



## Functional response of *Trichogramma brassicae* Bezdenko to *Anagasta kuehniella* (Zeller) and *Phthorimaea operculella* (Zeller) eggs

Nahid Vaez\*, Zahra Pourgoli

<sup>1</sup>Department of Plant Protection, Azarbaijan Shahid Madani University, Tabriz, Iran

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### Abstract

The parasitoid *Trichogramma brassicae* Bezdenko is used for inundative releases in biological control programs against lepidopteran pests in agro-ecosystems. Study on the functional response of natural enemies is one of the necessary features for evaluating the efficiency of a natural enemies and selection of them for biological control programs. The objective of the present study was to determine the suitability of *T. brassicae* for controlling the *Phthorimaea operculella* (Zeller), a serious pest of stored potatoes in Iran. This is the first study to determine the functional response of *T. brassicae* to vary eggs densities of *P. operculella*. In this study, laboratory tests were conducted to determine the functional response of *T. brassicae* to different egg densities (1, 2, 4, 8, 16, 32, and 64) of its factitious host, the Mediterranean flour moth, *Anagasta kuehniella* Zeller and its natural host, the potato tuber moth, *P. operculella* at  $26 \pm 1$  °C,  $65 \pm 5\%$  RH, and 16: 8 h photoperiod. A type III functional response was observed on both host with a direct density dependent mortality up to sixteen host eggs and an inverse one upward. Results show that the searching efficiency was the same on both hosts but handling time was higher on *P. Operculella* eggs. The maximum rate of parasitism ( $T/T_h$ ) on *A. kuehniella* and *P. operculella* eggs were 25.29 and 21.10 host eggs per day respectively. This study has revealed the potential capacity of *T. brassicae* as an efficient biological control agent to suppress potato tuber moth.

\*Corresponding Author: Nahid Vaez ✉ [naheedvaez@gmail.com](mailto:naheedvaez@gmail.com)

## Introduction

The potato tuber moth (PTM) or potato tuber worm (PTW), *Phthorimae operculella* (Zeller) (Lepidoptera: Gelechiidae), with a worldwide distribution (in more than 90 countries) is an oligophagous pest that attack to solanaceous crops, mainly potatoes in both field and storage (Sileshi and Teriess, 2001; Rondon, 2010; Golizadeh and Esmaeili, 2012). This pest reported in 1985 from Iran for the first time (Habibi and Hesani, 1991) and now there is in most parts of Iran (Khanjani, 2005). The main damage of PTM caused by the larvae which feed on potato leaves, stems, petioles, and more importantly potato tubers in the field and in storage. In addition to the direct damage caused by the larvae feed on the tubers, indirect damage creates due to entry of pathogenic agents such as bacteria and fungi into the tubers specially in storage (Capinera, 2001). The most common control method for *P. operculella* is the use of various pesticides (Dillard *et al.*, 1993; Khanjani, 2005; Vaneva-Gancheva and Dimitrov, 2013) and it should be noted that it is difficult to chemical control of this pest in storage. Recently, much attention is to biological control of *P. operculella* using bioinsecticides and natural enemies such as Braconids and *Trichogrammatids* either in the field or in potato storage (Prattisoli and Parra, 2000; Agamy, 2003; Sarhan, 2004; Keasar and Sadeh, 2007; Mandour *et al.*, 2008, 2009, 2012; Saour, 2009).

*Trichogrammatids* egg parasitoids are the most widely studied and successfully used natural enemies in the world regarding their short generation time, easy mass rearing in insectaries and voracious parasitizing habit on eggs of target hosts. These parasitoids can be easily reared on factitious hosts, such as *Anagasta kuehniella* (Zeller), *Sitotroga cerealella* (Olivier), *Plodia interpunctella* Hubner and etc. (Cônoli *et al.*, 2010). *T. brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) is the most widespread species of *Trichogramma* in Iran, which used to control of some serious pests (Ebrahimi, 1999).

One of the important factor influencing the efficacy of a parasitoid or predator in biological control programs is the functional response (Reay-Joens *et al.*, 2006). Holling (1959) distinguished three types of responses, which are characterized by the number of prey consumed increasing linearly (type I), hyperbolically (type II), or sigmoidal (type III). Determining functional response can be helpful in preliminary screening of natural enemies as well as predicting the results of their release in field (Vaez *et al.*, 2013). Different types of functional responses have reported for *T. brassicae* at different temperatures and relative humidities in Iran (Moezipour *et al.*, 2008). A type II functional response have been observed by Farrokhi *et al.* (2010), also a type III functional response have reported on *S. cerealella* (Arbab Tafti *et al.*, 2004), *A. kuehniella* and *P. interpunctella* eggs (Farazmand and Iranipour, 2006).

