Full Length Article

Parasitism Rate, Host Stage Preference and Functional Response of *Tamarixia radiata* on *Diaphorina citri*

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Abstract

The Asian citrus psyllid *Diaphorina citri* (Kuwayama) (Hemiptera: Psyllidae), is a serious pest and main threat to citrus production worldwide. The present study was carried out to assess the performance of *T. radiata*, as biological control agent of *D. citri*, under insectary condition. The result shows *T. radiata*, to preferred late (4th and 5th) instar nymphs than early (1st, 2nd and 3rd) instar nymphs of *D. citri*, with mean parasitism rate of 0%, 0%, 43.3%, 76.9% and 86.0% for 1st, 2nd, 3rd, 4th and 5th instar nymphs respectively. The binomial logistic regression analysis of *D. citri* nymphs attacked by *T. radiata*, as a function of host (*D. citri* nymphs) density offered, shows *T. radiata*, to display a functional response type II with attack rate of 39.99 and 34.04, and handling time of 0.60 and 0.71 for 4th and 5th instar nymphs of *D. citri* respectively. © 2014 Friends Science Publishers

Keywords: *Diaphorina citri*; Functional response; Malaysia; Parasitoid; *Tamarixia radiata*

Introduction

Control of several insect pests in citrus production areas of Malaysia depends to large extent on application of chemical insecticides (Ahmad *et al*., 2011). Whereas, resistance development have been observed frequently among pest population. Insect parasitoids have often been used to managed pest; parasitoids are vital agents of natural control of insect pest populations, and mostly are used in agriculture as biological control agents. Generally different techniques were used to evaluate the efficacy of natural enemies, which are classified into direct and indirect techniques. Direct techniques includes laboratory and field estimation of parasitism rate (Prasad, 1989), through parasitism of placed-out pray or biological check methods (Jervis, 2005). While the indirect techniques, include observation of pest population in the presence or absence of natural enemies, such as exclusion of natural enemies or removal of natural enemies.

The potential of a parasitoid can be evaluated under laboratory condition and it can give a good idea about efficacy of the parasitoid. However, efficacy of a parasitoids depends on its ability to locate suitably sized hosts within crop habitats and to kill larvae through host feeding (ingestion of the contents of host larvae) or parasitism (Ode and Heinz, 2002).

Functional response of a parasitoid is a major factor in regulating population dynamics of parasite-host intraction (Pervez and Omkar, 2005), it can be used to verify the efficiency of a parasitoid in managing host populations.

There are three types of functional response categorized according to the shape of the response curve (Holling, 1965), they can be differentiated by evaluating the coefficient of instantaneous search rate and handling time.

Given *T. radiata,* as a typical biological control agent for *D. citri*, considerable information is available on its developmental biology, rearing methodology and distribution in some parts of the world. However, information on its parasitism and functional response is lacking in Malaysia. Therefore, the objectives of the present study are, 1) to investigate, the parasitism rate and host preference of *T. radiata* on *D. citri*. 2) functional response of *T. radiata* on different larval stages of *D. citri*.

Materials and Methods

Sourcing and Rearing of *D. citri*

Colonies of Asian citrus psyllid *D. citri* were established on potted *Murraya paniculata* (Orange jasmine) plants with immature *D. citri* (nymphs) collected from *M. paniculata* plants in ladang 2 Universiti Putra Malaysia, using aspirator or cutting of *M. paniculata* shoots containing *D. citri* nymphs and quickly placing it in glass vial with ventilated cover.

These insects were taken to the glasshouse, thereafter ten nymphs of *D. citri* were introduced into a screen cage measuring 54x39x39 cm containing one *M. paniculata* plant with new flush of vegetative shoots, the insects were

allowed to remain on the *M. paniculata* plants in the cage until when they laid eggs on the new developing shoots, after that, they were removed from the cage to avoid overcrowding of nymphs. The F1 adults of *D. citri* from the cage *M. paniculata* plant were used to generate more colonies of *D. citri*. The colonies were maintained in screened cages in glasshouse and the plants were pruned and fertilized regularly to promote production of new leaves that is favoured for oviposition by adult female *D. citri* (Sule *et al.*, 2012), and glasshouse conditions (Temp. 30 ± 1^0C, RH 85% and L.D 12:12) were maintained throughout the rearing.

