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Research Article

Ecofriendly managing of *Helicoverpa armigera* in tomato field by releasing *Trichogramma evanescence* and *Habrobracon hebetor*

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Abstract: Health and environmental side effects of chemical insecticides and development of resistant population of *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) to pesticides have resulted in increasing demands for non-chemical control approaches against this pest. In this research, the efficacy of two biological control agents were studied under field condition. Treatments consisted of releasing *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae), *Trichogramma evanescence* Westwood (Hymenoptera: Trichogrammatidae) and combination of *H. hebetor* + *T. evanescence* (HABROBRACON-TRICO). The results revealed significant differences in the number of infested tomato fruits among treatments and harvesting times. The infested fruits was the lowest (2.68 ± 0.14%) in plots treated by HABROBRACON-TRICO. Moreover, the highest (3.36 ± 0.50%) and the lowest (2.88 ± 0.22%) damaged fruits was recorded in the second and fourth harvesting times, respectively. There was significant interaction between harvesting times and treatments. Regarding the tomato yield in treatments, findings revealed significant difference among treatments in both main harvesting times. However, there was no significant differences in total yield in treatments. It could be concluded that biological control agents can be used as a promising alternative for synthetic insecticides in control of *H. armigera* in tomato farms without significant crop losses.

Keywords: Biological control, Cotton bollworm, Tomato field, Chemical application

Introduction

The cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is one of the most destructive insect pests, causing high economic losses in a diverse array of agricultural host plant species (Kouhi *et al.*, 2014). Applying insecticides with different mode of action and from different classes, has not been able to control *H. armigera* (Downes *et al.*, 2017), frequently resulting in pest resistance and deletion of many biocontrol agents. So, lack of efficient and ecofriendly approaches is highly perceived for long lasting control of *H. armigera*. In recent years, there has been a growing interest toward

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the development of pesticides-free approaches such as cultural, physical, biological, varietal, biorational and genetic control measures (Subramanyam and Hagsturm, 2000; Phillips, 2006) amongst which, the use of natural enemies have been more promising (Scholler *et al.,* 1997; Scholler and Flinn, 2000).

In recent years, there has been tremendous increase in focusing on biological control agents either to produce healthy agricultural products or to descend drawbacks of insecticides application. *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) is an idiobiont ectoparasitoid with a cosmopolitan distribution that attacks the last larval stages of several species of pyralid and nuctoid moths (Benson, 1974). The females paralyze their host larvae first by stinging and then lay their eggs on or near the surface of paralyzed hosts (Antolin *et al.,* 1995). The paralyzed larvae are then consumed as food sources by developing larvae and also for the adult nutrition by parasitoids (Askari Seyahooei *et al.,* 2018). A lot of research has been carried out on different biological and behavioral aspects of *H. hebetor* (Al-Tememi, 2005; Gunduz and Gulel, 2005; Ghimire and Phillips, 2010; Askari Seyahooei *et al.,* 2018). Trichogrammatid wasps are another biocontrol agent for inundative releases which have been used widely against destructive lepidopteran pests such as *H. armigera* (Alba, 1991; Sing and Jalali, 1994; Sithanantham *et al.,* 2001).

To reach an efficient and reliable biological control, it is necessary that highly efficient strains of natural enemies are selected and tested in a given environment (Hassan, 1994; Pak, 1988). Therefore, native strains of biocontrol agents that generally well adapted to same conditions, are favored options for biocontrol programs in each region (Hommay *et al.,* 2002). Natural enemy richness is another important point which can affect the degree of success in biological control program. Although, the majority of studies show greater herbivore suppression with increased natural enemy richness, some negative interactions amongst natural enemies can disrupt biological control and reduce the herbivore suppression. Natural enemy interference can also lead to non-additive effects (Ferguson and Stiling 1996) in which neither positively nor is negatively interactive relation seen in biological program. Additive effects occur when different species of natural enemies are complementary and attack different life stages of a pest (Calvo *et al.,* 2009), or the same pest but in different parts of a plant (Onzo *et al.,* 2004; Gable *et al.,* 2012). Some interactions between natural enemy species can even be synergistic and occurs when one natural enemy alters the behaviour or feeding niche of prey making them more susceptible to attack by another natural enemy, known as 'predator facilitation' (Losey and Denno 1998; Sih *et al.,* 1998). So, study of natural enemies solely and in combination with each other can shed light on the unknown aspects of a biological control with more than one bio-control agent (Rocca and Messelink, 2017).

