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Research Article

Biological and population performance of *Plutella xylostella* (Lepidoptera: Plutellidae) on canola cultivars

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Abstract: For IPM programs, it is crucial to use plant cultivars that are less sensitive to pests or resistant to them. In this research, the susceptibility and resistance of 20 canola cultivars to the diamondback moth, one of the most destructive pests of canola worldwide, were evaluated under laboratory conditions based on the biological performance of the moth and the response of the secondary metabolites and protein of canola plants leaves. The biological performance of the pest was evaluated using larval development and pupal weight, oviposition preference, and the age-stage, two-sex life table. The highest intrinsic rate of increase (r), the net reproductive rate (R_0), developmental time, and lowest pupal weight were observed on the 1009 cultivar, while the lowest r, R_0, and highest pupal period were obtained on the RGS_{003} cultivar. Also, fecundity in RGS_{003} and Zarfam cultivars was lower than the other cultivars. Overall, study findings suggested that the Hyula50, Zarfam, and RGS_{003} cultivars would be suitable candidates for inclusion in integrated pest management programs against diamondback moth.

Keywords: diamondback moth, cultivar, resistance, pest management, plant-herbivore interaction

Introduction

The diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most destructive pest that causes damage to plants of the Brassicaceae, as well as Fabaceae, Chenopodiaceae, and some weeds belonging to the cruciferous families in the world (Talekar and Shelton, 1993; Asghari *et al*., 2009). Brassicaceae family, mainly cultivated crops such as canola, mustard, cabbage, cauliflower, broccoli, radish, turnip, kohlrabi, and more, are host plants of this insect pest (Kfir, 2005; Dosdall *et al*., 2011). Among these plants, canola is one of the most important sources of oil with low erucic acid (less than 2%), low glucosinolate (less than 30 µmol/g), and the healthiest edible oils in the world (Gunstone, 2011). However, *P. xylostella* larvae feed on the leaves, terminal buds, flowers, and canola pods during different physiological stages, reducing their yield (Mosiane *et al*., 2003). The larvae cause severe

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damage by feeding on the host plant during outbreak years (Zalucki *et al*., 2012).

The DBM was the first insect to resist *Bacillus thuringiensis* and DDT (Tabashnik *et al*., 1990; Talekar and Shelton, 1993). This insect is one species that has developed field resistance to different classes of insecticides such as organophosphates, carbamates, pyrethroids, organochlorines, spinosyns, nereistoxin analogs, neonicotinoids, *etc.* (Furlong, 2013). Furthermore, in Southeast Asia, farmers have sprayed a mixture of insecticides and increased their dosages, leading to environmental pollution and unhealthy products (Mosiane *et al*., 2003). Therefore, plant-based resistance can be a practical and alternative strategy to chemical control of pests, and to understand plant resistance, studying the mechanism of resistance would be useful (Sarfraz *et al*., 2006).

Plant resistance mechanisms include three categories: antixenosis, antibiosis, and tolerance. Antixenosis is a group of plant characteristics that affect herbivore behaviors, reducing the acceptance of a host to oviposition or feeding by a pest. Antibiosis includes the impacts of the host plant on the insect life history (i.e., development, fecundity, and survival), which are measured by the death of the early instars, small size or low weight, abnormal longevity, low food reserves, less fecundity, and abnormal behaviors. Tolerance refers to a plant's ability to resist or defend herbivorous damage (Wiseman, 1985). Based on many reports, knowledge of susceptibility or resistance of host plants to insect pests as well as the population growth performance of insect pests on host plants, is of great importance for IPM programs (Özgökçe and Atlihan, 2005; Razmjou *et al*., 2006; Atlihan *et al*., 2017; Qayyum *et al*., 2018; Satishchandra *et al*., 2019). Although there are numerous reports about host plant resistance to *P. xylostella* on various cultivated and wild Cruciferous plants (Soufbaf *et al*., 2010; Hariprasad *et al*., 2010; Fathi *et al*., 2011; Zhang *et al*., 2012; Kianpour *et al*., 2014; Fathipour *et al*., 2019, Jafary-Jahed *et al*., 2019), there is no evidence concerning the relationship between oviposition preference and the performance of this pest related toplant tolerance, host plant secondary metabolites, and proteins. Hence, we investigated oviposition preference and performance of *P. xylostella* on several different rapeseed cultivars cultivated in Iran to understand their relationships with these compounds.

Materials and Methods

Insect and canola plants

The primary colony of *P. xylostella* was collected from Babelan Field of the University of Mohaghegh Ardabili, Ardabil, Iran, in October 2019. This colony was reared in a wood cage (50 × 50 × 50 cm), covering the walls with a 50-mesh wire screen for ventilation. The twenty canola cultivars (*Brassica napus* L.) including Zarfam, RGS_{003}, Okapi, Opera, Hyula405, Nafis, Agamax, Nepton, Jolius, Nataly, 1008, Xpower, Hydromel, R15, Zafar, KS7, Safar, Hyula50, 1009 and Nima were obtained from the Plant and Seed Improvement Research Institute (Karaj, Iran). Canola seedlings were planted in plastic pots (12 cm high and 15 cm in diameter) containing a mixture of nearly 35% sand and 65% soil and were used in the initial screening test. Plants were grown in the greenhouse at 25 ± 5 ℃ with a natural photoperiod and 60 ± 10% RH. The experiments were conducted when plants were at six-leaf growth stage, and moths were fed on a mixture of 10% honey solution in the wood cage. To eliminate the influence of previous hosts, the moths in cages were kept in a growth chamber at 25 ± 1 ℃ and 60 ± 5% RH with a photoperiod of L16:D8-h for three generations before using them in the experiments. Based on the screening results, four relatively resistant cultivars, two moderately resistant, and two susceptible cultivars, including Zarfam, Jolius, Hyola50, 1009, Hydromel, R15, and $RGS_{003,}$ were selected for studies on the preference and performance of the DBM.