Major studies on *P. operculella* in Iran are about its biological parameters on various potato varieties (Moharamipour *et al.*, 1991; Mansouri *et al.*, 2010 a, b; Mansouri *et al.*, 2012; Golizadeh *et al.*, 2014) and research on biological control of PTM by natural enemies has not yet been documented in Iran. This is the first study intended to determine and compare the functional response of *T. brassicae* to vary eggs densities of *A. kuehniella* and *P. operculella* in the laboratory.

## Material and methods

### Insect stocks

*P. operculella*: The initial population of *P. operculella* was provided from available infested potatoes in the laboratory of the Department of Plant Protection, Azarbaijan Shahid Madani University, Iran and was reared in controlled condition of  $26 \pm 2^\circ\text{C}$  and a photoperiod of 16:8 (L: D) h in  $50 \pm 10\%$  relative humidity. For larvae feeding, the tubers of potato (Agria cultivar) was placed into rectangular containers (15×20 cm). After adult emergence, to obtain 0- 24 h old eggs, 20 male-female pairs of the newly emerged moths were maintained in transparent plastic cylindrical containers (with a

diameter of 14 and a height of 20 cm), covered with black paper. The top of containers was covered with mesh net and then reversed it and a filter paper was placed below it. The moths laid eggs on filter paper. After 24h, the filter paper was removed and eggs were used in the experiment. The adults were fed using a 20% honey solution in water (Maharjan and Jung 2011).

*A. kuehniella*: A primary culture of the flour moth, *A. kuehniella* was obtained from the Department of Plant Protection of Tabriz University and reared on wheat flour (cultivar Omid) in 20×14×6 cm rectangular containers under the conditions described above in the same location. To provide 0-24h old eggs, 20 pairs of emerged adults were transferred to cylindrical containers (18 cm in diameter and 30 cm in height). The top of the dish was closed by a 50 mesh cloth net and set upside down on a piece of paper. Deposited eggs (<24h old) after pasting on a thick paper using sucrose solution, used in functional response experiments.

*T. brassicae*: *T. brassicae* was obtained as parasitized eggs of cereal moth from Agriculture and Natural Resources Research and Education center of Golestan Province and was maintained in glass vials (1.5×10 cm) with hosts in an incubator. They had previously been reared on flour moth.

All insects colonies (hosts and parasitoid) were held in an incubator at  $26 \pm 2$  °C,  $65 \pm 10\%$  RH, and a photoperiod of 16: 8 h (L:D).

#### Functional response experiments

To determine the functional response, seven egg densities (1, 2, 4, 8, 16, 32 and 64) of fresh *A. kuehniella* and *P. operculella* eggs (<24 h old) were exposed to individual females of *T. brassicae* in 20 replications. In each replication host densities with the mentioned above, were stuck with a regular dispersion of eggs on a paper strip (1 × 5 cm) and a drop of 20% diluted honey solution as food for parasitoids. The strip was introduced into a glass vial (1.5 × 10 cm) containing a pair of ≤24 h old (1♀ and 1♂) parasitoids and was closed with cotton wool.

After 24 h, the adult parasitoids were removed and the host eggs were kept in an incubator under the same conditions (mentioned for the maintenance of the colonies) until they hatched. The number of *T. brassicae* adults per host egg (the number of black eggs black eggs), the emergence rate and the proportion of female/male (sex ratio) were determined.

#### Statistical analysis

Analysis of functional responses data comprises two distinct steps (Juliano, 2001). The first step is to determine the type (shape) of functional response curve using logistic regression analysis of the proportion of parasitized hosts ( $N_a/N_o$ ) vs. initial number of hosts ( $N_o$ ) by a following polynomial function (Juliano 2001):

$$\frac{N_e}{N_o} = \frac{\exp(P_0 + P_1 N_o + P_2 N_o^2 + P_3 N_o^3)}{1 + \exp(P_0 + P_1 N_o + P_2 N_o^2 + P_3 N_o^3)} \quad (1)$$

Where  $P_0$ ,  $P_1$ ,  $P_2$ , and  $P_3$  are the parameters to be estimated by using the CATMOD procedure in SAS (version 9.2). The sign of  $P_1$  and  $P_2$  can be used to distinguish the shape of the curves. A positive linear parameter ( $P_1$ ) indicates that functional response is type III, whereas the functional response is type II when linear parameter ( $P_1$ ) is negative. After determining the type of functional response, parameters (searching efficiency and handling time) must be estimated. A non-linear least square regression (NLIN procedure with DUD method in SAS) was used to estimate the mentioned parameters using Roger's (1972) random parasitoid equation (equation (2) for type-II and equation (3) for type-III functional responses).