Although laboratory and greenhouse studies are less time and cost consuming for selecting biocontrol agents, it has been shown that sometimes there is not strong enough relation between results gained in laboratories and fields (Calisi and Bentley, 2009). In an overall view, lack of success in biological control programs has often been caused by high mortality of natural enemies due to low adaptation of biocontrol agent to the climatic factors in newly released climates (Tran *et al.,* 1986; Tran and Hassan, 1986). Nevertheless, there are many well-known instances in which biocontrol agents have been able to control successfully lepidopteran pests attacking different vegetable host plants (see Li, 1994). Herein, we aimed to: study performance of two important biological control agents of *H. armigera* i.e., *H. hebetor* and *Trichogramma evanescence* Westwood (Hymenoptera: Trichogrammatidae) in field conditions and compare the ability of these biocontrol agents and synthetic insecticides in controlling *H. armigera*.

Materials and Methods

Farm selection

To implement the experiment, two neighboring tomato farms (2 and 8 ha under tomato

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cultivation) were chosen in Shamil district, in Hormozgan province southern Iran as an important tomato producing zone with GPS location (30º43´975˝N, 48º44´95˝E). A two hectare tomato field with accepted common chemical program for fruit borer control in the region was selected as control field (Table 1). The other, four treatments including release of two biocontrol agents individually and in combination with each other and a limited spraying of synthetic insecticides were implemented in separated plots (Table 1). The study was performed as split plot in time arranged in Randomized Complete Block (RCB) design. Each treatment was implemented in an isolated plot with coverage of 0.25 ha and replicated four times. To isolate the plots, an area of 0.5 ha of the filed was heavily sprayed by insecticides as guard distance. Number of infested tomato fruits by *H. armigera* in four harvesting dates (included two main harvesting sessions) was used as a criterion to compare efficiency of the treatments (Table 1).

Habrobracon hebetor and *T. evanescence* both were obtained from lines kept at the Plant Protection Department of Agricultural Organization of Hormozgan Province (Iran), where they have been used in a bio-control project to control *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) in tomato fields. *H. hebetor* was reared on the Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae). Rearing was performed using plastic basin containers (40 × 18cm) filled with 1,000g of a 2:1 mixture of wheat flour and rough wheat bran. The food was decontaminated at 60 °C for 2 d and then 0.2g of flour moth eggs were dispersed on top of the substrate. The plastic basin containers were covered with black sterile cotton cloth.

To obtain host eggs for preparing Tricho cards, 1.5g eggs of the cereal moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) was used to inoculate 1 kg decontaminated barley grains. After emergence of the moths, their deposited eggs on the cards were introduced to *T. evanescence*. The cards containing parasitized eggs were cut to pieces with 500-600 eggs as Tricho cards to use in fields.

Table 1 Treatments, augmentation or spraying date, harvesting date and list of the used insecticides in control of *Helicoverpa armigera*.

Treatments[1]	Augmentation date/Spraying date	Additional chemical spray
Trichogramma evanescence (TRICO)	2016-13-12	NACS[2]
	2017-28-1	
Habrobracon hebetor (HABROBRACON)	2016-19-12	NACS
	2017-28-1	
T. evanescence + *H. hebetor* (TRICO-HABROBRACON)	2016-13-12, 2017-28-1 (TRICO)	NACS
	2016-19-12, 2017-28-1 (HABROBRACON)	
Control with limited insecticide application (CON1)	2016-28-11, Indoxacarb + Fenpropathrin	Azoxystrobin 20% + Difenoconazole SC 12.5% and Copper oxychloride
	2016-4-12, Indoxacarb + Abamectin	
	2016-14-12, Profenofos + Abamectin	
Control according to the local accepted schedule (CON2)	2016-16-10, Indoxacarb + Fenpropathrin	NACS
	2016-25-10, Indoxacarb + Abamectin	
	2016-2-11 Imidacloprid	
	2016-25-11, Indoxacarb + Imidacloprid	
	2016-18-12, Indoxacarb + Fenpropathrin	
	2017-9-1, Indoxacarb + Abamectin	

[1.] Sampling dates for each treatment were 2016-25-12, 2017-5-1, 2017-12-1, 2017-2-2.
[2.] NACS: No additional chemical spray.

