}\text{C}$ . The greenhouse study showed that mean larval period on cabbage and rapa was significantly longer and shorter than on the other hosts, respectively. The pupal weight differed significantly at  $22.5^{\circ}\text{C}$  between chou moellier and other host plants. At  $24.3^{\circ}\text{C}$ , a significant pupal weight was found between rapa and cauliflower in comparison to cabbage and chou moellier.

**Keywords:** *Hellula undalis*, Abiotic factors, Temperature, Relative humidity, Development, Mortality, Host preference, Cruciferous.

### INTRODUCTION

The oriental cabbage webworm (OCWW), *Hellula undalis* Fabricius is one of the most important pests of cruciferous crops. Females of *H. undalis* live for 7-9 days and lay an average of 150 eggs per female (Sivapragasam, 2005). The mated females of OCWW have high reproductive potential. Females lay their eggs randomly in irregular groups or in chains or even singly on the leaves of host plants at a time. In the main fields of cruciferous plantation, eggs were mostly deposited on

the buds, stems and petioles of the leaves (Youssef *et al.*, 1973). After hatching, the whitish yellow larvae turn to greyish yellow. The majority of authors reported five larval instars for this pest. The first and second instars larvae mine in the leaves thus they are protected from pesticides. Later larval instars are found on the leaves, which can move from the leaves and feed on and cause death of the plant or prevent the plant development. Larval development is dependent on temperature, which is slowed down below  $20^{\circ}\text{C}$ , while the optimal temperature has been determined between 25 to  $35^{\circ}\text{C}$ .

Like larval development, the speed of pre-pupal and pupae development and longevity of OCWW adults depends on abiotic and biotic factors including temperature, relative humidity and food availability, respectively (Kalbfleisch, 2006). Sivapragasam (2005)

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demonstrated that OCWW larval and pupal developmental periods were 9.76, 12.57 and 6.3, 7.6 days in the laboratory and the field, respectively. OCWW pupates more frequently in the soil but sometimes larvae made a tunnel inside the plant stem, which prepare a suitable locality for pupation. The leaf of the plant may occasionally be used as a hideaway for pupation. The rate of development and survival of Lepidopteran larvae are considerably influenced by leaf structure of plant hosts (van Loon *et al.*, 1992).

The cultivated crops of Brassicaceae family such as cabbage, chou moellier, radish and turnip as well as Capparidaceae family were identified as host plants of OCWW (Mewis *et al.*, 2003). The plant family Capparidaceae contains specific secondary plant substances, such as mustard oils or glucosinolates (Redovnikovi *et al.* 2008). The content of mustard oil in these plants attracts the adult of Pyralid moth like *Hellula spp.* (Redovnikovi *et al.* 2008). Sivapragasam and Aziz (1990) reported the host preferences of OCWW to Chinese mustard followed by cabbage and radish. Several authors (Mewis *et al.*, 2001a, b, 2002) demonstrated that OCWW females differentiated among host plants for egg laying behaviour with regards to the glucosinolate contents of different host plants like Brassicaceae and Capparidaceae families (Kalbfleisch, 2006), which played a crucial role in the host plant detection for egg laying by some insect species. Many Lepidoptera showed a remarkable instinct for oviposition on the host plants that were used as food by their larvae (Renwick and Chew, 1994; Xue *et al.*, 2010).

Shortage of food availability, wet weather, pathogens and predators caused death to OCWW larval (Sivapragasam and Chua, 1997a; Mewis *et al.*, 2003). The larvae died under hot weather (over 32°C). Entomopathogenic virus might cause a delay in larval development. The OCWW Infected by virus in the egg

hatching stage, then the larvae would die when were in the late second instars (Mewis *et al.*, 2003). The high mortality of young larvae that was observed could be attributed only to the direct influence of weather.

In Iran, OCWW larvae have been found during the growing season, from September to May. However, the moths were particularly very active in November, December and January, and this was the time of maximum growth of the host crops. By the beginning of February, the evaporation rate could be increase by the increasing heat as well as the moisture content decreased. At this time, larvae were able to destroy their hosts. As mentioned above, during the larval development, moths were voracious which caused considerable damage between transplanting, and the heading stage of cabbage (Singh and Rose, 2009). Because of damage by a single larva boring through the growing shoot of the cabbage plant in the pre-heading stage, either death or production of multiple heads that were not marketable may occur, and then there was no economic threshold level to initiate insecticide treatments (Sivapragasam, 2005).