$$N_a = N_o \{1 - \exp[a(T_h N_a - T)]\} \quad (2)$$

$$N_a = N_o \{1 - \exp[(d + b N_o)(T_h N_a - T)/(1 + c N_o)]\} \quad (3)$$

Where  $N_a$  is the number of hosts attacked,  $N_o$ , the initial number of hosts,  $T$ , the time of exposure (24 h),  $a$ ,  $b$ , the rate of successful attack,  $T_h$ , the handling time and  $c$  and  $d$  are constants. Parameters  $d$  and  $c$  were excluded successively from analyses in non-significant circumstances. Curves were drawn by Excel.

## Results

According to the results presented in Table 1, the linear coefficient of logistic regression was positive both in hosts that imply the *T. brassicae*'s functional response to *P. operculella* and *A. kuehniella* eggs were type-III. Functional response curves of the *T. brassicae* female adults to various densities of two host eggs are shown in Fig. 1. This suggests that parasitism acts as a positive density dependent

mortality factor at low host densities and as a negative density dependent factor at higher host densities (Fig. 2).

Results of nonlinear least square regression indicated that parameters *c* and *d* were not significantly different from zero; therefore, we eliminated them from the model and a reduced model was used.

**Table 1.** Logistic regression analyses of proportion of parasitized *Anagasta kuehniella* and *Phthorimaea operculella* eggs by *Trichogramma brassicae*.

Host species	Coefficient	Estimate	SE	$\chi^2$	P- value
<i>Anagasta kuehniella</i>	Intercept	0.9740	0.2678	13.23	0.0003
	N <sub>0</sub>	0.0514	0.0412	1.55	0.2125
	N <sub>0</sub> <sup>2</sup>	-0.00410	0.00157	6.86	0.0088
	N <sub>0</sub> <sup>3</sup>	0.000046	0.000015	8.94	0.0028
<i>Phthorimaea operculella</i>	Intercept	0.0330	0.2406	0.02	0.8908
	N <sub>0</sub>	0.1430	0.0381	14.07	0.0002
	N <sub>0</sub> <sup>2</sup>	-0.00732	0.00147	24.87	<.0001
	N <sub>0</sub> <sup>3</sup>	0.000077	0.000015	27.78	<.0001

Estimated *b* values and host handling times on *A. kuehniella* were  $0.0046 \pm 0.001 \text{ h}^{-1}$  and  $0.95 \pm 0.056 \text{ h}$ , and on *P. operculella* were  $0.0045 \pm 0.001 \text{ h}^{-1}$  and  $1.14 \pm 0.071 \text{ h}$  respectively. The maximum number of attacks is limited by an upper asymptote value

defined by the ratio of  $T/T_h$ . So the maximum parasitism rate by the *T. brassicae* on *A. kuehniella* and *P. operculella* were 25.29 and 21.10 host eggs per day respectively (Table 2).

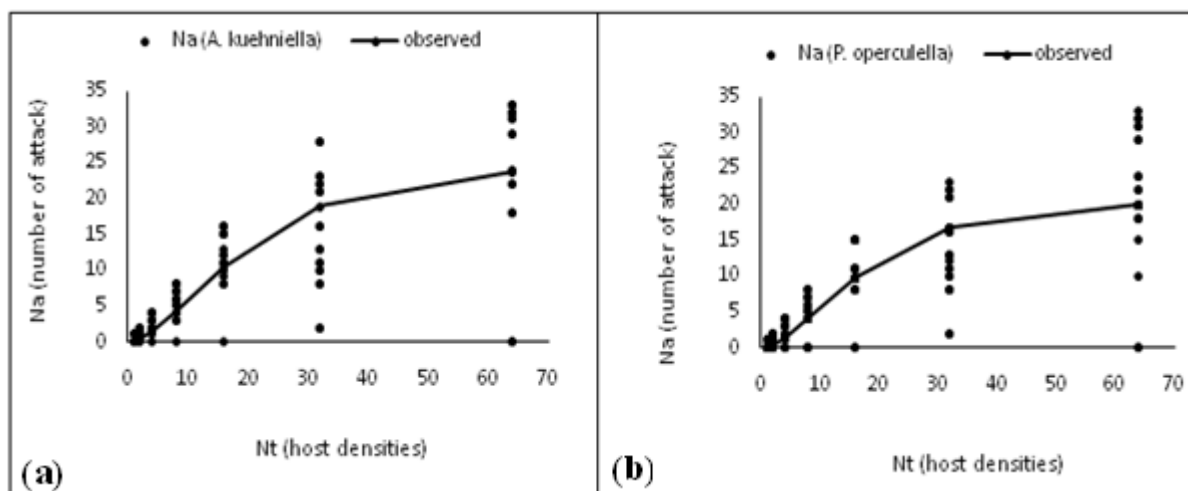
**Table 2.** Parameter (means  $\pm$  SE) estimates of the functional responses of *Trichogramma brassicae* to *Anagasta kuehniella* and *Phthorimaea operculella* eggs.