Sourcing and Rearing of *T. radiata*

Tamarixia radiata colonies were established in laboratory from mummified larvae of *D. citri* collected from *Citrus suhuiensis* trees and *M. paniculata* plant in ladang 2 Universiti Putra Malaysia and Universiti Putra Malaysia mosque, by cutting shoots containing mummified larvae of *D. citri* and placed it in plastic cup containing moist sponge at the bottom with ventilated cover. These mummified larvae were taken to the insectary for rearing.

After emergence of adults *T. radiata*, they were reared on potted *M. paniculata* plants placed in a screen cage measuring 55 x 40 x 35 cm. Approximately 50 ovipositing *D. citri* females were released into the cage and allowed to oviposit for 4 days and then removed from the cage. After that, adults *T. radiata* were released into the cage when the psyllids population reached the third instars. At the interval of each 3 days, paper coated with a mixture of honey and yeast extract in the ratio of 60:40 were hung on the plants as source of food for adult wasps, as recommended by Chien *et al.* (1994). In addition water was sprayed with a squeeze bottle on to the plant foliage at regular intervals through the cage top to form fine water droplets from which the parasitoids will drink (Skelley and Hoy, 2004). The first generation adults obtained from the cage *M. paniculata* plant were used to generate more colonies of *T. radiata*.

Parasitism Bioassay

Parasitism rate of *T. radiata* was determined using no-choice test, by placing 10 nymphs of each growth stage of *D. citri* nymphal instars using camel hair brush, on 5 cm tall *M. paniculata* plant with flush leaves placed in a plastic cup measuring 7 cm height x 7 cm upper diameter x 5 cm lower diameter. Thereafter, 1 pair of adult *T. radiata* was introduced into the plastic cup containing the *M. paniculata* plant and psyllid nymphs, the open end of the cup was covered with white muslin cloth and tied very well with rubber band, and the experimental set up was replicated 5 times.

After 48 h the parasitoids were removed from the cup and the experimental set up was held at ambient temperature (29 ± 1^0C) and relative humidity (85% RH) in insectary for at least 10 days to allow adults of *D. citri* and *T. radiata* to

emerge. Later the cups were open and the number of *D. citri* and *T. radiata* adult that emerge were counted and percent parasitism of each nymphal stage was calculated using the formula below as describe by Qureshi *et al.* (2009).

$$\text{Parasitism rate (\%)} = \frac{No.of\ emerged\ parasitiods}{No.of\ emerged\ host + No.of\ emerged\ parasitiods} \times 100$$

Nymph-stage Preference

Preference of *D. citri* nymphs by *T. radiata* was determine by conducting choice test with 5 replication between susceptible nymphal stages in the previous experiment, the combination of the choice test are 3rd and 4th instars nymphs, 3rd and 5th instars nymph and 4th and 5th instars nymphs. Eight nymphs of each nymphal stage were individually transferred with camel hair brush onto *M. paniculata* plant with flush leaves in a screen cage measuring 7 cm height x 7 cm upper diameter x 5 cm lower diameter. A newly mated female *T. radiata* was introduced into each of the screen cage for 24 h to oviposit and then removed by coxing between two camel hair brushes. The nymphs were left on the plant in cage for another 24 h, thereafter leaves having nymphs on them were removed from plant and placed in plastic cup on paper towel over a moist sponge to maintain the freshness of the leaves for adult parasitoid to emerge and the open end of the cup was covered with white muslin cloth and tied very well with rubber band.

Functional Response

The functional response of *T. radiata* on 3 different nymphal stages of *D. citri* was studied separately using bioassay protocol similar to that described by Yingfang and Fadamiro (2010) with minor modification. The parasitism arena consist of *M. paniculata* shoots with 4 non matured fully expanded leaves, placed on a moist paper towel over a wet sponge, inside a transparant plastic cup measuring 7 cm height x 7 cm upper diameter x 5 cm lower diameter. Nymphal instar 3rd, 4th and 5th were introduced seperately onto the leaves of the Murraya inside the plastic cup at densities of 2, 5, 10, 15, 20, 40, 80, 100 and 120, each density has five replications. Single 2-3 days old newly mated female *T. radiata* was introduced, and the open end of the cups were covered with white muslin cloths and tied very well with rubber band. The *T. radiata* was removed after 24 h and the number of parasitized nymphs was estimated after 7 days by counting mummified nymphs.

