TRITROPHIC INTERACTIONS OF NINETEEN CANOLA CULTIVARS- *CHROMATOMYIA HORTICOLA* – PARASITOIDS IN ARDABIL REGION

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ABSTRACT: The leafminer, *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae), is one of the most important pest of canola, *Brassica napus* L., crop in Ardabil region. In this research, the parasitoid species of the leafminer larvae were collected and identified in the experimental field of canola in the University of Mohaghegh Ardabili during 2008 and 2009. Then the percentage of abundance of each of parasitoid species was determined in the experimental field. In 2008 and 2009, *Diglyphus isaea* (Walker) had the highest abundance (48.3% and 42.8%, respectively) and *Chrysocharis Pubichornis* (Zetterstedt), *Dacnusa sibirica* Telenga and *Pediobius* sp. had lower abundance, respectively. Also, the density of larvae and the percentage of parasitized larvae were determined on nineteen canola cultivars in the experimental field. The density of larvae was lower on Opera, Hyola401 and RGS003 than on the other tested cultivars during 2008 and 2009. The percentage of parasitized larvae on Opera, hyola401 and RGS003 was significantly higher than on the other tested cultivars. Therefore, it could be concluded that using of Opera, Hyola401 and RGS003 cultivars might be increased the efficiency of parasitoids in control of the larvae of *C. horticola*.

KEY WORDS: *Chromatomyia horticola*, parasitoid, identification, canola cultivars, interaction, Ardabil.

The leafminer, *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae) is an important pest of canola, *Brassica napus* L., crop in Ardabil region, Iran (Saljoqi et al., 2006; Fathi, 2010). This insect is polyphagous with worldwide distribution (Gençer, 2005). The leafminer females create leaf punctures by ovipositior for feeding or oviposition. Adult females insert their eggs into the leaf tissue. The larva consumed mesophyll parenchyma tissue and creates continuous mines in leaves. In addition to reducing the level of photosynthesis in the infested plant, leafminers have also been shown to transmit plant pathogens (Parrella et al., 1985; Parrella, 1987; Capinera, 2008).

The parasitoid species of *C. horticola* have been reported in previous studies (Gençer, 2005). The leafminer parasitoids mainly belong to Braconidae, Eulophidae and Pteromalidae. Eulophids include more than 80% of leafminer parasitoids (Gençer, 2004, 2005; LaSalle & Parrella, 1991). Use of biocontrol agents has an important role in the IPM of leafminers. But, the control level by biocontrol agents is not sufficient to achieve adequate control of leafminers (Capinera, 2008). Therefore, further research is required to investigate the potential of using resistant cultivars in combination with biocontrol agents in the integrated management of leafminers. Therefore, study of interactions of canola cultivars - *C. horticola* - parasitoids can potentially improve pest management programs.

Canola cultivars, namely Talayh, Jewel, PF/7045/91, Licord, SIM043, SIM046, Elvis, Zarfam, RGS003, Opera, Okapi, Ebonite, Elite, Orient, Option500, Hyola60, Hyola308 Hyola401, and Adder have recently been introduced in Ardabil. Currently, Zarfam, Okapi and Ebonite are being planted on vast acreage compared with other cultivars tested in this research. In an earlier research (Fathi, 2010) the resistance of some canola cultivars was evaluated to *C. horticola*. But, our literature review indicated that no study have been done on interactions of canola cultivars - *C. horticola* – parasitoids. Therefore, the purpose of this research is (a) identify of the parasitoid species of *C. horticola* in canola field in Ardabil region and (b) study of tritrophic interactions of *C. horticola* – parasitoids on studied canola cultivars.

