Indian Journal of Ecology (2022) 49(5): 1764-1769
DOI: https://doi.org/10.55362/IJE/2022/3731

Manuscript Number: 3731
NAAS Rating: 5.79

Host Stage Preference, Functional Response and Biological Parameters of *Aphelinus asychis* Walker on *Myzus persicae* (Sulzer) in Bell Pepper

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Abstract: *The parasitoid, Aphelinus asychis* Walker (Aphelinidae: Hymenoptera), is an important naturally occurring solitary koinobiont endo-parasitoid of green peach aphid, *Myzus persicae* (Sulzer) (Aphididae: Hemiptera). To supplement the augmentative or conservation biological control of *M. persicae* in capsicum, the host stage preference, functional response, and biological parameters of this parasitoid on *M. persicae* was studied. The parasitoid was able to parasitize all the nymphal stages of the aphid with preference to the second instar (51%) followed by the first instar (46%). The biological parameters of the *A. asychis viz.* total development, pre-oviposition, oviposition, post-oviposition periods, sex ratio (F: M), and fecundity were 16.23±0.45, 1±0.00, 6.6±0.40, 1.6±0.40 days, 1:0.97, and 58.8 eggs female^{-1}, respectively. The parasitoid when offered with different densities *viz.* 10, 15, 20, 25, and 30 of the second instar nymphs of *M. persicae* exhibited a Type-II functional response. Theoretically, parasitoid could parasitize a pinnacle of 18.25 aphids within 24 h. The present study indicates that the parasitoid has a potential for supplementing the augmentative and/or conservation biocontrol of the pest. The study also underscores the scope of optimizing the second instar nymphs of the aphid for mass production of the parasitoid.

Keywords: *Aphelinus asychis, Myzus persicae,* Relative preference, Oviposition period, Sex ratio, Functional response

Myzus persicae (Sulzer) (Aphididae: Hemiptera) commonly known as green peach aphid is a cosmopolitan and major pest of bell pepper, *Capsicum annuum L*. The aphid sucks the sap from copious portions of the plant causing leaf curling, distortion, discoloration, and premature senescence (Castle and Berger 1993, Syller 1994, Kumar et al 2019). The pest depreciates the yield and quality of bell pepper through direct feeding as well as contaminating the foliage by honeydew deposits on which sooty mold builds and represses photosynthesis (Cloyd and Sadof 1998). Additionally, the aphid transmit above 150 viral diseases (Castle and Berger 1993, Syller 1994). The ability to develop insecticide resistance, wide host range, and high biotic potential makes the aforementioned pest all the more challenging to manage (Yano 2003, Ralec et al 2010). Hence, there is an urgent need to generate alternatives for insecticides to regulate the pest. Biocontrol is one such an option in which use of chemicals is negligible, or only selective insecticides are used (Hoy 1993).

Aphelinus asychis Walker is a polyphagous, solitary, koinobiont endo-parasitoid that attacks above 40 aphid species, counting M. *persicae* (Li et al 2007, Byeon et al 2011, Gavkare et al 2013, Kumar et al 2019). In addition to parasitism, *A. asychis* annihilates the aphids through host-feeding which enhances its longevity, survival, and ovigenesis (Bai and Mackauer 1990). The host instar selection phenomenon is embraced by a parasitoid that influences the potential aphid population density and reduces their numbers (Hagvar and Hofsang 1991). The host suitability is a linear function of the host size, although it is dependable on hosts that do not feed, and not the perpetual state in aphid parasitoids (Askew and Shaw 1986, Sequeira and Mackauer 1992). According to optimal foraging theory the host selection and acceptance assist in enhancing the profits to the coming generation of parasitoids (Pyke 1984). *A. asychis* females distinguish between parasitized and unparasitized hosts (Mackauer 1982, Wahab 1985) which makes them effective biocontrol agents. According to Gavkare and Kumar (2012), *A. asychis* can result in 35 to 40 per cent parasitism in *M. persicae* under protected conditions. Although there are quite a few reports on the parasitization potential and biological parameters of the parasitoid on *M. persicae* (Takada 2002, Tatsumi and Takada 2005, Wang et al 2016), little has been reported with respect to the Indian populations of this parasitoid. Searching for local effective strains of the natural enemies has become vital, especially after the implementation of the Nagoya Protocol on access and benefit-sharing (Smith et al 2018). Before urging the parasitoid for field releases, it is crucial to scrutinize its biology and biotic potential. Consequently, we studied the relative preference, functional response, and biological parameters of *A. asychis* against *M. persicae* on bell pepper to supplement the biocontrol of the pest.