Larval development and pupal weight

The larval development and pupal weight were studied in the laboratory using the method of Wearing et al. (2003). One hundred pairs of moths (< 24 h old) were collected randomly from the colony and were fed with 10% honey solution on

cotton for 24h in an oviposition cage. A Canola plant in the 3 or 4-leaf stage was transferred to the cage as an ovipositional substrate. After 12 hours, 100 eggs were collected and transferred to each leaf of 20 canola cultivars with a wet brush in a colorless plastic container (12 × 10 × 4 cm) covered with a 50-mesh wire screen. After the emergence of *P. xylostella* larvae and until the emergence of pupae, each treatment was checked daily and recorded. The pupae were weighed after 24h by a sensitive scale (0.0001 gr) (Sartorius GCA803S, Germany) and were kept in a Petri dish with a label until the adult emerged. Then adult sex determination was based on the end of the abdomen. The end of the male abdomen has valvae, but the female has papillae anale.

Oviposition preference

In a free-choice experiment, 40 days after planting canola cultivars, the plants were transferred to a growth chamber at 25 ± 1 ºC, 60 ± 5% RH, and a photoperiod of L16:D8-h. One pot per cultivar was randomly selected and placed on a circular platform (diameter 40 cm) inside a colorless nylon cage (60 × 60 × 60 cm) covered with a 50-mesh wire cloth. Ten pairs of DBM adults (one day old) were released in the center of the cage and fed on a mixture of 10% honey solution. After 48 hours, the eggs laid on each cultivar were counted.

In a no-choice test, each canola cultivar was placed in the growth chamber in a colorless plastic cylindrical cage (diameter 20 cm and height 35 cm covered with a 50-mesh wire cloth). A pair of DBM adults (one day old) was released in each cage with a 10% honey solution for adults. After 48 hours, the number of eggs laid on each cultivar was counted and recorded.

Life table parameters of *P. xylostella*

One hundred pairs of *P. xylostella* were reared per canola cultivar in cages (50 × 50 × 50 cm) with food provided. After ten h, the eggs were collected by brush and transferred to a plastic leaf cage (10 × 12 × 4 cm) covered with a 50-mesh wire cloth in the growth chamber. Seventy eggs were used to initiate the experiments per cultivar. Every egg was kept in a plastic leaf cage separately in a growth chamber. Leaf cages were checked daily for egg hatching, larval and pupal development time, larval survival rate, and adult emergence. Adult sex determination was based on the end of their abdomen, and the number of each sex was recorded on each cultivar. A pair of newly emerged moths were transferred to a clip cage (diameter 9 cm and height 13 cm) covered with a 50-mesh wire screen to check for fecundity. One leaf of each cultivar was placed in a cage for laying eggs, so the petiole of leaves was wrapped in a piece of wet cotton. The number of eggs laid and survival of adults were observed twice each day until the female died. During the observation period, dead males were replaced by recruiting live ones from the rearing culture to keep females and males together until the end of the experiment. The experiments were conducted in a growth chamber at 25 ± 1 ºC, 60 ± 5% RH, and a photoperiod of L16:D8-h.

Secondary Metabolites and protein

Flavonoids

For the preparation of leaf extract, a leaf sample (0.1 gr) was crushed with 5 ml methanol (methanol/acetic acid, 85:15) in a porcelain mortar and stored for 24 h and then, the samples were centrifuged at 4300 g for 15 min. The supernatant was transferred to another falcon. 500 µl of the extract was added to falcon containing 1500 µl of aluminum chloride 10%. Then, 1500 µl of potassium acetate 1M was added. After half an hour of keeping the reaction mixture in the dark at room temperature, total flavonoids were determined at 415 nm based on a standard quercetin curve using a spectrophotometer (spectrophotometer, Jenway 6705, UK) (Woisky and Salatino, 1998).

Phenol

Phenol content was measured by Folin–Ciocalteu Reagent (FCR) using a spectrophotometer (Jenway 6705, UK) (Slinkard and Singleton, 1977). 500 µl of the leaf extract was poured into a test tube by adding 1000µl of Folin–Ciocalteu Reagent, which was diluted with water to a ratio of 10:1. After 10 minutes, 1000 µl of 5% sodium bicarbonate solution was added. The absorbance

was evaluated in a 765-nm wave versus Blanc (acidified methanol with equal proportions Folin–Ciocalteu and 5% sodium bicarbonate) by spectrophotometer.