MATERIAL AND METHODS

Preparation of the experimental field

The experimental field in a 1000 m^2 was selected in the Agricultural Research Station of the University of Mohaghegh Ardabili, Ardabil, Iran (elevation of 1332 m; longitude 48° 17' E; latitude 38° 15' N) in 2008 and 2009. Seeds of cultivars, namely Talayh, Jewel, PF/7045/91, Licord, SIM043, SIM046, Elvis, Zarfam, RGS003, Opera, Okapi, Ebonite, Elite, Orient, Option500, Hyola60, Hyola308, Hyola401, and Adder were obtained from the Seed and Plant Improvement Institute of Karaj (Iran). All tested cultivars were planted in October-2007 and 2008 to vernalize during winter in a field, following a randomized complete block design with four blocks for each cultivar. The distance of 2 m between blocks was selected for sampling. The row to row and plant to plant distances were maintained at 75 cm and 20 cm, respectively. The field was managed according to the local practice with weekly flood irrigation and hand weeding. No insecticides were applied to the plants. Dithane fungicide (Mancozeb, Rohm and Haas Co., Philadelphia, PA) was used to prevent foliar disease before inflorescence emergence. Also, nitrogen fertilizer (100 kg ha^{-1}) was used in rosette stage in may-2008 and 2009.

Identification of parasitoid species and their abundance

In experimental canola field, the leaves infested to leafminer larvae observed on plants of each cultivar in each block were collected and transferred to the laboratory. These larvae were maintained in the growth chamber at 23°C±1 and 50±5 RH and 14:10 h (L:D) until adult emergence of wasps or leafminer. The species of parasitoid of leafminer larvae in each sample were separated and identified under stereomicroscope by their morphological characters (Darvas et al., 1999; Noyes, 2003; Gençer, 2004, 2005, 2009). Then the number of each of parasitoid species in each sample was recorded. These data was used for calculation of the percentage of abundance of each species.

The larval densities and parasitism rates

The density of *C. horticola* larvae and the percentage of parasitized larvae of leafminer were investigated on 19 cultivars of canola in the experimental canola field. Sampling on each cultivar was carried out every three days starting from the stem extension stage and continued until the late seed development stage between 10:00 and 11:00. On each sampling date, 4 plants from each of the 19 cultivars per block were selected randomly and the number of mines contains leafminer larvae per leaf were recorded using a 20X hand lens. The leaves infested to leafminer larvae observed on 4 plants of each cultivar in each block were collected and transferred to the laboratory. These larvae were maintained in the growth chamber at 23°C±1 and 50±5 RH and 14:10 h (L:D) until adult emergence of wasps or leafminer. The numbers of adult *C. horticola* and parasitoid species were recorded to determine the percentage of parasitized larvae in the experimental field. Also, mines without leafminer larvae were not account in calculation of the percentage of parasitized larvae. One of the suitable indicators for evaluating the performance of parasitoids on different plant cultivars is comparison of rate of parasitized larvae of leafminer on different cultivars of canola (Rauf et al., 2000; Civelek, 2002; Chen et al., 2003; Gençer, 2004, 2005, 2009).

Data analysis

Prior to analysis, data were log-transformed to correct the heterogeneity of variance whereas data on parasitism rate were arcsine-transformed. Data on the abundance of each of parasitoid species were analyzed using one-way ANOVA. Data on larval densities and rates of parasitism in two years were analyzed by a combined analysis, completely randomized block design in two years (PROC GLM, SAS Institute, 2005). The differences among treatment means were compared using Tukey's HSD test (PROC ANOVA, SAS Institute, 2005).

RESULTS

Identification of parasitoid species and their abundance

In this research, *Diglyphus isaea* (Walker), *Chrysocharis pubicornis* (Zetterstedt) and *Pediobius* sp. (Hym.: Eulophidae), and *Dacnusa sibirica* Telenga (Hym.: Braconidae) were identified as parasitoid of *C. horticola* larvae in canola fields of Ardabil region. The percentage of abundance of each of parasitoid species was significantly different compared with each other in two years (df=3, 108; F=3.36; P=0.0026) (Table 1). In 2008 and 2009, *D. isaea* had the highest abundance (48.3% and 42.8%, respectively) among the parasitoid species of *C. horticola* in Ardabil region. The abundance of other parasitoid species decreased in the following order: *Chrysocharis pubicornis*, *Dacnusa sibirica* and *Pediobius* sp. in both years (Table 1).