Statistical Analysis

Datasets collected on parasitism rate and nymphal preference experiments were analyzed using analysis of variance with computer software (SAS 9.0 for windows) statistical package. Treatments means with significant differences were compared at 0.05% level of probability using Duncan multiple range test (DMRT).

The type (shape) of *T. radiata* functional response was determined by performing a binomial logistic regression analysis of host attacked as a function of host density offered as described by Timms *et al.* (2008) and Juliano, (2001).

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

Na is the number of killed hosts, *No* is the number of host offered (density), P_0 is the intercept (constant), P_1, P_2 and P_3 are the linear, quadratic and cubic coefficients respectively, related to the slope of the curve. After determining correct shape of the functional response, parameter *a* (attack constant) and b (asymptote of functional response curve or maximum number of hosts parasitized) were estimated using non-linear least squares regression procedure in SAS computer software and the values were used in Holling (1965) disc equation

$$N_e = \frac{aNT}{1 + aNT_h}$$

Where, *Ne* = the number of host parasitized by a parasitiod per unit time, *a* attack rate of a parasitiod, *N* is the initial number of host provided to parasitiod at the onset of the experiment, *T* is the total exposure time in the experiment and T_h is the handling time for each host attacked. The handling time (T_h) which is the time required to handle a host i.e. identify, pursue and attack a host = *b/a*, and maximum attack rate (*a*) which represent the highest number of hosts that could be attacked during the exposure period of the experiment = T/T_h.

Results

Tamarixia radiata Parasitism

Parasitisation rate of *D. citri* nymphs by adult *T. radiata* are shown in Table 1. The results indicate that parasitisation rate differed significantly within the different *D. citri* nymphal stages (F = 111.23, P = 0.0001). High number of 5th instars nymphs were parasitized by adult *T. radiata* compare to the other nymphal stages, however this number did not differ significantly with the number of 4th instars nymphs parasitized (Table 1). Furthermore, the result shows that 1st and 2nd instars nymphs were not parasitized.

Nymph-stage Preference

Preferences of *T. radiata* for different nymphal stages of *D. citri* are shown in Table 2. The result shows that, *T. radiata* oviposited on both nymphal instars offered in all the three host stage combinations. Significantly low number of 3rd instars nymphs were preferred for oviposition by *T. radiata* when assayed with 4th and 5th instars nymphs (Table 2). But, when 4th and 5th instars nymphs were assayed together, there was no difference statistically in preference shown by *T. radiata* to the 4th and 5th instars nymphal stages of *D. citri*.

Table 1: Host stage parasitism rate of *Tamarixia radiate* for 24 h, under insectary condition (Temp. 29±1°C, RH 85% and L:D 12:12)

Host stage	Mean ± SE
1st Instars	0c
2nd Instars	0c
3rd Instars	43.3±4.1b
4th Instars	76.9±4.7a
5th Instars	86.0±6.0a

Means followed by same letters within same column are not significantly different at P= 0.05% level of probability according to DMRT test

Table 2: Host stage preference of *T. radiata* when offered same ratio of different *D. citri* nymphs for 24 hours under insectary condition (Temp. 29±1°C, RH 85% and L:D 12:12)

Host stage combination	Host stage	Mean ± SE
3rd and 4th Instars	3rd Instars	4.2 ± 0.39b
	4th Instars	6.8 ± 0.48a
3rd and 5th Instars	3rd Instars	3.6 ± 0.53b
	5th Instars	7.6 ± 0.65a
4th and 5th Instars	4th Instars	7.4 ± 0.21a
	5th Instars	7.8 ± 0.26a

Means followed by same letters within same column are not significantly different at P= 0.05% level of probability according to DMRT test

Table 3: Estimates of coefficients in a binomial logistic regression of the proportion of host parasitized on total hosts for the three nymphal instars of *D. citri* under insectary conditions