Anthocyanin

The anthocyanin was measured according to Hara et al. (2003). The leaf extract (3ml) was kept in the dark for about 12 h. The color absorption at 550 nm was read by a spectrophotometer. The following formula was used for calculating the anthocyanin concentration: $A_\lambda = \varepsilon CI$, in which A_λ is the absorption rate read at wavelength λ, ε is extinction coefficient (33000 cm M), C is anthocyanin concentration, and I is the width of the cuvette (cm).

Protein

0.1 gr of plant sample crushed with 3ml of buffer phosphate (50 mM, PH: 7.8) in the porcelain mortar. The extract was centrifuged at 12000g for 15 min, and the supernatant was collected. Then 100 µl of supernatant was added to 3 ml Coomassie Brilliant Blue and vortexed, and the color absorption at 559 nm was read by a spectrophotometer after 20 min (Bradford, 1976).

Statistical analysis

The Kolmogorov-Smirnov test was used to evaluate the normality of data distribution. The means of screening test data were compared with Tukey HSD post hoc analysis (SPSS 16 software) (SPSS Inc., Chicago, IL, USA). Relative cultivar resistance (R_c) was calculated through the following formula (Wearing et al., 2003):

$$R_i = (d_i - \bar{d}_s) + (\bar{w}_s - w_i)$$
$$R_c = {(\sum_i^n R_i)}/{n}$$

Where; R_i is the relative resistance of larva i, d_i is the number of days it takes to pupation for larva i, $\bar{d}_s$ is The average days it takes for each sex (larvae i) to pupate for all cultivars tested, w_s is the mean weight (mg) of the pupae of each sex (larva i), for all cultivars tested, w_i is the weight (mg) of the pupa from larva I, and n is the number of larvae that turn into pupae in one cultivar.

Analysis of life table data was performed by TWOSEX-MSChart program (Chi, 2020b) based on age-stage two-sex life table theory (Chi and Liu, 1985; Chi, 1988) and the population parameters (r = intrinsic rate of increase, λ = finite rate of increase, R_0 = net reproductive rate, and T = the mean generation time) were estimated accordingly. The variances and standard errors of biological and life table parameters were calculated by using the Bootstrap method with 100000 resampling, and differences in the life-table parameters were compared using the paired Bootstrap test ($P < 0.05$) (Efron and Tibshirani, 1993; Huang and Chi, 2012; Akköprü *et al*., 2015).

The oviposition period, between the first and last oviposition, has been extensively employed in demographic studies. A long oviposition period may not always indicate a high fecundity, as the number of days an insect has produced eggs is not equal to this time frame. To demonstrate the actual number of days that a female has produced eggs, the parameter oviposition days (O_d), rather than the oviposition period, was utilized in this study. The oviposition days (O_d) were calculated as indicated by Chen *et al*. (2018).

$$O_d = \sum_{x=1}^{N_{fr}} D_x \Big/ N_{fr}$$

Where N_{fr} represents the total number of reproductive females or females that laid eggs, and D_x indicates the number of oviposition days of the xth female.

Predicting population growth patterns as well as the stage structure in the long and short term is made possible by population projection, which makes use of essential characteristics, including developmental rate, fecundity, and survival rate. Therefore, based on the data collected on each canola cultivar, the population growth of the pest was projected using the computer program TIMING-MSChart (Chi 2020a).

$$P(t) = \sum_{j=1}^{m} \left(\sum_{x=0}^{\infty} c_{xj} n_{xj,t} \right)$$

One-way ANOVA was used to compare the effects of canola cultivars on the oviposition preference of *P. xylostella* in free-choice and no-choice tests (SPSS 16.0 software; SPSS Inc., Chicago, IL, USA).

The analysis of secondary metabolites was performed by factorial procedure at a 5% significance level by Duncan's test. In addition, the correlation between larval development time and plant metabolites was calculated using SPSS 16.0 software.

Results

Larval development and pupal weight

The pupal weight, developmental time of *P. xylostella*, and relative resistance of canola cultivars against this pest are shown in Table 1. Feeding on different canola cultivars had significant effects on the larval developmental time and pupal weights of *P. xylostella*. The R_C value ranged from -2.5 to + 2.5 for the larvae that survived the pupal stage. The results indicated that the longer development time and higher pupal weight resulted in a higher R_C value. Rc values of larvae of *P. xylostella* grown on the cultivars 1009, R15, Jolius, Hyula 50, Zarfam, and 1008 (0.2706, 0.2705, 0.2705, 0.2701, 0.2700, and 0.2700, respectively) were higher than those on the other tested cultivars. The shortest developmental time of *P. xylostella* was obtained on Hydromel and Opera with the shortest R_c -0.897 and -0.610, respectively.

The highest female pupal weight was observed on Zafar and Hyula420 cultivars, and the longest female developmental time was obtained on Jolius, 1008, Zarfam, 1009, R15, and Hyula50 cultivars, while the shortest developmental time was observed on Hydromel. In addition, the weight and developmental times of females and males differed on the same cultivars. Females weighed more than males. The similarity of R_c values in canola cultivars (R15, Jolius, Hyula50, Zarfam, 1009, and 1008) showed that DBM was affected by similar characteristics in the host plant.