Host species	Type	r <sup>2</sup>	Parameters	Estimate	Asymptotic 95% CI	
					Lower	Upper
<i>Anagasta kuehniella</i>	III	0.86	<i>b</i>	$0.0046 \pm 0.001$	0.0010	0.0046
			<i>T<sub>h</sub></i>	$0.95 \pm 0.056$	0.8368	0.9489
<i>Phthorimaea operculella</i>	III	0.81	<i>b</i>	$0.0045 \pm 0.001$	0.0010	0.0045
			<i>T<sub>h</sub></i>	$1.14 \pm 0.071$	0.8063	1.1374

Based on model estimations in all densities, mortality rate of the *A. kuehniella* and *P. operculella* by *T. brassicae* was the same.

Subsequently, functional response curves of the *T. brassicae* on two hosts were nearly parallel (Fig. 1).

In both hosts of the parasitoids, host mortality was directly density-dependent till it reached a density of sixteen, while it was inversely density dependent at 32 and 64 densities.



**Fig. 1.** Functional response of *Trichogramma brassicae* to different initial densities of *A. kuehniella* (a) and *P. operculella* (b).

The searching efficiency was the same on both hosts but the handling time was higher on *P. operculella*. Our results indicated that change in host, could not change the type of functional response of *T. brassicae* and for each two host, it was type III.

### Discussion

To determine the efficiency of a parasitoid, the functional response studies are useful to provide the first step for comparing the efficiency of different species (Overholt and Smith, 1990) and also provide valid information on host-finding abilities of candidate natural enemies (Munyanza and Obrycki, 1997).

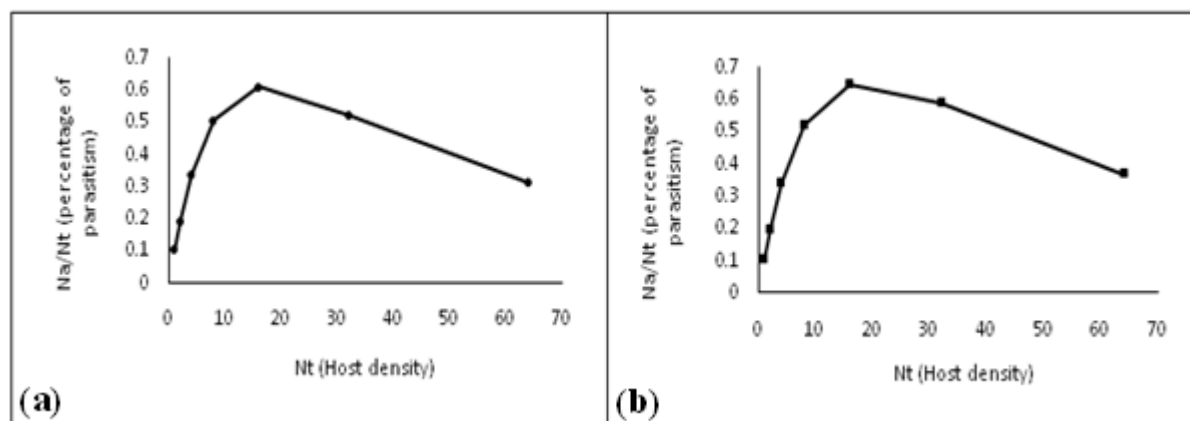
Functional response shape and parameters can be affected by a series of factors such as temperature, rearing conditions (temperature and humidity), species/strain of parasitoid and also by type, size and physiological state of host (Juliano and Williams, 1985; Wang and Ferro, 1998; Moezipour *et al.*, 2008; Vaez *et al.*, 2013). According scientist reports, type I, II and III functional responses have been seen for *Trichogramma* species in laboratory studies. A type I response has been reported for *Trichogramma minutum* Riley on *A. kuehniella* eggs by Mills and Lacan (2004). Also Kfir (1983) reported the type II response for *Trichogramma pretiosum* Riley with *P. operculella* eggs.