Nymphal stage	Coefficients	Estimate	SE	t	P-value
3rd Instars	P_0 (Constant)	0.984	0.058	17.047	0.000
	P_1 (Linear)	-2.798	0.001	-7.585	0.000
	P_2 (Quadratic)	3.109	0.003	-5.720	0.001
	P_3 (Cubic)	-1.249	0.005	-3.742	0.10
4th Instars	P_0 (Constant)	1.086	0.036	30.086	0.000
	P_1 (Linear)	-2.086	0.001	-9.973	0.000
	P_2 (Quadratic)	1.252	0.001	-11.101	0.000
	P_3 (Cubic)	-0.101	0.003	-5.132	0.002
5th Instars	P_0 (Constant)	1.093	0.057	19.351	0.000
	P_1 (Linear)	-0.013	0.001	-9.770	0.000
	P_2 (Quadratic)	1.856	0.002	6.733	0.000
	P_3 (Cubic)	2.632	0.005	2.667	0.037

Table 4: Estimates of functional response parameters of *T. radiata* on different nymphal instars of *D. citri* from linearization of Holing's type II model

D. citri nymphal stage	Attack rate (*a*)	Handling time (T_h)	R^2
3rd Instars	21.62	1.11	0.923
4th Instars	39.99	0.6002	0.886
5th Instars	34.04	0.7051	0.878

Functional Response

Functional responses of *T. radiata*, to different densities of the three different nymphal stages of *D. citri*, are shown in
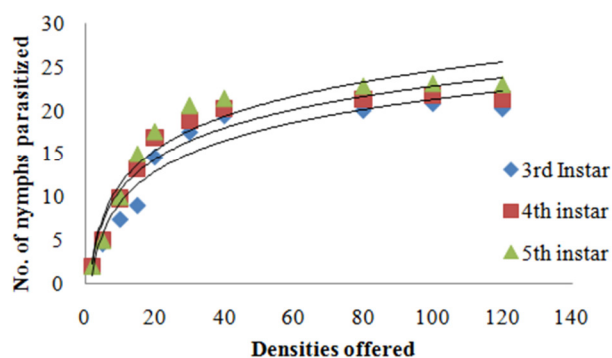
Fig. 1: Mean number of *D. citri* nymphs parasitized by *T. radiata* in relation to nymphal densities under insectary condition (Temp. 29±1^{0}C, RH 85% and L:D 12:12).
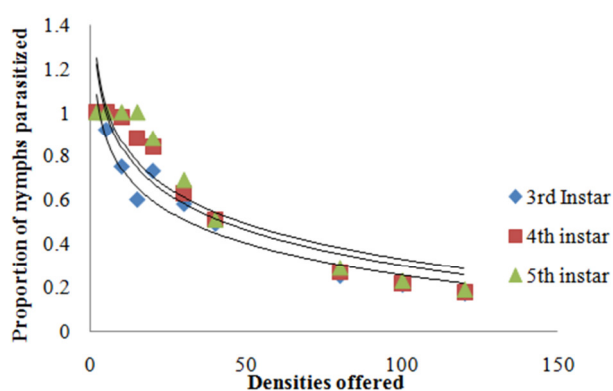

Fig. 2: Proportion of *D. citri* nymphs parasitized by *T. radiata* in relation to nymphal densities under insectary condition (Temp. 29±1^{0}C, RH 85% and L:D 12:12).

Fig. 1 and 2. The average number of *D. citri,* nymphs parasitized by *T. radiata,* in all the three nymphal stages increase at a decreasing rates until when it reach an upper plateau with increase in host densities during the 24 h exposure period (Fig. 1). When the density of *D. citri* nymphs were increased from 15 to 20 nymphs, number of 3rd, 4th and 5th instars nymphs parasitized increased from 9 to 14.6, 13.2 to 16.8 and 15 to 17.6 respectively, also when the density was increased from 40 to 80 nymphs, number of nymphs parasitized increased from 19.4 to 20, 20.2 to 21.2 and 21.4 to 22.8 in 3rd, 4th and 5th instars nymphs respectively.

While, the proportion of parasitized 3th, 4th and 5th instars nymphs of *D. citri* by *T. radiata* decreased with increased in density of hosts (Fig. 2), i.e. when the density of *D. citri* nymphs were increased from 20 to 30 nymphs, the proportion of 3rd, 4th and 5th instars nymphs parasitized decreased from 0.73 to 0.58, 0.84 to 0.63 and 0.88 to 0.69 respectively, also when the density was increased from 80 to 100 nymphs, proportion of nymphs parasitized decrease from 0.25 to 0.21, 0.27 to 0.22 and 0.29 to 0.23 in 3rd, 4th and 5th instars nymphs respectively.