Oviposition preference

There was no significant difference between eight canola cultivars in terms of the mean number of eggs laid per cultivar in the free-choice test (F = 3.501; df = 7, 24; $p > 0.05$), while a significant difference was observed in the no-choice test (F = 1.416; df = 7, 24; $p < 0.05$). The highest number of eggs were obtained on the Opera cultivar (51.50 eggs) and the lowest on Hyula50 (26 eggs). In addition, the median number of eggs in Zarfam, Hyula50, Hydromel, Jolius, R15, and 1009 were 31.0, 32.50, 33.25, 36.0, 38.0, and 41.17, respectively.

Table1 Mean (± SE) of larval development day and pupal weight of *Plutella xylostella*.

Canola cultivar	Development time (day)		Pupal weight (mg)		R_c (Relative cultivar resistance)		
	Male	Female	Male	Female	Male	Female	Total
Nafis	9.9 ± 0.1^{ab}	9.93 ± 0.071^{a}	56 ± 0.00014^{ab}	65 ± 0.0002^{b}	0.146 ± 0.085^{ab}	0.1982 ± 0.071^{ab}	0.1701 ± 0.056^{ab}
Agamax	9.8 ± 0.1^{abcd}	9.74 ± 0.104^{abc}	59 ± 0.00014^{ab}	64 ± 0.0002^{b}	0.088 ± 0.122^{ab}	0.0065 ± 0.104^{abc}	0.0365 ± 0.079^{abc}
Nepton	10 ± 0^{a}	9.82 ± 0.095^{a}	55 ± 0.00014^{ab}	61 ± 0.0002^{b}	0.271 ± 0.000^{a}	0.0935 ± 0.095^{ab}	0.1703 ± 0.056^{ab}
Jolius	10 ± 0^{a}	10 ± 0^{a}	54 ± 0.0001^{ab}	59 ± 0.00016^{b}	0.271 ± 0.000^{a}	0.2702 ± 0.000^{ab}	0.2705 ± 0.000^{a}
Nataly	9.8 ± 0.1 ^{abc}	9.81 ± 0.101^{abc}	54 ± 0.0001^{a}	62 ± 0.00014^{b}	0.056 ± 0.114^{ab}	0.0824 ± 0.101^{abc}	0.0703 ± 0.074^{ab}
1008	10 ± 0^{a}	10 ± 0^{a}	56 ± 0.00019^{ab}	64 ± 0.0002^{b}	0.270 ± 0.000^{a}	0.2697 ± 0.000^{ab}	0.2700 ± 0.000^{a}
Xpower	10 ± 0^{a}	9.85 ± 0.082^{a}	61 ± 0.00017^{ab}	62 ± 0.00011^{b}	0.270 ± 0.000^{a}	0.1199 ± 0.082^{ab}	0.1699 ± 0.056^{ab}
Hydromel	9 ± 0.2132 ^{f}	8.83 ± 0.167^{e}	56 ± 0.0009^{ab}	62 ± 0.00016^{b}	-0.730 ± 0.213^{cd}	-0.8968 ± 0.167^{e}	-0.8299 ± 0.130^{e}
Okapi	9.6 ± 0.1 ^{cd}	9.29 ± 0.125^{cde}	57 ± 0.00013^{ab}	65 ± 0.00017^{b}	-0.167 ± 0.128^{abc}	-0.4447 ± 0.125^{ade}	-0.2966 ± 0.092^{c}
Zarfam	10 ± 0^{a}	10 ± 0^{a}	57 ± 0.00012^{a}	62 ± 0.00015^{b}	0.270 ± 0.000^{a}	0.2699 ± 0.000^{ab}	0.2700 ± 0.000^{a}
1009	10 ± 0^{a}	10 ± 0^{a}	51 ± 0.00016^{b}	57 ± 0.00015^{b}	0.271 ± 0.000^{a}	0.2704 ± 0.000^{ab}	0.2706 ± 0.000^{a}
R15	10 ± 0^{a}	10 ± 0^{a}	52 ± 0.00019^{b}	58 ± 0.00011^{b}	0.271 ± 0.000^{a}	0.2703 ± 0.000^{ab}	0.2705 ± 0.000^{a}
Nima	9.7 ± 0.1^{abcd}	9.73 ± 0.097^{abc}	57 ± 0.00017^{ab}	66 ± 0.00015^{a}	0.020 ± 0.164^{ab}	-0.0033 ± 0.097^{abc}	0.0030 ± 0.082^{abc}
Zafar	9.8 ± 0.0^{abcd}	9.53 ± 0.133^{abcd}	54 ± 0.00015^{ab}	70 ± 0.0039^{a}	0.137 ± 0.091^{ab}	-0.2010 ± 0.132^{abcd}	0.0318 ± 0.085^{abc}
Ks7	9.9 ± 0.0^{ab}	9.78 ± 0.101^{abc}	56 ± 0.00021^{ab}	64 ± 0.0014^{b}	0.187 ± 0.083^{a}	0.0475 ± 0.101^{abc}	0.1034 ± 0.069^{a}
Safar	9.4 ± 0.1 ^{de}	9.47 ± 0.133^{bcd}	57 ± 0.00021^{ab}	65 ± 0.00027^{b}	-0.396 ± 0.126^{bc}	-0.2637 ± 0.134^{bcd}	-0.3300 ± 0.091^{d}
Hyola 420	9.7 ± 0.1^{bcd}	9.57 ± 0.111^{abcd}	58 ± 0.0002^{ab}	70 ± 0.00022^{a}	-0.063 ± 0.167^{ab}	-0.1590 ± 0.111^{abcd}	0.1302 ± 0.091^{bc}
RGS_{003}	9.7 ± 0.1 ^{abcd}	9.79 ± 0.114^{abc}	54 ± 0.00016^{ab}	63 ± 0.00014^{b}	0.021 ± 0.11^{ab}	0.0557 ± 0.114^{abc}	0.0370 ± 0.079^{abc}
Opera	8.6 ± 0.4^{ef}	9.12 ± 0.176^{de}	62 ± 0.00024^{ab}	61 ± 0.00018^{b}	-1.13 ± 0.40^{d}	-0.6103 ± 0.176^{de}	-0.6969 ± 0.16^{de}
Hyula 50	10 ± 0^{a}	10 ± 0^{a}	57 ± 0.00021^{ab}	61 ± 0.00018^{b}	0.27 ± 0.00^{a}	0.2700 ± 0.000^{ab}	0.2701 ± 0.000^{a}