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Female adults of *Habrobracon hebetor* was released at a rate of 250 adults/0.25 ha as a replication. Biocontrol agent was released two times during the cultivation season. In the second releasing program, *T. evanescence* was released twofold of the first release (i. e., 0.5g/replicate). To compare the results inferred from releasing biological control agents with chemical application, two check treatments were established in which number of times of chemical application varied. The first check treatment consisted of a representative and reasonable application of chemical pesticide, i.e., three times over the whole period of experiment and the second treatment was representative of the farmer's chemical application pattern i.e. six times (Table 1).

Data Analysis

The normality of data was checked by kurtosis and skewness tests in SPSS prior to analysis. One way analysis of variance (ANOVA) was used to test significant differences between treatments and then means were separated by least significant difference (LSD) test. Statistical analysis was performed by SAS version 9.1.3.

Results and Discussion

In the present study we addressed the possibility of managing *H. armigera*, by releasing two well-known hymenopteran parasitoids in an augmentation plan. *H. armigera*, also known as cotton bollworm, is one of the most destructive pests on agricultural crops almost all over the world (Europe, Africa, Asia, Australia, South America and likely North America) (Kriticos *et al.*, 2015; Downes *et al.*, 2017). Management of *Helicoverpa* in crops is complicated by the variability in their infestation levels between regions and different years. Also, the infestation levels of *H. armigera* are affected by several factors like climate, host plant abundance, its quality and movements of moths into and out of the crops (Rochester *et al.*, 1996). Biological control agents are amongst the safe and ecofriendly approaches which produce a permanent trend of pest control. *T. evanescence* and *H. hebetor* both are the main parasitoids of *H. armigera* that parasitize the egg and last larval instars of *H. armigera*, respectively. The capability of *H. hebetor* in simultaneous application with chemicals to sustain its efficiency (Faal-Mohammadali *et al.*, 2014) shows high flexibility of these bio-control agents to involve in integrated pest management programs.

Results of the current study revealed a strong effect for the biological agent in controlling *H. armigera*. We found significant differences among control methods which have been defined as treatments ($F = 10.5$; $df = 4, 12$; $p < 0.01$) and among various harvesting dates ($F = 3.49$; $df = 3, 36$; $p < 0.05$). In comparing treatments, TRICO-HABROBRACON and CON2 with the lowest (2.68 ± 0.14%) and the highest (3.68 ± 0.51%) damage in fruits were ranked as the most and least effective treatments (Table 2).

Also, the highest (3.36 ± 0.50%) and the lowest (2.88 ± 0.22%) infested fruits was recorded at the second and fourth harvesting times, respectively (Table 2). The high efficiency of biocontrol treatments in the last harvesting date may be stemming from augmentation of parasitism produced by new generations of the released parasitoids. We also found significant interaction between harvesting dates and treatments ($F = 15.2$; $df = 12, 36$; $p < 0.01$). As mentioned, the treatments had significantly different impacts on *H. armigera* in which TRICO-HABROBRACON and CON2 with the lowest (2.68 ± 0.14%) and the highest (3.68 ± 0.51%) damage in fruits were the most and least effective treatments, respectively (Table 2).

In each harvesting date, there was significant difference among treatments. Except the first harvesting date in which TRICHO or HABROBRACON release caused an effective damage control, in other harvesting dates, the combined treatment of TRICO-HABROBRACON was more effective than separate release of *H. hebetor* or *T. evanescence* (Table 2). This is a positive sign of complementary effects that occurred by releasing

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an egg parasitoid combined with a larval parasitoid in the field. This study indicates, simultaneous releasing of *Trichogramma*, and *Habrobracon*, enhances the net result by reducing the pest population comported with releasing any of the two solely. *Trichogramma* parasitizes the eggs and reduces the pest density for *Habrobracon*, resulting in more efficient and meaningful control of *H. armigera* even compared with insecticide application. Accompaniment effect of natural enemies in a co-releasing program has been documented by Rocca and Messelink (2017) who showed more efficient control of foxglove aphid, *Aulacorthum solani* (Kaltenbach) (Hemiptera: Aphididae), when it was targeted by two natural enemies including a parasitoid, *Aphidius ervi* Haliday (Hymenoptera: Braconidae), and a predator, *Micromus variegatus* (Fabricius) (Neuroptera: Hemerobiidae).