The infested host plants by OCWW might increase their net above ground in the response to defoliation. Various mechanisms including intrinsic mechanisms such as photosynthetic enhancement (Oosterheld and McNaughton, 1988; Trumble *et al.*, 1993) and extrinsic mechanisms like food type recycling (Holland *et al.*, 1992) have been identified that could contribute to such compensatory growth. Therefore, shoot growth could lead to increase root growth (McNaughton *et al.*, 1998) and all well-nourished plants had a higher capability of compensation than food type were limiting (Meyer, 1998, 2000). If Brassica plants grow in fertile soils, they will be able to maintain leaf growth rates when infested with larvae of *H. undalis* (Mochiah *et al.*, 2011).

In view of the importance of larval OCWW damage,

our research was conducted to investigate the influence of abiotic factors and host preference on development and mortality of *H. undalis* larvae under laboratory and greenhouse conditions. Furthermore, the effect of these parameters on OCWW pupae was studied.

## MATERIALS AND METHODS

### Experiment 1: Effect of temperature and relative humidity on larval mortality

There were twelve treatments including three temperatures of  $15\pm 0.5^{\circ}\text{C}$ ,  $20\pm 0.5^{\circ}\text{C}$  and  $25\pm 0.5^{\circ}\text{C}$  and four relative humidities (RH) at each temperature namely; 13-20%, 32-34%, 43-45% and 59-61%. The treatments were maintained using a saturated salt solution for each RH. Each temperature was kept constant in four bottles with the same RH and fresh air was bubbled through cotton and glycerol and then through the saturated solutions. The air was ultimately passed into a container (8" x 7"), in which its bottom was filled with one inch of solution. To maintain the solution, water was added to them to balance what was evaporated. For this experiment, three-days old larvae were collected from a stock maintained in the insectary, and 16 larvae were used for each treatment. The larvae were placed on a chou moellier leaf and were kept in a 3.5" x 3.5" jar, which was covered by a piece of muslin and kept on a platform inside the container. The chou moellier leaves were changed every morning so that there was no shortage of fresh food for the larvae. To avoid the occurrence of pathogenic disease among the larvae, the jar was changed daily. More precautions were done via washing the jars thoroughly and rinsed with 0.5% sodium hypochlorite to protect against any pathogenic and fungi contamination.

Daily counts were made of the dead larvae. The experiment was conducted twice so 32 larvae were finally used in each treatment, and two runs of the

experiment were treated as an experiment, since no significant differences were observed between them.

### Experiment 2: Effect of temperature and relative humidity on pupal weight

Under same conditions as mentioned in experiment 1, the effects of temperature and RH on pupal weight were studied. In this experiment, the pupae were weighed immediately after they had pupated using an analytical scale (Phoenix, GH-202).

### Experiment 3: Effect of different host plants on larval and pupal development

Larvae of *H. undalis* were reared in a greenhouse on six-week-old plants of four different crucifers namely cabbage (*Brassica oleracea* var. *capitata*), cauliflower (*B. oleracea* var. *botrytis*), chou moellier (*B. oleracea* var. *acephala*) and rapa (*B. rapa* var. *rapa*). Each larva was placed on a healthy and inner leaf on the plant after it had hatched and was enclosed in a leaf cage of 1.25 inch diameter which was sufficiently large so that even the fully developed larva was able to move around easily inside. In the early instars (first and second), the larva ate very little, so that there was no need to shift the leaf cage daily.

Leaf cages were used on a fresh portion of the host plants, and then fifth instars shifted to them. When the larva pupated, the pupa was removed and its weight was recorded in milligram. Eighty larvae were used for this experiment, so there were twenty for each plant host. Five samples of each host plants were used. To keep the plants healthy from pathogenic fungi, they were carefully washed.

