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Research Article

Dispersal of *Trichogramma brassicae* in tomato field

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Abstract: A study was carried out to map dispersal of *Trichogramma brassicae* Bezd. on tomato in a 0.5 ha field. First, 1, 5, 10, 15, 20 and 25 meter distances from the central point of the field were marked in form of squares. On each square, 8 points were selected and a wooden stake of 1.5 m length was vertically secured into the soil at each point equal to plant height and bearing a yellow sticky trap (YST, 10 × 20 cm) on the top. Moreover, three egg batches each containing ~200 fresh *Sitotroga cerealella* Oliv. eggs on cardboards (SEC) were stapled on top leaf of three plants around the wooden stake arranged in a 0.75 m arch. Evaluation was done two days before and 2, 4, 6 and 8 days after a release of 50000 newly emerged *T. brassicae* (~1:1 Male:Famale) from the central point. The number of *T. brassicae* trapped by YST, and number of parasitised eggs on SEC were counted under a stereomicroscope and noted down. The coefficient of fitting YST data to the model (R^2) for all 4 sampling dates was as high as 86%. The average diffusion coefficient was equal to 16.89 m^2/day for four times sampling. In general, the distance encompassing 98% of trapped *T. brassicae* predicted by the model was up to maximum 29.9 m from the central point 8 days after the release. The mean percent parasitism was 44.7, 5.5, 5 and 0.9 per SEC during 2, 4, 6 and 8 days after release, respectively. It is concluded that, the farther the distance from the releasing point and the longer the time elapsed after release, the less number of wasps were recaptured. Therefore, multiple releasing points are required to achieve even distribution and enough population of parasitoids in the field.

Keywords: biological control, *Helicoverpa armigera*, augmentative release, diffusion model

Introduction

Biological control has been a unique way to avoid pesticides hazards on human and animal health and environment (Bale *et al*., 2008). It is actually the development of a natural way to employ parasitoids, predators and antagonistic microbial agents. One of the worldwide used biocontrol agents has been *Trichogramma* species (Li, 1982). Based on a new survey, there are 11 species of the wasp defined in Iran (Ebrahimi, 1999), amongst which, *T. brassicae* Bezd. is commonly mass reared and released in rice, corn, tomato, cotton and soybean fields to control serious stem, fruit, boll and pod borers (Shirazi *et al*., 2010).

T. brassicae, being the dominant species in Iran (Ebrahimi, 1999), has been the core of biocontrol reseasrches including evaluation

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against *Helicoverpa armigera* on tomato (Shirazi *et al*., 2010). Despite the vast published material on *Trichogramma*, there are rare studies to point the basic behavioral and ecological aspects of the wasp (Hassan, 1994). For example, knowing the dispersal potential of the wasp would help the prediction of the pest control after a mass release (Smith, 1996; Orr *et al*., 2000). Some studies have proved a great area of dispersal for *Trichogramma* sp. after only one release from 1 point in the field (Wright *et al*., 2001; Wang *et al*., 1999). However, Shirazi *et al*. (2010) and Pezeshk *et al*. (2010) have reported a reversed relationship between plant area and searching ability and dispersal of *T. brassicae* in corn.

Rudd and Gandour (1985) introduced a model for dispersal estimation of an insect in the field (diffusion model). Chapman (2007) employed the model to study the dispersal of *T. ostriniae* (Peng & Chen) in the field to optimize its application in bell pepper for *Ostrinia nubilalis* (Hub) control. Similarly, the present study was undertaken to find out the direction, distance, area and timing of *T. brassicae* dispersal in tomato field after releasing from a central point. The information may improve biological control programs against tomato fruit borer in Iran.

Materials and Methods

Parasitoid preparation: The *T. brassicae* population was taken as about 5000 parasitized *Sitotroga cerealella* Oliv. eggs from Biological Control Research Dept. (Amol Lab), Iranian Research Institute of Plant Protection. It had been reared for 13 generations before. Then a colony was established at 25 ± 1 ºC, 65 ± 5 % RH and 16:8 h L: D. The wasp was reared for 4 generations on the same host and quality control test including purity of the species, longevity, fecundity, percent emergence, percent mortality and malformed adults were measured before release.