Mean value in the same column that do not share the same letter are significantly different ($P \leq 0.05$).

Immature stages development

Developmental time for *P. xylostella* eggs, larvae, and pupae on eight canola cultivars are presented in Table 2. Our results revealed that feeding on different canola cultivars resulted in variations in the developmental time of egg, larval, and pupal stages and, consequently, the total developmental time. The moth's total developmental time significantly differed among the cultivars tested; it was the shortest on cultivar 1009 and the longest on cultivar R15 (Table 2). The immature survival rate of *P. xylostella* was significantly affected by feeding on different canola cultivars; the highest survival rate was obtained on cultivar 1009 and the lowest on cultivar RGS_{003} (Table 2).

Adult longevity, oviposition days, and fecundity

Adult longevity and reproductive potential of adults varied among eight canola cultivars tested (Table 3). The longevity value of adult male and female was the highest on cultivar R15, while the APOP duration was the shortest on this cultivar and the Opera cultivar. The TPOP values obtained in different canola cultivars were similar except for the Jolius cultivar, which obtained the shortest TPOP duration. TPOP, as opposed to APOP, is a crucial metric. Since it includes the entire preadult period, TPOP represents the impact of the first reproductive age on population growth (Gabre et al., 2005). The most significant number of oviposition days (Od) was obtained on Opera; however, the values obtained on cultivars R15 and Jolius were statistically similar to those obtained on Opera. Feeding on different canola cultivars affected the fecundity of the pest. The total number of eggs laid in 1009, Hydromel, R15, Jolius, and Opera cultivars were similar; however, they were statistically higher than those obtained on the other three cultivars (RGS_{003}, Hyula 50, and Zarfam) forming the same group (Table 3).

Life table parameters of *P. xylostella*

Rearing on different canola cultivars considerably affected the life table parameters, reflecting the pest's population growth performance (Table 3). The net reproductive rate (R_0), intrinsic rate of increase (r), and finite rate of increase (λ) values obtained on cultivar 1009 were higher than those obtained on the other cultivars (Table 4). However, the values of the parameters above obtained on Hydromel, Opera, R15, and Jolius cultivars statistically were in the same group with 1009. The lowest values of these parameters were on cultivar RGS_{003}, which was in the same statistical group as Zarfam and Hyula50. The mean generation time (T) obtained on 1009, Hydromel, and Opera cultivars was significantly shorter than those obtained on Hyula 50, Zarfam, and RGS_{003}.

The projected population growth showing the potential of *P. xylostella* fed on different canola cultivars is illustrated in Fig. 1. The results indicated that the pest population growth was highest on 1009 and Hydromel. The predicted population growth on Hyula50 Zarfam and RGS_{003} cultivars was considerably lower than those obtained on the other cultivars.

Secondary metabolites and protein in canola plant leaves

The studied cultivars showed a significant difference in phenol, flavonoid, and anthocyanin on uninfected canola leaves ($P < 0.01$), while no significant differences appeared for protein. On the other hand, no significant differences were observed for flavonoid, anthocyanin, and protein on infected canola leaves ($P > 0.01$), but there was a considerable difference for phenol. In addition, the interaction of secondary metabolites of canola cultivars with *P. xylostella* was significant in all cases except for phenol.

The highest total anthocyanin and the lowest total flavonoid and protein content were observed in the R15 cultivar. Also, the highest flavonoid and lowest anthocyanin were observed in the Hyula50 cultivar. Finally, the highest total protein content was observed in Zarfam (Tables 5 and 6).

Table 2 Mean (± SE) developmental time (day) and survival rate of *Plutella xylostella* reared on eight canola cultivars.