The larval densities and parasitism rates

The density of *C. horticola* larvae differed significantly among the 19 canola cultivars for both study years (df=18, 108; F=3.62; P=0.0001) (Fig. 1). The density of larvae was lower on Opera, Hyola401 and RGS003 than on the other tested cultivars during 2008 and 2009. For other cultivars the density of larvae decreased in the following order: Talayh, Elvis, Ebonite, Hyola60, Okapi, Licord, PF/7045/91, Adder, Hyola308, SIM043, Jewel, Elite, Orient, SIM046, Option500 and Zarfam in 2008; and Elvis, Adder, Talayh, Hyola60, Licord, PF/7045/91, Hyola308, Jewel, Ebonite, Zarfam, Option500, SIM046, Okapi, Orient, SIM043 and Elite in 2009 (Fig. 1).

The percentage of parasitized larvae of *C. horticola* was significantly different among the 19 canola cultivars tested (df=18, 108; F=5.81; P=0.0001) (Fig. 2). In 2008, the parasitism rate was higher on Opera, Hyola401 and RGS003 than on other tested cultivars; however parasitism rates decreased in the following order for other cultivars: Okapi, Ebonite, Elvis, Zarfam, Licord, Option500, Orient, Talayh, Elite, Jewel, SIM046, Hyola60, Hyola308, Adder, SIM043 and PF/7045/91 (Fig. 2). In 2009, the parasitism rates decreased in the following order: Opera, RGS003, Okapi, Hyola401, SIM043, Elite, Ebonite, Elvis, Zarfam, Orient, Hyola308, Option500, SIM046, Licord, Hyola60, Talayh, Adder, Jewel and PF/7045/91 (Fig. 2).

DISCUSSION

The *Diglyphus isaea* had highest abundance among the parasitoid species of *Chromatomyia horticola* in canola fields in Ardabil region. This parasitoid species has been reported as the dominant parasitoid of *C. horticola* larvae in Turkey (Uygun et al., 1995; Gençer, 2004, 2005; Cikman et al., 2006; Gençer, 2009).

The results of field experiments indicated that the density of *C. horticola* larvae was lower on Opera, RGS003 and Hyola401 among the 19 canola cultivars tested during 2008 and 2009. Also, the parasitism rate of *C. horticola* larvae was higher on Opera, Hyola401 and RGS003 respectively among the tested cultivars in 2008 and 2009. The low density of larvae and high parasitism rate on Opera, Hyola401 and RGS003 cultivars should result in lower pest damage and higher crop yield. These results indicated that different levels of resistance in cultivars have different effects on level of parasitism. Difference in leaf trichome density that could be related to these interactions was not discernible between 19 studied cultivars of canola. In most cases, the difference in the rate of parasitized larvae on different host species was associated with the cuticle thickness and volatile profiles of the tested species (Fagoonee & Toory, 1983; Parrella et al., 1983; Knodel-Montz et al., 1985; Minkenberg & Fredrix, 1989; Minkenberg & Ottenheim, 1990; Carolina et al., 1992; Erb et al., 1993; Wei et al., 2000). Our findings are consistent with previous studies. Fathi et al. (2010) concluded that Opera, RGS003 and Hyola401 exhibited antibiosis and reduced developmental rate, fecundity and survival of *C. horticola*. Plants responsible for antibiosis are known to have an indirect effect by increasing the exposure of the insect to its native natural enemies as a result of prolonged developmental time.