As similar results, Karimian (1998) and Farrokhi *et al.* (2010) reported the type II response for *T. brassicae*. Nikbin *et al.* (2014) reported a type III functional response for the one-day-old females of *T. brassicae* and a type II for two- to nine-day-old ones. In contrast, in some studies type- III functional response was observed in these parasitoids (Fathipour *et al.*, 2002, 2003; Arbab Tafti *et al.*, 2004; Reay-Jones *et al.*, 2006; Farazmand and Iranipour, 2006; Vaez *et al.*, 2013) that is similar to present study. In some studies, both types of functional response (II and III) were observed in different treatments. Wang and Ferro (1998) reported a type-II functional response for *Trichogramma ostrinae* Pang et Chen parasitizing *Ostrinia nubilalis* (Hubner) at low temperatures and a type-III at high temperatures. Moezipour *et al.* (2008) reported a type-II functional response of *T. brassicae* to *S. cerealella* at 25 °C and a type III at 20 and 30 °C. Their results indicated that there is a significant difference in functional response of *T. brassicae* at different temperatures.

The scientists suggested that increased foraging behavior at high temperatures may have accelerated the learning process of *Trichogramma* wasps in discriminating between parasitized and unparasitized eggs, resulting in a type III functional response (Guo, 1986; Zhang *et al.*, 1983; Wang and Ferro, 1998). In our study a type-III functional response was observed

for *T. brassicae* both on *A. kuehniella* and *P. operculella* eggs, that showing an initial increase in the rate of parasitism in a 24-hour period experiment. Our results revealed that the type of host has no important effect on the functional response of *T. brassicae*. The efficacy of *Trichogramma* spp. during

the first 24 h after their release is crucial, because inundative releases of parasitoids should have an immediate effect in the field. In present study, although the shape of the functional response curve did not change on two hosts, the height of the curve declined with *P. operculella* eggs.



**Fig. 2.** Attack rate of parasitized *A. kuehniella* (a) and *P. operculella* (b) eggs by *T. brassicae*.

The handling time and the attack rate or searching efficiency are a good indicators of the parasitism rate (Atlihan and Guldal, 2009). In present study the searching efficiency, however, was not affected by host type. Handling time is the time spent in activities such as catching, overcoming, exploitation and digesting of a prey or finding, drilling and parasitising a host. In this study, the time spent for parasitizing on *P. operculella* eggs was 1.2 times longer compared with *A. kuehniella* eggs.

This may suggest that parasitic wasps developed in potato tuber moth eggs were weaker than other host. In our study, the maximum rate of parasitism ( $T/T_h$ ) was higher on *A. kuehniella* eggs, because handling time ( $T_h$ ) was lower. It has been stated that the asymptote is determined by either the handling time ( $T_h$ ), egg limitation or egg retention of female parasitoids (Hassell, 1978, Hassell; Waage, 1984). Many factors affect searching efficiency and handling time of parasitoids including temperature and relative humidity (Shojaei *et al.*, 2006; Moezipour *et al.*, 2008), density and strain (Tahriri *et al.*, 2007; Farrokhi *et al.*, 2010), as well as host age and density (Chen *et al.*, 2006).

Fathipour *et al.* (2002) reported searching efficiencies of *T. pinto* on *A. kuehniella* and *S. cerealella* eggs were 0.48 and 0.16  $h^{-1}$ , respectively and handling times were 0.965 and 1.169 h, respectively.

Searching efficiency and handling time of *T. brassicae* in Farazmand and Iranipour (2006) study, for *A. kuehniella* were 0.0042  $h^{-1}$  and 0.601 h respectively and for *P. interpunctella* were 0.0042  $h^{-1}$  and 1.344 h respectively. In Arbab Tafti *et al.*'s (2004) study, the searching efficiency and handling time of *T. brassicae* on *S. cerealella* were 0.168  $h^{-1}$  and 1.468 h respectively. Both parameters were lower in Moezipour *et al.* (2008) study in all sets of temperatures and moistures.

In conclusion, this study has improved our knowledge on *T. brassicae* and its hosts (*A. kuehniella* and *P. operculella*) interactions in the laboratory and has illustrated the potential capacity of *T. brassicae* as an efficient biological control agent to suppress potato tuber moth. *T. brassicae* by showing a type III functional response has better opportunities to regulate *P. operculella* population.



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