Except TRICO-HABROBRACON, the effect of different harvesting dates was significant on efficiency of each treatment (Table 2). We also found a significant interaction between treatments and harvesting date. The treatments were also compared in terms of tomato yield and significant differences were found among them ($F = 14.9$; $df = 4, 12$; $p < 0.01$). Accordingly, in the first main harvesting time, the maximum production was observed in CON2 with 441 ± 18.8 kg and the other treatments had no significant differences. In the second main harvesting date, the maximum tomato yield belonged to biological control treatments which were significantly higher than chemical control treatments. However, the treatments showed no significant difference when total tomato yield of both harvesting dates was taken into account (Table 3). Many instances exist on the successful application of natural enemies on different crops in which no significant decrease in the production were found between application of chemical and biocontrol agent (see de Freitas Bueno *et al.*, 2011).

Table 2 Efficacy of control methods and harvesting times on the number of fruits infested by *Helicoverpa armigera*.

Treatments	Infested fruits per plot (%) (Mean ± SE)				
	2016-25-12	2017-05-01	2017-12-01	2017-02-02	Total mean
TRICO-HABROBRACON	3.00 ± 0.35b (A)	2.70 ± 0.84b (A)	2.70 ± 0.55b (A)	2.30 ± 0.19c (A)	2.68 ± 0.14d
TRICO	2.10 ± 0.41c (C)	4.60 ± 1.36a (A)	3.50 ± 0.76a (B)	3.30 ± 0.72a (B)	3.38 ± 0.51ab
HABROBRACON	1.80 ± 0.68c (C)	4.50 ± 1.29a (A)	3.60 ± 1.07a (B)	2.40 ± 0.36bc (C)	3.08 ± 0.60bc
CON1	3.20 ± 0.28b (AB)	2.20 ± 0.42b (C)	2.50 ± 0.68b (C)	3.30 ± 0.92a (A)	2.80 ± 0.27cd
CON2	5.10 ± 0.76a (A)	2.80 ± 0.71b (B)	3.70 ± 0.55a (B)	3.10 ± 0.58ab (B)	3.68 ± 0.51a
Total mean	3.04 ± 0.58BC	3.36 ± 0.50A	3.20 ± 0.25AB	2.88 ± 0.22C	

Means followed by the same letters in each column (small letters) and in each row (capital letters) are not significantly different (LSD test, $P < 0.05$).

Table 3 The effect of different treatments against *Helicoverpa armigera* on tomato yield in the first and second harvesting dates.

Harvesting times	Tomato yield (Kg/plot) (Mean ± SE)				
	TRICO-HABROBRACON	TRICO	HABROBRACON	CON1	CON2
First time	319 ± 13.0b	304 ± 19.1b	326 ± 10.3b	314 ± 6.2b	441 ± 18.8a
Second time	812 ± 105.0ab	882 ± 76.0a	782 ± 12.0abc	678 ± 33.0bc	598 ± 25.0c
Total mean	1131 ± 107.0ab	1186 ± 68.0a	1109 ± 15.0ab	991 ± 33.0b	1039 ± 21.0ab

Means followed by the same letters in each row are not significantly different (LSD test, $P < 0.05$). First and second time indicate dates of 2016-25-12 and 2017-05-01, respectively.

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This finding demonstrates a reasonable efficacy for the biological control agents almost equal to chemicals application. This is very important in view of pesticide-free vegetables production which is mainly used as fresh and daily salad and it is crucial to be free of any hazardous chemicals. However, one of the main obstacles on the way to widely applying natural enemies is the cost-effective application which still remains uneconomical for many biocontrol agents and consequently is less used by the farmers. Therefore the main finding of this research is that biological agents have similar efficacy as conventional chemicals.