Field preparation: Sterling cultivar of tomato was transplanted in 5000 m^2 around mid May 2013. Plants were 30 cm apart on rows in furrow irrigation system (ridges with 120 cm width and furrows with 80 cm width) with 7 days interval. There was no tomato field around the experimental area. Later on, 2500 m^2 was selected in the center of the field. From the central point, squares with 1, 5, 10, 15, 20 and 25 m sides were marked off. On the periphery of each square, 8 equidistant points (4 on the corners and 4 in the middle of the sides) were marked and a wooden stick (1.5 m long) was secured into the soil at each point equal to plant height and bearing a yellow sticky trap (YST, 10 × 20 cm) on the top. Moreover, three egg cards each containing ~200 fresh *S. cerealella* eggs (SEC) were stapled on top leaf of 3 plants around the wooden stick arranged in a 0.75 m arch. Points were numbered clockwise 1 to 8 from west (1) to southwest (8). Similarly, letters from A to F were assigned to each square from the largest to the smallest one (Fig. 1).

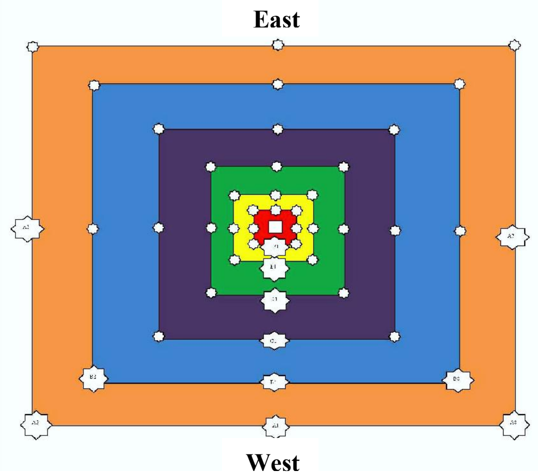

Figure 1 Squares: white = Release center, red = 1 × 1 m, yellow = 5 × 5 m, green = 10 × 10, purple = 15 × 15 m, blue = 20 × 20 m and orange = 25 × 25 m. Distance from release center to outer square on mid wall of all directions equals to 1, 5, 10, 15, 20, 25 m and on all 4 corners equals to 1.40, 7, 14.10, 21.20, 28.30 and 35.30 m. A1 is located on the mid wall of 25 × 25 square at west direction and F8 on the north west of 1 × 1 square.

Experiment implementation: Evaluation was done two days before (for natural parasitism estimation) and 2, 4, 6 and 8 days after a release of 50000 newly emerged *T. brassicae* with ~1:1 F: M sex ratio. At each evaluation interval, new YST and SEC were replaced and old ones were taken to lab for further examination. The number of *T. brassicae* trapped by YST, and number of parasitised eggs on SEC were counted under a Stereomicroscope and noted down.

Statistical method: The data was normalized (log10 (x + 1)) and fitted to a model proposed by Rudd and Gandour (1985) using SAS software (SAS, ver. 9.1). Table 1 has summarized formulae and equations used in this study. Meteorological statistics were taken from a nearby local synoptic weather station. Graphs were prepared using Excel software (MS Office, 2007).

Table 1 Formulae and equations used in this study (Rudd and Gandour 1985).

No	Formula /Equation	Consideration
1	$\frac{\partial y}{\partial t} = \frac{D\partial^2 y}{\partial x^2} - my$	Diffusion Model which considers only 1 dimension in the field for ease of calculations. It calculates changes in number of insects (*y*) within certain distance (*x*) during time after release (*t*) from a central point. Two important postulates: a) Wasps move random and b) There is no directed preference for the released insects.
2	$y(x,t) = y_0 \frac{e^{mt} e^{-(\frac{x^2}{4Dt})}}{2\sqrt{\pi Dt}}$	When there is no external force for wasp movement in the field, then, y_0 is the number of released population. The rest is as above formula.
3	$y(x,t) = y \frac{e^{mt} e^{-(\frac{(x-vt)^2}{4Dt})}}{2\sqrt{\pi Dt}}$	If there is effective wind equation 2 is modified as 3.
4	$y_i = Ae^{-Bx_i^2}$	To estimate the parameters of the model, first all captured wasps on YST from all directions and distances are arranged in an excel sheet. The NLin proc is used in SAS to fit the data to the model. In equation 4, (y_i) is the number of dispersed individuals at the distance of (*xi*). Constants A and B are the estimation of individual at the distance *x=0* and the ratio of density decrement of the wasp population with the distance from release point.
5	$D = \frac{1}{4Bt}$	Based on information obtained from equation 4, the dispersal coefficient (*D*) is calculated.
6	$\chi 98=2^{\sqrt{4Dt}}$	As the dispersal variance is equal to *4Dt*, then, the distance encompassing 98% of population is calculated through formula 6.
7	$m = \frac{-\ln(\frac{2A\sqrt{\pi Dt}}{y^0})}{t}$	Referring to equation 2, the mortality or migration can be achieved as *m*.
8	$y(t) = y_0 e^{-mt}$	The amount of the wasp remained in the field (*yt*) based on *m* (7) will be predicted.
9	$r^2 = 1 - \frac{SSe}{SST}$	r^2 is determination coefficient obtained as 1 deducted from sum square of error over total sum square.