Two experiments were performed for this study. Experiment "A" was conducted in March 2008 while the mean temperature of the glass house was  $22.5^{\circ}\text{C}$ . Experiment "B" was conducted during May 2008 with a mean temperature of  $24.3^{\circ}\text{C}$  at the glass house. The period of larval development and the pupal weight were recorded in a same method as explained above.

### Data Analysis

Data analysis was calculated using a completely randomized factorial design with three replications, which its factors included temperature and RH % treatments. The means were separated using Duncan's multiple range tests at the 5% probability level. Differences between treatments were analyzed using ANOVA with statistical software SPSS 16.0, IBM (www.ibm.com).

## RESULTS AND DISCUSSION

### Effect of temperatures and relative humidities on larval mortality

Table (1) shows the results of OCWW larval mortality at different temperatures and relative humidities. The maximum percentage of mortality was observed at  $15\pm 0.5^\circ\text{C}$ . These results were significant at  $P=0.05$  level. The mortality at different temperatures varied significantly. At  $15\pm 0.5^\circ\text{C}$ , the larvae were less active, and they ate much less food. The smaller larvae, particularly in the first instar, hardly tolerated such a low temperature. It was hypothesized that the larvae were not able to convert nitrogen from leaves of host plants (Freitas *et al.*, 2005) when probably the metabolic activities were low.

It was concluded that when the maximum level of temperature was above  $15^\circ\text{C}$ , the mortality of OCWW declined. However, by rising the daily temperature, the rate survival of larvae increased. From this study, it was found that when the average daily temperature was about  $25^\circ\text{C}$ , the percentage of larval mortality decreased. The relatively higher larval survival at  $25^\circ\text{C}$  could be due to the quality of food and the occurrence of thermotaxis activities during the larval period which is needed further investigations in the future. As stated by Sivapragasam (2005), the faster rate of *H. undalis* larval development was observed at  $28\pm 1^\circ\text{C}$  and  $80\pm 5\%$  RH.

In our study, at  $25^\circ\text{C}$ , OCWW completed its larval period faster than  $15^\circ\text{C}$  (Table 1). Singh and Rose (2009) stated that the average total larval duration of *H. undalis* at room temperature on the heart of cabbage was  $15.63\pm 1.82$  days but on cauliflower it required only 10-13 days. The larval diet as well as an optimal temperature in larval period seems to be adequate to determine the duration of larval development under controlled conditions.

At each given temperature, different relative humidities produced a significant effect on the mortality. Furthermore, among the different temperatures tested, the same relative humidity showed no significant difference (Table 1). Interaction temperatures and the same relative humidity showed the efficiency of low temperature with a range of 13-44% of humidity for causing the maximum percentage of larval mortality. These findings confirmed the influences of temperature and relative humidity on mortality in insect species (Speight *et al.*, 2008). Gullan and Cranston (2010) stated that at low relative humidity, larval development might be retarded.

Our results showed that the maximum percent of larval survival was observed at  $25^\circ\text{C}$  and a range of 43-60% RH. Temperature is also involved in the larval development. We found the mean larval development lasted about 32, 22 and 14 days at 15, 20 and  $25^\circ\text{C}$  under RH ranged between 13-61%, respectively. Variation in development time from first to fifth larval instars showed a direct correlation with temperature. The larvae grown at lower temperature take a comparatively longer period to complete their development. It has been suggested that the larval period is longer at lower temperature, and then the larvae consume more food (Kalbfleisch, 2006).

Sivapragasam (2005) determined five larval instars of OCWW. The mean larval period was significantly shorter in the laboratory as compared with the field (9.76

and 12.57 days, respectively). Larval survival was higher in the field (92.5%) than in the laboratory (68.2%). In the laboratory, the mean pupal duration was significantly longer (7.6 days) than in the field (6.3 days). The results showed that moths were more active in the plot of cabbage and cauliflowers (Sivapragasam, 2005).

Moreover, Wali *et al.* (2000) studied the effect of high humidity and temperature on the economic character of silkworm *Bombyx mori*. Their findings showed that the mean temperature of 30° C with low RH decreased larval weight (13%), cocoon weight (24%) and fecundity (48%) in *B. mori*.