Results of the present study unveiled the high capability of biocontrol agents as an efficient alternative for synthetic insecticides which will allow production of healthy agricultural products with little crop loss. We strongly suggest avoiding simultaneous application of biocontrol agents and synthetic insecticides against *H. armigera* in the same farm because the biocontrol agents may suffer from chemical application and result in failure of biological control program. In some tomato farms of Hormozgan province, *H. hebetor* and *T. evanescence* have been annually released against *H. armigera,* under supervision of Plant Protection Organization but since in these farms, broad spectrum synthetic insecticides are used at the same time, a high number of the released wasps are usually annihilated. Lack of efficient population of natural enemies with high adaptation is another challenge that hinders success of biological control procedure. Results indicated that proper control of *H. armigera* could be obtained by combining two important parasitoid wasps to produce healthy agricultural products and decrease insecticide application.

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کنترل کرم میوه‌خوار گوجه‌فرنگی، *Helicoverpa armigera* توسط زنبورهای پارازیتوییید *Trichogramma evanescence* و *Habrobracon hebetor* در قالب برنامه رهاسازی اشباعی

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چکیده: اثرات مخرب حشره‌کش‌های شیمیایی بر محیط‌زیست، انسان و هم‌چنین ظهور پدیده مقاومت در آفاتی مانند *Helicoverpa armigera* Hubner (Lepidoptera: Noctuidae) باعث شده تا تقاضا برای به‌کارگیری روش‌های غیرشیمیایی علیه این آفت افزایش یابد. در پژوهش حاضر، کارایی دو عامل بیولوژیک در کنترل *H. armigera* در قالب برنامه رهاسازی اشباعی در مزارع گوجه‌فرنگی بررسی شد. تیمارها شامل به‌کارگیری زنبورهای *Habrobracon hebetor* (Say) (Hymenoptera: Braconidae) و *Trichogramma evanescenc* Westwood (Hymenoptera: Trichogrammatidae) به‌تنهایی و در تلفیق با یک‌دیگر (*H. hebetor*+ *T. evanescence*)، تیمار کنترل یک (کاربرد محدود سموم شیمیایی) و تیمار کنترل دو (کاربرد سموم شیمیایی براساس عرف منطقه) بود. نتایج به‌دست آمده نشان داد که بین تیمارهای مختلف و هم‌چنین زمان‌های مختلف برداشت گوجه‌فرنگی (چین برداشت گوجه‌فرنگی) از نظر تعداد میوه آلوده تفاوت معنی‌داری وجود داشته کم‌ترین درصد میوه آلوده (۰/۱۴ ± ۲/۶۸) در تیمار *T. H. hebetor* + *evanescence* مشاهده شد. مقایسه میانگین تعداد میوه آلوده در زمان‌های مختلف برداشت گوجه‌فرنگی نشان داد که بیش‌ترین و کم‌ترین درصد میوه آلوده به‌ترتیب در چین‌های دوم (۰/۵۰ ± ۳/۳۶) و چهارم (۰/۲۲ ± ۲/۸۸) برداشت مشاهده شد. نتایج هم‌چنین نشان داد که اثر متقابل زمان برداشت و تیمار معنی‌دار است. ازنظر تأثیر تیمارها بر میزان عملکرد گوجه‌فرنگی، نتایج نشان داد که در هر یک از دو چین اصلی برداشت گوجه‌فرنگی، بین تیمارهای مورد مطالعه از نظر عملکرد تولید گوجه‌فرنگی اختلاف معنی‌داری وجود دارد ولی در مجموع دو چین برداشت گوجه‌فرنگی، اختلاف معنی‌داری بین تیمارهای کنترل بیولوژیک و کنترل شیمیایی مشاهده نشد. با این نتایج می‌توان اظهار کرد که کنترل بیولوژیک می‌تواند به‌عنوان یک جایگزین کارآمد و نویدبخش سموم شیمیایی در مبارزه علیه *H. armigera* در مزارع گوجه‌فرنگی بدون هیچ‌گونه کاهش عملکرد محصول به‌کار گرفته شود.

واژگان کلیدی: کنترل بیولوژیک، کرم میوه‌خوار گوجه‌فرنگی، مزرعه گوجه‌فرنگی، کنترل شیمیایی

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