Canola cultivar	n	Egg	n	Larva	n	Pupa	n	Total	Preadult survival
1009	67	3.0 ± 0.0b	57	8.75 ± 0.1b	57	3.54 ± 0.26ab	57	15.3 ± 0.28c	0.85 ± 4.36a
Hydromel	70	3.46 ± 0.06a	59	7.58 ± 0.12b	59	4.00 ± 0.3a	70	15.05 ± 0.37ab	0.84 ± 4.33a
Opera	70	2.9 ± 0.04bc	55	8.25 ± 0.38b	55	4.4 ± 0.31ab	55	15.56 ± 0.65ab	0.79 ± 4.91ab
R15	70	3.0 ± 0.0b	51	9.2 ± 0.13a	51	3.86 ± 0.42ab	51	16.06 ± 0.44a	0.73 ± 5.31ab
Jolius	70	3.03 ± 0.02b	53	8.11 ± 0.13b	53	3.79 ± 0.08a	59	14.94 ± 0.15b	0.76 ± 5.11ab
Hyula50	70	2.89 ± 0.04c	63	9.11 ± 0.16a	53	3.79 ± 0.09a	53	15.89 ± 0.19b	0.76 ± 5.14ab
Zarfam	68	3.04 ± 0.04b	53	8.38 ± 0.12b	53	3.77 ± 0.10a	53	15.25 ± 0.12b	0.78 ± 5.01ab
RGS003	70	3.0 ± 0.0b	49	9.92 ± 0.12a	49	3.18 ± 0.06b	49	16.10 ± 0.12b	0.70 ± 5.47b

Mean values in a column followed by different letters are significantly different (P < 0.05, paired bootstrap test).

Table 3 Mean (± SE) longevity, oviposition period, and fecundity of *Plutella xylostella* reared on eight canola cultivars.

Canola cultivar	n	Male longevity (d)	n	Female longevity (d)	APOP (d)	TPOP (d)	Oviposition days (d)	Fecundity (eggs per female)
1009	26	31.35 ± 0.26b	31	32.52 ± 1.10abc	0.65 ± 0.14bc	15.97 ± 0.50ab	9.58 ± 5.65bcd	170.74 ± 9.92a
Hydromel	28	30.29 ± 0.20c	31	33.48 ± 2.02ab	1.03 ± 0.20ab	16.45 ± 0.67ab	10.06 ± 0.34bc	174.45 ± 11.592a
Opera	25	30.84 ± 0.39bc	30	34.67 ± 2.20ab	0.57 ± 0.10c	16.6 ± 1.17ab	11.43 ± 0.51a	159.90 ± 10.60a
R15	20	32.75 ± 0.27a	31	38.55 ± 3.20a	0.52 ± 0.12c	16.97 ± 0.69a	10.55 ± 0.56ab	171.1 ± 12.18a
Jolius	23	31.22 ± 0.32b	30	32.83 ± 0.28ab	0.77 ± 0.16bc	16.1 ± 0.18b	10.23 ± 0.50abc	166.8 ± 17.16a
Hyula50	23	31.91 ± 0.41bc	30	31.50 ± 0.29c	0.97 ± 0.11b	16.8 ± 0.26a	8.57 ± 0.36de	113.3 ± 7.79b
Zarfam	23	30.22 ± 0.31c	30	34.5 ± 1.83c	1.43 ± 0.21a	16.93 ± 0.25a	9.2 ± 0.37cde	116.73 ± 10.01b
RGS003	21	31.90 ± 0.59ab	28	31.82 ± 0.52bc	1.00 ± 0.24abc	16.82 ± 0.30a	8.00 ± 0.56e	117.43 ± 6.24b

Mean values in a column followed by different letters are significantly different *(P* < 0.05, paired bootstrap test). APOP, adult pre-oviposition period; TPOP, total pre-oviposition period).

Table 4 Mean (± SE) Life-table parameters of *Plutella xylostella* reared on eight canola cultivars.

Canola cultivar	R_0 (Offspring/individual)	r (d^{-1})	λ (d^{-1})	T (d)
1009	79.0 ± 11.4a	0.230 ± 0.008a	1.258 ± 0.011a	19.03 ± 0.29b
Hydromel	77.3 ± 11.5ab	0.229 ± 0.010a	1.257 ± 0.012a	19.04 ± 0.34b
Opera	68.5 ± 10.5abc	0.221 ± 0.009ab	1.247 ± 0.011a	19.13 ± 0.31b
R15	75.8 ± 11.5ab	0.221 ± 0.009ab	1.248 ± 0.011a	19.55 ± 0.27ab
Jolius	71.5 ± 12.2abc	0.218 ± 0.010abc	1.244 ± 0.012ab	19.56 ± 0.22ab
Hyula 50	48.6 ± 7.5c	0.196 ± 0.009bcd	1.216 ± 0.01bc	19.84 ± 0.22a
Zarfam	51.5 ± 8.2bc	0.196 ± 0.009bcd	1.217 ± 1.011bc	20.01 ± 0.38a
RGS_{003}	47.0 ± 7.3bc	0.192 ± 0.008d	1.211 ± 0.010c	20.09 ± 0.17a

Mean values in a column followed by different letters are significantly different (P < 0.05, paired bootstrap test). R_0, net reproductive rate; r, intrinsic rate of increase; λ, finite rate of increase; T, mean generation time.