**Table 1. Larval mortality of *Hellula undalis* at different temperatures and relative humidities under laboratory conditions.**

Tem. (°C)	RH1 (%)	LM (%)	days to death	Larval period	RH2 (%)	LM (%)	days to death	Larval period	RH3 (%)	LM (%)	days to death	Larval period	RH4 (%)	LM (%)	days to death	Larval period
15	13	74	b 10.6	34( <i>a</i> )	34	84	a 11.8	33( <i>a</i> )	44	84	a 10.2	31( <i>a</i> )	61	57	c 10.3	31( <i>a</i> )
20	20	54	a 6.8	23( <i>b</i> )	32	53	ab 7.4	22( <i>b</i> )	44	50	b 6.9	22( <i>b</i> )	61	31	c 7.3	21( <i>b</i> )
25	17	37	a 5.8	14( <i>c</i> )	33	31	ab 6.5	15( <i>c</i> )	43	12	c 5.5	15( <i>c</i> )	60	15	c 7.0	15( <i>c</i> )

Tem.: Temperature, LM: Larval mortality, RH.: Relative humidity.

The **small** and **capital** letters indicate differences at  $P=0.05$  between relative humidities of each temperature and between same relative humidity within different temperatures, respectively. The same letters indicate that the means are not significantly different at  $P<0.05$ .

The *small italic* letters in brackets indicate differences at  $P=0.05$  between larval period at different temperatures.

### Effect of temperatures and relative humidities on pupal weight

The pupae had higher body weights at  $20\pm 0.5^{\circ}\text{C}$  than  $25\pm 0.5^{\circ}\text{C}$  and followed by  $15\pm 0.5^{\circ}\text{C}$  (Table 2). Non-significant variations were observed between the pupal weights at different RHs percent within each temperature. The results showed significant variation in pupal weight between different temperatures within the same relative humidity. Maximum pupal weight was

observed at  $20^{\circ}\text{C}$  with a range of 44-61% relative humidity. As stated before, maximum percent of larval survival at  $25^{\circ}\text{C}$  and relative humidity ranged between 43-60% may provide a favorable condition to pupate by OCWW. The fast emergence of the adult was observed in heavier pupas rather than lighter ones. We found the mean pupal period lasted about  $17.36\pm 0.41$  days at  $20^{\circ}\text{C}$  and relative humidity ranged between 43-60%.

**Table 2. Mean pupal weight of *Hellula undalis* at different temperatures and relative humidities under laboratory conditions.**

Tem. (°C)	RH1 (%)	MPW	RH2 (%)	MPW	RH3 (%)	MPW	RH4 (%)	MPW
15	13	a	34	a	44	a	61	a
		C	145.4	B	148.4	C	147.5	C
20	20	b	32	b	44	ab	61	a
		A	155.2	A	157.2	A	160.2	A
25	17	b	33	b	43	a	60	ab
		B	148.2	C	153.7	B	150.6	B

**Tem.:** Temperature, **MPW:** Mean pupal weight, **RH.:** Relative humidity.

The **small** and **capital** letters indicate differences at  $P=0.05$  between relative humidities of each temperature and between same relative humidity within different temperatures, respectively. The same letters indicate that the means are not significantly different at  $P < 0.05$ .

Shirai and Yano (1994) reported a hibernation step in the last larval instar or pupal stage, which its duration depends on temperature. Sivapragasam and Chua (1997b) found the development pupal period of OCWW as  $9.33 \pm 0.19$  days on leaf and  $8.59 \pm 0.28$  days on shoot of cabbage at  $29.1^\circ\text{C}$  and 71.8% RH. In another study, Sivapragasam (2005) determined this period about 7.6 days which at  $28 \pm 1^\circ\text{C}$  and  $80 \pm 5\%$  RH was significantly longer ( $P < 0.05$ ) than in the field (6.3 days). Singh and Rose (2009) reported the pupal duration of OCWW in  $6.50 \pm 0.50$  days at room temperature and relative humidity about 60-70%. Like larval development, the pupal development depends on temperature while development below  $20^\circ\text{C}$  would be slowed down in comparison to higher temperatures (Kalbfleisch, 2006) as was confirmed in our study. This indicates the effect of relative humidity on survival and development OCWW pupa.