Results of the binomial logistic regression analysis (Table 3) for all the three nymphal stages, shows negative coefficients for P$_1$ (linear term), and estimated handling time of *D. citri* 3rd, 4th and 5th instars nymphs by *T. radiata* were 1.11, 0.6002 and 0.7051 respectively, with the attack rate of 21.62, 39.99 and 34.04 on 3rd, 4th and 5th instars nymphs respectively (Table 4).

Discussion

Lack of parasitisation of 1st and 2nd instars nymphs in the present study may be associated with body size of the nymphs, which are relatively small compared to the remaining nymphal instars and could not support the full development of the parasitoids. Female *T. radiata* are reported to attack *D. citri* during the psyllids 3rd, 4th or 5th instars nymphal development (Skelley and Hoy, 2004), by ovipositing particularly underneath the fourth and fifth instar nymph. According to Mann and Stelinski (2010), *T. radiata* has a short generation time and high reproductive rate in the laboratory, yet no much work have been done in laboratory to evaluate *T. radiata* parasitism rates on different *D. citri* nymphal stages.

Nevertheless, the values of parasitism of 4th and 5th instar nymphs observed in the present study under insectary condition (29±1º and 85% RH) are similar to those obtained by Gomez-Torres *et al.* (2012), who studied life table of *T. radiata* on *D. citri* at different temperatures, they reported a parasitism rate of 77.24% at a temperature of 26.3ºC. Similar results have been obtained for other species of the family Eulophidae. According to Wang *et al.* (1999), temperatures ranging from 20 to 30ºC favor the parasitism and reproduction of members of Eulophidae. Those authors found that the number of hosts parasitized by eulophid females, and the eulophid fertility rates were higher at temperatures above 25ºC than at other temperatures studied.

Effectiveness of *T. radiata* in managing psyllid populations under field conditions has shown variable results. In Malaysia for instance, Osman and Quilici (1991), reported parasitisation rates ranging up to 28% in 4th and 5th instar nymphs and Lim *et al.* (1990) reported parasitisation rate of up to 36%. Field parasitism rates of of *D. citri* by *T. radiata* within Florida and other adjoining regions have been lower, rarely exceeding 20%, in spring and summer, rising to 39-56% in the fall compared with rates reported for previous biocontrol programs by using *T. radiata* in other places such as Reunion Island, Guadaloupe, and Puerto Rico (Hall *et al.*, 2008, Qureshi *et al.*, 2009). Yet high (64-100%) mortality of *T. radiata* in Florida has been associated with predation (killing and eating potential competitors) by coccinellid species (Qureshi *et al.*, 2009, Michaud, 2004), thus reducing populations of *T. radiata*. In addition, extreme climatic conditions of Florida and the intense use of insecticides in controlling *D. citri* populations are presumed to be responsible for low parasitoid populations in Florida (Michaud, 2004).

The total number of host parasitized varied between host stages, when *T. radiata* was given a choice between equal number of each nymphal instars, it consistently show higher preference for 4th and 5th instar nymphs than 3rd instar nymph. Our observed preference of the two nymphal instars over the other is related to difference in the body size of the nymphal stages. The *T. radiata* have preferred the 4th and 5th instars nymphs, because they are larger, more easy to locate and can provide more fitness for the parasitoid. Different studies also suggest that large hosts may generate superior parasitoid fittness (Zain-ul-Abdin *et al*., 2012). A similarly results were obtained by Hoy *et al*. (2006) and Chien *et al*. (1991) in which they reported female *T. radiata* to showed considerable preference for 5th-instar nymphs of *D. citri*. According to Mackauer (1983), host stage preference is not constant, but influence by test duration and parasitoid functional responses to densities of host offered and it may depends on several factors.