MATERIAL AND METHODS

Insect Cultures

Myzus persicae: Pure culture of the green peach aphid, *M. persicae* was maintained on bell pepper seedlings raised in plastic pots (10cm diameter). The aphid colonies were consolidated from the field and released on bell pepper plants. Before using the green peach aphids in experiments, the aphids were reared for two generations. The exhausted and dried bell pepper plants were changed systematically with fresh plants to assure a sufficient number of aphids for examination during the research.

Aphelinus asychis: The mummified aphids were collected from bell pepper plants nurtured inside the polyhouse and placed inside the glass tubes (15cm×2.5cm) for the development of the adult parasitoid. The freshly developed parasitoids were introduced onto the bell pepper seedlings *grown* in the plastic pots infested with *M. persicae* and covered with glass chimney (10cm×14.5cm). Honey solution (30%) provided as food to the adult parasitoids. Glass chimney top was covered with muslin cloth and tied with a rubber band. The parasitoid was established for one generation before using in the experiments. The adults of the parasitoid, *A. Asychis,* were identified by employing the taxonomic identification key formulated by Takada (2002).

Relative preference of *A. asychis* to parasitize different stages of *M. persicae:* The relative preference of *A. asychis* to oviposit nymphal instars of the green peach aphid was studied in a choice experiment at 25±0.5°C, 70±5% RH, and 14L:10D photoperiod. In the experiment, each pair of *A. asychis* was provided concurrently with twenty individuals each of the first, second, third, and fourth instar nymphs of *M. persicae* on capsicum seedling. For this, the instar wise aphids were carefully transferred with a fine camel hair brush on to the capsicum seedling and allowed to settle for 24 h. One pair of the parasitoid was allowed to mate for 24 hour and then the mated female was confined in the glass chimney and allowed to parasitize for 24 hour and thereafter the female was removed from the glass chimney. There were ten replications. After mummification, the mummies were counted, removed, and retain in the glass vial for the development of the adult parasitoid.

Functional response of parasitoid: Functional response of *A. asychis* to the second nymphal instar, the most preferred stage of *M. persicae* was studied on bell pepper seedlings covered with glass chimney at 25±0.5°C, 70±5% RH, and 14L:10D photoperiod. The honey solution (30%) was implemented inside a glass chimney as food for the parasitoid. The second nymphal instar of *M. persicae* were randomly assigned the bell pepper seedlings at 10, 15, 20, 25, and 30 densities. A single mated female (24 h old) was confined in the each glass chimney for parasitization. Each density was replicated ten times. After 24 hours, the female parasitoid was taken out from the glass chimney and the aphids were reared for mummification. Data on parasitized aphids in each density were recorded.

Developmental biology of the parasitoid, *A. asychis:* Development biology of *A. asychis* was studied on the second nymphal instar of *M. persicae* infesting the bell pepper seedlings raised in pots covered with glass chimney at the same environmental conditions described earlier. Twenty numbers of the second nymphal instar of *M. persicae* were restrained in potted seedlings covered with the glass chimney. In each glass chimney, a female parasitoid from the stock culture was released for parasitization for 24hour and thereafter the female parasitoid was removed. The aphids were retained for mummification. Then these mummified aphids were transferred individually in glass vials and inspected every day for adult formation. On emergence, the adult parasitoids were sexed and each pair was released into the glass chimney containing the bell pepper seedling infested with 2^{nd} instar nymphs of the aphid and 30 percent honey in the cotton swab. After 24 hour, the old batch of the aphids was substituted with a new one and this process proceeded till the mortality of the last parasitoid. The data on total development, pre-oviposition, oviposition, post-oviposition periods, sex ratio, and fecundity (mummified aphids) were observed.