Results

Quality control statistics of the population employed in the study have been summarized in Table 2. It is quite clear that the important biological attributes of *T. brassicae* used in the experiment including mortality (less than 15% within 4 days after emergence), sex ratio (> 50% female) and adult emergence (> 90%) were above standards defined for the mass produced population of the species in Iran (Table 2).

The SEC did not contain any parasitisation within 2 days at the time the experiment was carried out. Therefore, it is concluded that all counted wasps on YST belonged to the population released in the field. Table 3 has depicted the results obtained by fitting data to the equation 4 in Table 1.

The data was normalized through log10 (x + 1). The goodness of fit for first sampling date (2 days after release) was relatively high (> 72%) and very high at second and third sampling dates (90 and 90%, respectively). However, the determination coefficient of pooled data was acceptable (r^2 = 0.86) which proved the good fit of data to the model (Table 3). Similarly, the diffusion coefficient (*D*) was 43.5540 m/day and gradually declined to the end of experiment to 15.39 m/day. Generally, the average dispersal was determined as 16.89 m/day (Table 3).

The distance encompassing 98% of dispersed *T. brassicae* population except in the first sampling date (2.1324 m), was somewhat equal in the second, third and fourth counts (47, 46 and 44 m, respectively). Anyhow, the mean distance was equal to 30 m for all 4 sampling dates (Table 3). Adding to that, the density of remaining population in the field at each sampling date (Y_t) was a very small value compared with the released density. However, it was 530.92 wasps as average of all four sampling dates recorded (Table 3).

The relationship between 6 distances and number of wasps recaptured by YST from release point for 8 different directions were bar charted in Fig. 2. As it is shown, there was a uniform dispersal of the wasp from 1st (except for W direction which had unusually high counts of wasps) to 4th distance (1 to 15 m) with somehow increasing trend in all directions. Beyond 15 meters, sporadic counts were recorded irrelevant to the geographical directions (Fig. 2).

The information obtained from SEC for all distances with respect to sampling dates and 8 directions was compiled in Table 4. Considering the mean parasitisation, it was demonstrated that total number of parasitized eggs counted on SEC decreased as distance increased from release point. It was 44.77, 5.5, 5 and 0.9 parasitised eggs/SEC for 1st, 2nd, 3rd and 4th sampling dates of all 8 directions on each date, respectively (Table 4).

The wind as the most important meteorological parameter effective on dispersal was on average 10 m/s during the 8 days of experiment.

Table 2 Quality control statistics of *Trichogramma brassicae* measured at 25 ± 1 °C, 65 ± 5 RH and 16: 8 h L: D.

Attribute	Unit	Observed	Standard*
Emergence rate	%	90.6	> 85
Sex ratio	% females	50.6	> 50
Adult abnormality	%	4.3	< 5
Fecundity	eggs/female/ life span	40.2	> 40
Mortality within 4 days	%	15.0	< 20

*(Courtesy of Anonymous, 2004).

Table 3 Parameters and coefficients calculated by fitting number of recaptured *Trichogramma brassicae* by yellow sticky traps (YST) to diffusion model (Rudd and Gandour, 1985) after release of 50000 adults from a central point in tomato field.

Sampling date	t^a (day)	A^b	B^b	R^{2*}	D^c m/day)	$X_{98\%}{}^d$	m^e (no.)	Y_t^f (no.)
1st	2	68.6803	0.87960	0.72	43.5540	2.13249	2.97703	129.76
2nd	4	3.0667	0.00178	0.90	39.8089	47.40455	1.49373	128.80
3rd	6	2.4746	0.00179	0.91	23.5404	46.88072	1.03037	103.06
4th	8	1.4445	0.00201	0.85	15.3940	44.60997	0.84673	57.16
Mean of pooled data		20.0989	0.00450	0.86	16.8918	29.81424	0.56814	530.92

[a] Days after release.
[b] Coefficients of the model (Eq. 4).
[c] Diffusion coefficient (Eq. 5).
[d] Distance encompassing 98% of population (Eq. 6).
[e] Mortality / emigration rate (Eq. 7).
[f] Number of wasps remained at certain time (Eq. 8).
*Calculated based on Eq. 9.