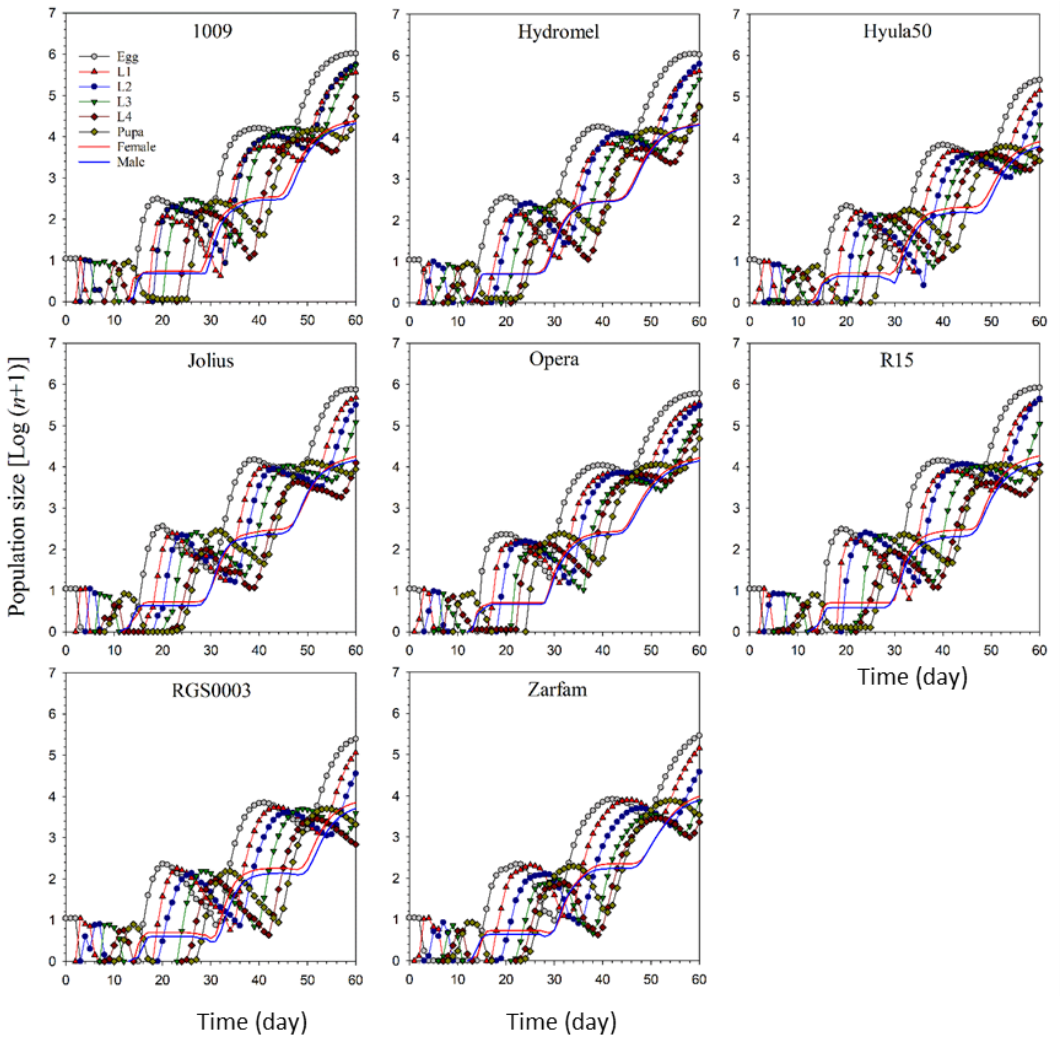

Figure 1 Population growth and stage structure of *Plutella xylostella* on eight canola cultivars.

Discussion

The present study investigated whether resistant or non-preferable canola cultivars exist for *Plutella xylostella* among the cultivars tested. Our experiments showed that cultivars 1009, Jolius, R15, Zarfam, Hyula50, and 1008 had the highest resistance with the highest value of RC (Table 1). On the other hand, Opera and Hydromel cultivars had the lowest R_C value and were more sensitive than the other cultivars. Scientists believe that oviposition preference is related to plant physical and chemical traits (Ramaswamy *et al*., 1978). Females are attracted by volatiles, and the secondary metabolite is involved in host detection (Honda, 1995). In the correlation study using the Pearson correlation coefficient, no significant correlation was observed between the mean number of eggs (in choice-test) and primary (protein), as well as secondary plant compounds (phenol, flavonoid, anthocyanin) ($P > 0.05$). Differences in our results may be due to other plant chemical compounds, appearance, and physiological structure of plants. In addition, observations showed that larvae had longer developmental time when fed on leaves with higher anthocyanin. In other words, cultivars with higher anthocyanin showed resistance to *P. xylostella* with increasing larval development time (Sharma and Agrawel, 1982; Maskato *et al*., 2014).

Table 5 Mean squares from analysis of variance of secondary metabolites and protein.

Treat	df	Mean squares			
		Flavonoid	Phenol	Anthocyanin	Protein
Cultivar	7	341.71**	3836.95**	211.16**	37.43 ns
Insect stress (IS)	1	20.36ns	72996.50**	18.85 ns	95.20 ns
Cultivar × IS	7	201.32*	772.876ns	108.67**	199.03**
Error		48	48	48	
cv		63	63	63	

ns., * and ** indicate non significance, significant at %5 probability level and significant at %1 probability level, respectively.