Our findings suggesting that using of resistant cultivars in integrated with biocontrol agents could play an important role in the IPM of *C. horticola*. The results of this study indicated that Opera, RGS003 and Hyola401 cultivars had lower larval density and higher parasitism rate by parasitoids. Therefore, these cultivars could increase the efficacy of *D. isaea* in control of *C. horticola* larvae. These results can be useful in decreasing the current dependence on using of chemical insecticide. The present findings in combination with our earlier results suggest that using Opera, RGS003 and Hyola401 integrated with *D. isaea* should result in increased efficacy of this parasitoid and lead to effective and more sustainable management of *C. horticola* in the region.

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Table 1. Mean (±SE) of the density and percentage of abundance of each of parasitoid species of *Chromatomyia horticola* during seasonal growth in 2008 and 2009.

species	The population density		The percentage of abundance	
	2008	2009	2008	2009
Eulophidae				
Diglyphus isaea	9.79 ± 0.38 a (A)	8.79 ± 0.49 a (B)	48.3 ± 4.4 a (A)	42.8 ± 3.1 a (A)
Chrysocharis pubicornis	5.05 ± 0.39 b (A)	5.26 ± 0.33 b (A)	24.9 ± 3.9 b (B)	25.6 ± 3.6 b (B)
Pediobius sp.	1.58 ± 0.17 d (B)	2.36 ± 0.19 d (A)	7.8 ± 1.9 c (C)	11.5 ± 2.3 c (C)
Braconidae				
Dacnusa sibirica	3.84 ± 0.37 c (A)	4.11 ± 0.34 c (A)	18.7 ± 3.3 b (B)	20.0 ± 4.2 b (B)

Means followed by different small letter within and capital letter between columns of both years are significantly different at P≤0.05.

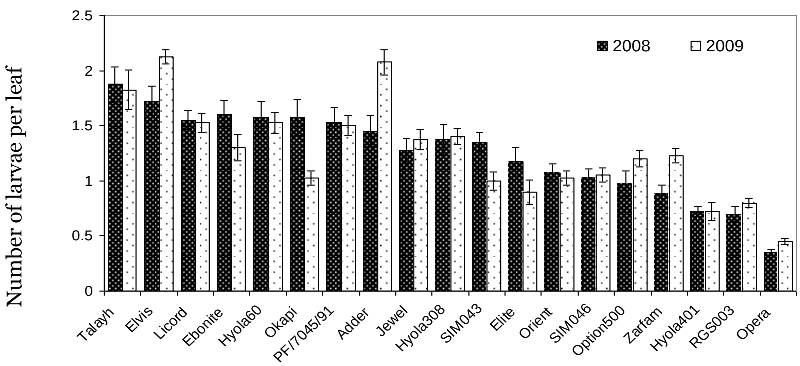

Figure 1. The densities of larvae (mean ± SE) of *C. horticola* on 19 cultivars of canola in the fields during 2008 and 2009.

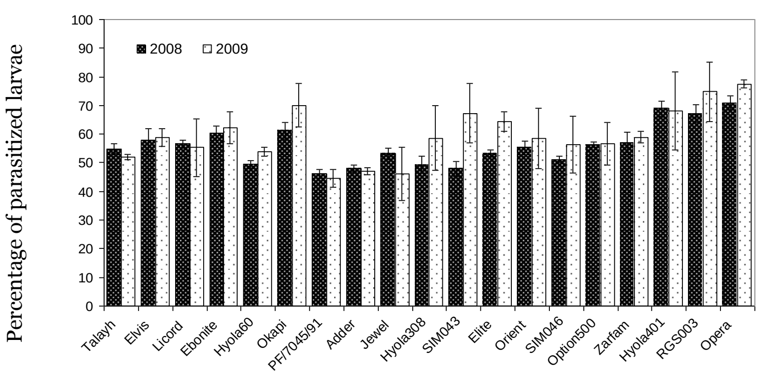

Figure 2. Mean (± SE) percentage of parasitized larvae of *C. horticola* on 19 commercial cultivars of canola in the fields during 2008 and 2009.