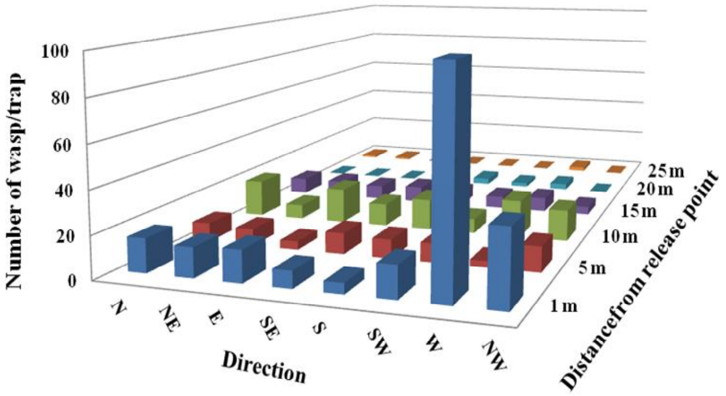

Figure 2 Cumulative number of *Trichogramma brassicae* counted on yellow sticky traps at different direction and distances after releasing 50000 adults from a central point and 4 times sampling in tomato field.

Table 4 Mean number of parasitised eggs of *Sitotroga cerealella* presented to 50000 *Trichogramma brassicae* released from a central point and sampled after 2, 4, 6, and 8 days in tomato field.

Sampling dates	Time elapsed (days)	Directions								Total average
		N	NE	E	SE	S	SW	W	NW	
1st	2	0	9.3	8.0	0	10.0	2.3	5.0	10.2	44.8
2nd	4	1.6	0.6	0.6	0.6	0.3	0.9	0.9	0	5.5
3rd	6	2.3	0	0.3	0.9	0	0.9	0.3	0.3	5.0
4th	8	0.3	0	0	0	0.3	0	0	0.3	0.9

Discussion

One of the important considerations for *Trichogramma* sp. application at field level would be the availability of information on its dispersal after release which depends on different parameters (Wang *et al*., 1997; Kuhar *et al*., 2004; Hoffmann *et al*., 2002).

For modeling the dispersal of an insect based on the idea of Rudd and Gandour (1985), there are some assumptions to be made namely, that insects move randomly, no external force could affect their behavior and they would not interfere with each other. Therefore, adding a linear approach to them for facilitating the calculations and considering the one dimension of insect movement (only horizontal) in the field has justified the employment of Diffusion Model in insect dispersal studies (Rudd and Gandour, 1985; Chapman, 2007; Kuhar *et al*., 2004).

Obtained dispersal coefficient (*D*) proved a decline in *T. brassicae* density with respect to time elapsed after release and distances from release point. It is believed that this coefficient varies among insects depending on insect species, gender and behavior in the field (Rudd and Gandour, 1985). Findings of the present study on *D* is in accordance with what Chapman (2007) reported while studying *T. ostriniae* dispersal in sweet potato. Moreover, it was demonstrated that during the first 2 days after release, wasps dispersed very slowly ($X_{98\%}$~2 m). The same finding was verified by Chapman (2007) for the first 48 h sampling. This could be due to time required for the wasps to get accustomed to new environment. In general, based on calculations of recaptured number of 50000 wasps released from

1 point in 2500 m^2, about 98% of the population was dispersed in 900 m^2 of the area (36% of the total arena) which is at par with research of Greatti and Zandigiacomo (1995), Wright *et al*. (2001) and Fournier and Boivin (2000), respectively, on *T. brassicae*, *T. ostriniae* and *T. pretiosum* dispersal in the field.

From another point of view, considering the approach in *T. brassicae* mass release for pest control, longevity of the parasitoid would play an important role in its uniform dispersal with enough density throughout the target field. Although the strain of *T. brassicae* used in our experiment passed the QC criteria of acceptable standards, the field condition may impose higher disappearance (mortality/emigration) to the wasp (Attaran, 2002; Wang and Ferro. 1998). Results on m and Y_t parameters obtained in this research have been emphasized by other scientists (Kanour and Burbutis 1984; Burbutis *et al*., 1977; Shirazi *et al*., 2010).