Table 6 Mean (± SE) comparison of interactions of cultivars and insects based on secondary metabolites and protein.

Cultivar	Interaction	Anthocyanin	Flavonoid	Protein
1009	Without insect stress	1.7273de	47.7572abc	9.5000abc
	Insect stress	2.5000de	45.8461bcd	9.1133bcd
Hydromel	Without insect stress	17.8106ab	52.1194ab	1.0400ab
	Insect stress	6.4750cde	39.4158cd	8.0133d
Opera	Without insect stress	9.0984cd	47.7572abc	9.6533abc
	Insect stress	12.2499bc	49.1394abc	9.7633abc
R15	Without insect stress	21.3409a	39.4398cd	1.0090ab
	Insect stress	7.6742cde	25.0165e	9.5500abc
Jolius	Without insect stress	1.9166de	46.6995abcd	9.3700abc
	Insect stress	9.3863cd	51.4711ab	9.8333abc
Hyula50	Without insect stress	0.8181e	50.9085abc	9.1400abcd
	Insect stress	2.5984de	57.8533a	1.0433a
Zarfam	Without insect stress	1.5908de	39.5721cd	9.8666abc
	Insect stress	3.3484de	52.7211ab	8.5800cd
RGS003	Without insect stress	2.3106de	35.5456d	9.2366abc
	Insect stress	3.6969de	46.4350abcd	9.7166abc

Means followed by the same letters in each column are not significantly different (Tukey's test, $p < 0.005$).

The results showed that rearing on different canola cultivars affected the life table parameters of the moth. The lower net reproductive rate (R_0), intrinsic rate of increase (r), and finite rate of increase (λ), as well as the longer mean generation time (T) obtained on Zarfam, Hyula50, and RGS_{0003} might be attributed to the longer developmental time and lower fecundity of the pest on these cultivars. The parameters r and λ reflect the effects of developmental time, survival rate, first reproductive age, fecundity, and proportion of reproductive females. Therefore, even a slight reduction in these parameters might cause a pest's population to change drastically; this can be observed in the values obtained for RGS_{003}, Zarfam, and Hyula50 (Atlihan and Chi, 2008; Chang *et al*., 2016; Atlihan *et al*., 2017; Bussaman *et al*., 2017). The high r value showed they are more suitable hosts than the other cultivars.

The increase in anthocyanin, flavonoid, and protein occurred in more cultivars after insect feeding (Tables 5, 6). The damage to the vegetation caused by feeding leads to a host plant response with an accumulation of secondary metabolites (Kaur *et al*., 2017). Heng-Moss *et al*. (2004) reported no difference in the protein profiles of plants infested with insects and non-infested controls. Ni *et al*. (2001) reported that

feeding of cereal aphids significantly increased total protein content compared to control cereals. Increased expression of specific plant proteins may increase plant resistance to stress. The lack of phenol content in the interaction between cultivar and insect nutrients in this experiment was due to the calculation of phenol content based on gallic acid, and the measurement of other phenols in the plant may have different results.

Conclusions

According to these results, RGS_{003} cultivar can be considered as the most resistant cultivar. Resistant and relatively resistant cultivars can be used in integrated pest management strategies (Du *et al*., 2004). Therefore, our results can be significant in designing integrated pest management of *P. xylostella* in Iran.

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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عملکرد زیستی و جمعیتی شبپره پشت‌الماسی *Plutella xylostella* (Lepidoptera: Plutellidaae) روی ارقام کلزا

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چکیده: استفاده از ارقام مقاوم برای برنامه‌های کنترل تلفیقی آفات بسیار مهم است. در این پژوهش، حساسیت و مقاومت بیست رقم کلزا به شبپره پشت‌الماسی، یکی از مخرب‌ترین آفات کلزا در سراسر دنیا، در شرایط آزمایشگاهی براساس عملکرد بیولوژیکی شبپره و پاسخ متابولیت‌های ثانویه و پروتئین برگ‌های کلزا مورد ارزیابی قرار گرفت. عملکرد بیولوژیکی آفت با استفاده از رشد لارو و وزن شفیرگی، ترجیح تخم‌گذاری، مرحله سنی و جدول زندگی دوجنسی ارزیابی شد. بیشترین نرخ ذاتی افزایش جمعیت (r)، نرخ خالص تولیدمثل (R_0)، طول دوره رشدونمو لارو و کم‌ترین وزن شفیرگی در رقم 1009 مشاهده شد درحالی‌که کم‌ترین r و R_0، طولانی‌ترین دوره شفیرگی در رقم RGS_{003} به‌دست آمد. هم‌چنین، باروری حشرات کامل در ارقام RGS_{003} و Zarfam کم‌تر از سایر ارقام بود. یافته‌های کلی نشان می‌دهد که ارقام Zarfam، Hyula50 و RGS_{003} کاندیدهای مناسبی برای گنجاندن در برنامه‌های مدیریت تلفیقی آفات علیه شبپره پشت‌الماسی می‌باشند.

واژگان کلیدی: شبپره پشت‌الماسی، ارقام، مقاومت، مدیریت آفات، تعامل گیاه-گیاهخوار