#### **Effect of different host plants on larval and pupal development**

Mean larval period and pupal weight reared on different

host plants are given in Tables (3). The result of experiment **A1** and **A2** revealed a significant difference that showed at both  $22.5^\circ\text{C}$  and  $24.3^\circ\text{C}$ , mean larval period on cabbage was significantly longer than on the other hosts. Our results are in agreement with the results of El-Sherif *et al.* (1976) who reported that the effects of four different food-plants on the development of *H.undalis* in Egypt. Their results demonstrated that cabbage was the most suitable food-plant followed by cauliflower, radish and garden rocket (*Eruca sativa*), respectively. However, Peter *et al.* (1987) found that the development of this pest were preferred as a good food plant on cauliflowers in comparison to radishes, cabbages and knolkhol.

In our study at  $24.3^\circ\text{C}$ , mean larval period on rapa were significantly lower than other host plants whereas at  $22.5^\circ\text{C}$  no significant differences were found between rapa, chou moellier and cauliflower (Table 3). Youssef *et al.* (1973) did one of basic study on biology of *H. undalis* under the laboratory conditions on leaves and leaf-petioles of cabbage, radish, turnip, garden rocket,

and cauliflower. They explained the influence of the host plant on the duration of the larval period at 26.7°C and relative humidity 63%. In line with our finding, OCWW larvae fed on cabbage, cauliflower, turnip, radish and garden rocket lived for 17.8, 12.3, 13.6, 14.5 and 13.7 days, respectively.

As stated by Kinjo and Arakaki (2002) the development of cucumber moth larvae (*Diaphania indica*) slowed down at high temperatures, and the development time at high temperature (35°C) was significantly greater than low temperature (30°C). However, we found the opposed results with their statement. In a comparison between our results and Youssef *et al.* (1973) findings which showed the development time of OCWW at low temperature (22-24°C) was greater than high temperature (26.7°C).

Mohamed *et al.* (2013) confirmed that the different species of plant hosts might play a credible role on the survival rate, the rate of development and the number of eggs laid by the adults of pests. Awmack and Leather (2002) explained the reasons for such variation. They stated that host plant quality and its components like secondary metabolites, nitrogen and carbon is known as key determinants, which directly affects the fecundity and reproductive strategies of insects as well as their development periods. Sivapragasam *et al.* (1994) reported that when temperatures below 20°C, OCWW infestation were transient because of low development rate of plant species. The highest Glucosinolates (GS) content as a secondary plant metabolite might encourage the larvae to feed on young plant hosts such cabbage in comparison to rapa during this time (Mewis *et al.*, 2002; Sivapragasam and Chua, 1997b).

Furthermore, table (3) presents the results obtained from the pupal weight on the host plants. The results of

experiment **B1** revealed that the pupal weight differed significantly at 22.5°C ( $P=5\%$ ) between chou moellier and other host plants. under this temperature, pupa gain more weight on this plant rather than others. No significant differences were found between cauliflower, rapa and cabbage. At 24.3 °C (experiment **B2**), a significant pupal weight was found between rapa and cauliflower in comparison to cabbage and chou moellier (Table (3)). The reason why rapa and cauliflower were more attractive for OCWW to obtain weight is ambiguous. Pupa gain and lose the weight on chou moellier at 22.5°C and 24.3°C, respectively. It was concluded that the quality of food during larval and pupal development was protracted at the lower temperature. Therefore, if the food values of cauliflower, cabbage and rapa leaves were equal, the pupae fed on chou moellier leaves should be heavier, since at 22.5°C was found. A possible explanation for significant differences between pupal weight at 22.5°C and 24.3°C might be that positive influence of low temperature on plant growth and releasing plant volatile. Our results showed that shorter larval period on the host plants; provide sufficient time for pupae to feed enough during its development.

In India, the biology of *H. undalis* on cauliflowers in the laboratory showed that the larval, prepupal and pupal periods were took a range of 6.00 to 18.33, 1.00 to 3.37 and 4.10 to 19.75 days, respectively (Singh *et al.*, 1990). We found pupal periods on the host plants varied between  $17\pm 2.1$  and  $18\pm 0.7$  days at 22.5°C and 24.3°C, respectively. The pupal period showed no significant differences on the different host plants. It is possible to hypothesize that these conditions are less likely to reveal significant differences between pupal duration on the host plants, which might be needed to investigate in future study.