The effect of wind speed on *Trichogramma* dispersal efficiency has been debated by different researchers. If there is wind around 5 m/s, *T. pretiosum* and *T. evanescens* would change their movement same to the wind direction (downwind) (Fournier and Boivin, 2000). Moreover, the reduction in pest parasitism was observed upwind in the field (Greatti and Znadigiacomo, 1995; Hsiao, 1981; Smith, 1988; Fournier and Boivin, 2000). However, with the dominant south west wind in the region with around 10 m/s during the field test, except for high counts at 1 m distance in west point, there was more or less uniform dispersal based on counts in all directions and distances. The similar conclusion applies to host egg parasitisation as it was higher upwind (1 and 5 m distances) compared with that of downwind distances.

Finally it could be concluded that one release point would not be enough for effective dispersal of *T. brassicae* in a certain area of tomato field. Adding to that, the distance encompassing 98% of trapped wasps may imply that about 50 release points per one hectare of tomato would be enough to cover a uniform distribution of parasitoid for effective pest control. Considering the environmental conditions and quality control measures may also enhance the successful reduction of pest population in biocontrol programs of tomato fruit borer using the tiny friend.

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مطالعه‌ی پراکنش زنبور *Trichogramma brassicae* در مزرعه‌ی گوجه‌فرنگی

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چکیده: مطالعه‌ای به‌منظور تعیین نحوه و میزان پراکنش زنبور پارازیتویید *Trichogramma brassicae* Bezd. در یک مزرعه‌ی گوجه‌فرنگی با استفاده از مدل پخش انجام شد. ابتدا، مربع‌های به اضلاع ۱، ۵، ۱۰، ۱۵، ۲۰، و ۲۵ متری از مرکز زمین به سمت خارج مشخص شدند. روی هر مربع، ۸ قیّم چوبی ۱/۵ متری در ۴ نقطه در گوشه‌ها و ۴ نقطه در وسط اضلاع، هم ارتفاع با بوته نصب و یک تله‌ی زرد چسبنده (۱۰ × ۲۰ سانی‌متر) به سمت مرکز زمین، روی آن قرارداده شد. به‌علاوه، ۳ دسته‌ی تخم تازه‌ی (۲۰۰ عددی) *Sitotroga cerealella* Oliv. روی نوارهای مقوایی به برگ بالایی سه بوته‌ی گوجه‌فرنگی اطراف قیّم به شکل یک کمان ۰/۷۵ متری متصل شد. دو روز قبل و ۲، ۴، ۶، و ۸ روز بعد از رهاسازی حدود ۵۰۰۰۰ زنبور تازه ظاهر شده‌ی (۰-۲۴ ساعت طول عمر و نسبت جنسی ۱:۱ نر:ماده) *T. brassicae* از مرکز زمین، تعداد زنبورهای چسبیده به تله‌های زرد و تخم‌های پارازیته به تفکیک با استفاده از استریومیکروسکوپ شمارش شد. هیچ زنبوری در ارزیابی ۲ روز قبل از رهاسازی در مزرعه‌ی آزمایشی دیده نشد. ضریب تبیین مدل (R^2) و میانگین ضریب پخش زنبور (D) برای ۴ مرحله نمونه‌برداری بعد از رهاسازی به‌ترتیب ۸۶٪ و ۱۶/۹۸ متر/ روز بود. به‌طور کلی، براساس برآورد مدل، فاصله‌ی در برگیرنده‌ی ۹۸٪ از زنبورهای شکار شده در تله‌ها طی ۸ روز بعد از رهاسازی، حداکثر برابر با ۲۹/۹ متر از مرکز زمین بود. میانگین پارازیتیسم به‌ترتیب ۴۴/۷، ۵/۵، ۵ و ۰/۹ درصد در ۲، ۴، ۶ و ۸ روز بعد از رهاسازی بود. نتایج مشخص کرد که با توجه به افزایش فاصله از نقطه‌ی رهاسازی و گذشت زمان، تعداد زنبورهای شمارش شده روی تله‌ها کاهش می‌یابد. بنابراین برای دستیابی به توزیع یکنواخت زنبور در مزرعه و وجود جمعیت کافی در زمان معین برای کنترل آفت هدف، نقاط رهاسازی متعدی در گوجه‌فرنگی مورد نیاز است.

واژگان کلیدی: کنترل بیولوژیک، *Helicoverpa armigera*، رهاسازی اشباعی، مدل پخش