Decreasing in proportions of attacked host with increasing host density is common for arthropods parasitiods (El-Basha *et al*., 2012; Fernandez-arhex and Corley, 2003). The accelerated decline in the proportion of *D. citri* nymphs parasitized by *T. radiata* in relation to the density of the host in the present study fitted the description of a type II functional response. Results of the binomial logistic regression analysis confirmed this observation (Table 3) for all the three nymphal stages, by having significantly negative coefficients of P_1 (linear term), suggested that the slope of the functional response curves were declining, which is characteristics of a type II functional response. Functional responses type II were reported previously in laboratory experiments for a number of parasitoids (Kafle *et al*., 2005; Patel *et al*., 2003). The type of functional response of natural enemy (parasitoid/predator) could be affected by many factors i.e. host plant, temperature, type of pray/host and host/pray growth stages (Mohaghegh *et al*., 2001). Zohdi and Talebib (2010), shows *Tetrastichus gallerucae* (Hymenoptera: Eulophidae) functional response to be type II functional response when fed on eggs of elm leaf beetle *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae). In this context, Wang *et al*. (2006) in a laboratory experiment reported that *Citrostichus phyllocnistoides* (Narayanan) (Hymenoptera: Eulophidae) reveal a type III functional response, when fed with adults citrus leaf miner, *Phyllocnistis citrella* Stainton (Lepidoptera: Phyllocnistidae).

Although natural enemies which demonstrate functional response type III are generally regarded as competent biological control agent (Pervez and Omkar, 2005), nevertheless, many reports have shown that, a lot of the natural enemies have exhibited a type II functional response on their pray/host when release as biological control agents (Yingfang and Fadamiro, 2010; Timms *et al*., 2008; Fernandez-arhex and Corley, 2003). Furthermore, the magnitude of functional responses are been determine with attack rate and handling time of the natural enemy.

Handling time in insect parasitoid is defined as duration of time between two ovipositions, and is a good indicator of efficacy of parasitoid, as it reflects the total time taking in searching, probing, oviposition and resting. Estimated handling time of *D. citri* 3rd, 4th and 5th instars nymphs by *T. radiata* in the present study were 1.11, 0.6002 and 0.7051 respectively, with the attack rate of 21.62, 39.99 and 34.04 on 3rd, 4th and 5th instars nymphs respectively (Table 4). Whereas, it's searching efficiency (P_2 coefficient) on 3rd, 4th and 5th instars nymphs ware 3.109, 1.252 and 1.856 / arena / h (Table 3). Although, there was no relevant data to compare our results with, nevertheless, these handling times seems to be longer (1.11 h for 3rd instar nymph, 0.6002 h for 4th instar nymph and 0.7051 h for 5th instar nymphs). These longer handling time obtained in the present study, may be explain by the fact that, the duration of the experiment (24 h) was longer the real period when the parasitoids were active i.e., during day time when light was available (12 h), as such it resulted into overestimation of the handling time. According to Chong and Oetting (2007), time taken to handling a host by a parasitoid when derived from functional response models usually is been overestimated since the parasitoid did not spend all the available time in foraging but often engage in other activities such as searching, feeding, grooming and resting.

Optimum foraging performance of a parasitoid is affected by the number of hosts it encounter and attacks, its egg load, or other physiological variables (Godfray, 1994). The attack rates of *T. radiata* on 3rd, 4th and 5th instars nymphs of *D. citri* obtained in the present study suggest that, the efficiency of the parasitoid may be restricted by the egg load (Castillo *et al*., 2006). According to Heimpel *et al*. (1996) most parasitoids often experience egg depletion daily, particularly if their parasitism rate exceeds the daily egg maturation rate. When a parasitoid perceive a depletion in its egg load, it becomes increasingly selective with respect to the hosts they accept for oviposition, which may lead to a longer searching of host and a shorter handling time, as withness in the present study.

In conclusion, studies on parasitoid performance (parasitism rate, host preference and functional response) could provide insights into the behavior/ interactions of the parasitoid in relation to it host. Findings fom the present study shows that, *T. radiata* preferred to parasitized nymphs from the late (3rd, 4th and 5th instar nymphs) nymphal instars and the rate of parasitism may reach up to 77 and 86% for 4th and 5th instar nymphs respectively. The functional response type II exhibited by *T. radiata* indicated a negative density-dependent mortality in the *D. citri* nymphs populations. However we should bear in mind that, the functional response was measured in insectary, as such the pattern observed may be an experimental artefact, not necessarily reflecting the true effect of parasitoid on its host population in the field. Nevertheless, this results demostrate the potentials of *T. radiata* in regulating population of *D. citri* nymphs and will contribute to the establishment of a

biological control program for *D. citri,* as a component of IPM in different citrus-growing regions of Malaysia.

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