**Table 3. Mean larval period and pupal weight of *Hellula undalis* reared on different host plants.**

Measurement	Tem. (°C)	Host plants			
		Cabbage	Cauliflower	Chou moellier	Rapa
Mean larval period (days ± SE)					
Experiment A1	22.5	20.0±0.38 <i>a</i> A	18.6±0.58 <i>b</i> A	18.8±0.50 <i>b</i> A	18.8±0.50 <i>b</i> A
Experiment A2	24.3	18.8±1.06 <i>a</i> A	17.3±1.24 <i>b</i> A	17.2±0.40 <i>b</i> A	15.6±1.02 <i>c</i> A
Mean pupal weight (weight ± SE)					
Experiment B1	22.5	148.1±7.14 <i>bc</i> B	160.06±9.44 <i>b</i> B	184.2±5.74 <i>a</i> B	155.2±5.68 <i>b</i> B
Experiment B2	24.3	126.7±10.46 <i>b</i> A	134.5±11.82 <i>ab</i> A	123.3±7.52 <i>b</i> A	138.7±7.46 <i>a</i> A

Tem.: Temperature.

The *small* and *capital* letters indicate differences at  $P=0.05$  between host plants at each experiment and on same host plants between temperatures, respectively. The same letters indicate that the means are not significantly different at  $P<0.05$ .

### CONCLUSION

In conclusions, the findings suggested that in general OCCW larvae that fed on cabbage had higher weight as compared with larvae that fed on other host plants. The data clearly show that OCCW performed differently in larval and pupal development and survival, pupal weight when they were affected under different temperatures. Temperature played a vital role and a direct correlation with the growth and mortality rate of OCWW. The data

clearly showed that OCWW performed differently in larval and pupal development, survival and weight on the host plants. It was therefore concluded that content of secondary metabolites in the studied host plants might impress the findings. Therefore, future studies should focus on detection of such metabolites of host plant species for the development of resistance plant species in integrated pest management of *Hellula* spp.

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## تأثير العوامل غير الحيوية وتفضيل العائل على حياتية دودة الملفوف الشرقي الغازلة *Hellula undalis* (حرفشية الأجنحة: Pyralidae)

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### ملخص

بسبب أهمية حشرة *Hellula undalis* (حرفشية الأجنحة: Pyralidae) كأفة خطيرة لنباتات العائلة الصليبية في إيران، ولعدم توفر معلومات كافية، تم القيام بهذه الدراسة للبحث في حياتية هذه الحشرة تحت ظروف المختبر والدفينة على عدة عوامل في الأعوام 2007-2008م. أظهرت النتائج أن النسبة المئوية العظمى لموت اليرقات وحياتها تمت مشاهدتها على درجات حرارة مئوية  $15 \pm 0.5$ ، على التوالي. وفي كل درجة حرارة، فإن اختلاف الرطوبة النسبية تسببت في تأثير معنوي على الموت، بينما كان تأثير الرطوبة النسبية على درجات حرارة متفاوتة لم يكن معنوياً على درجة حرارة مئوية  $20 \pm 0.5$ . وكانت العذارى أقل وزناً من على درجة حرارة مئوية  $25 \pm 0.5$ . الدراسة داخل الدفينة أظهرت أن متوسط العمر اليرقي على الملفوف، كولورابي (*rapa*)، كان أطول وأقصر معنوياً، على التوالي، منه على المحاصيل الأخرى. اختلف وزن العذراء معنوياً على درجة حرارة مئوية 22.5 بين كرنب أبو ركية (*Choumoellier*) والمحاصيل الأخرى. كما أظهرت النتائج أنه على درجة حرارة مئوية 24.3 كان وزن العذارى أكبر معنوياً على محاصيل كولورابي، الزهرة، كرنب أبو ركية.

**الكلمات الدالة:** *Hellula undalis*، عوامل غير حيوية، درجة الحرارة، الرطوبة النسبية، التطور، نسبة الموت، تفضيل العائل، الصليبيات.

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