Adult longevity: The adult longevity of *A. asychis* was ascertained by employing diverse foods *i.e.* honey solution (10, 30, and 70%), distilled water, and the aphid nymphs individually in separate test tubes. The honey solution and distilled water were rendered in cotton swabs and green peach aphid on the leaves. The five treatments were replicated four times. The adult parasitoids were inspected every day until they had died.

Data analysis: The data recorded on different parameters were subjected to one-way analysis of variance (ANOVA) using online software OP-STAT followed by calculation of critical difference (CD) at p= 0.05 to differentiate the significantly different means (Sheoran et al 1998). Data recorded in the functional response experiment were initially subjected to logistic regression between the proportion parasitized host (Na/N) and the host density offered (N) for determining the type of functional response as given below:

$$\frac{Na}{N} = \frac{\exp(P^0 + P^1 + N + P^2N^2 + P^3N^3)}{1 + \exp(P^0 + P^1 + N + P^2N^2 + P^3N^3)}$$

Where P_0= Intercept

P_1 =Linear coefficient

P_2 = Quadratic coefficient

P_3 = Cubic coefficient

Na = Number of parasitized host

N = Number of hosts offered

The linear coefficients (P_1) from the logistic regression equation significant negative or positive indicate Type-II or Type-III, respectively (Juliano 2001). If P_1< 0, it depicted Type -II functional response whereas if P_1> 0, it indicated a Type- III functional response.

The functional response parameters were assessed by applying the Holling disc equation for the type II functional response as determined by the logistic regression (Holling 1959) as under:

$$Na = \frac{aNT}{1 + aT_h N}$$

Where Na = Number of parasitized host

N = Number of the host offered

T = Total time available for the parasitoid

a = Attack rate (searching efficiency)

T_h = Handling time

RESULTS AND DISCUSSION

Relative preference of *A. asychis* to parasitize different nymphal instars of green peach aphid: In a choice experiment, different nymphal stages of *M. persicae* were offered simultaneously to *A. asychis.* The parasitoid preferred the second stage (51%) followed by the first (46%) and the third (44%) (F_{cal} =7.853; df = 3, 36; P < 0.001) while the fourth instar nymphs were the least preferred stage (33%) (Table 1).

Functional response of *A. asychis* parasitoid to varied host densities: The logistic regression analysis between the parasitized host (Na/N) and host density offered (N) yielded a significantly negative linear coefficient (P_1=-0.671) confirming a Type - II functional response of the parasitoid to the second nymphal instar of the pest (Table 2). The number of second stage nymphs of green peach aphid parasitized by the parasitoid differed significantly (F =8.614; df = 4, 45; P < 0.001) at different densities. These values were 5.6, 7, 8.5, 11.1, and 9.9 at 10, 15, 20, 25, and 30 host densities, respectively (Table 3, Fig. 1). The number of hosts parasitized increased with the increase in the host density until the aphid density of 25, but at a decelerating rate while the proportion of the hosts parasitized declined with the increase in the host density (Fig. 1). The attack rate (a) and handling time (T_h) was 0.034 ± 0.004 h^{-1} and 1.32 ± 0.23 h, respectively. The estimated theoretical maximum parasitism rate (T/T_h) over the 24h period was 18.25, while the attack rate per handling time (a/T_h) was 0.03. The data fit well the Holling disc equation (R^2 =0.768) (Table 4).

Developmental biology of *A. asychis* on second instar of *M. persicae:* The parasitoid development from egg to emergence of adult occurred in 16.23 days. The oviposition to mummification and pupal period was 6.70 and 9.53 days, respectively. *A. asychis* adults had a pre-oviposition, oviposition, and post-oviposition periods of 1.00, 6.60, and 1.60 days, respectively. The parasitoid laid on an average 58.80 eggs per female in the 2nd instar nymph of *M. persicae* and the sex ratio (F: M) of the parasitoid was 1:0.97 (Table 5).

Adult longevity: Adult longevity was ascertained on diverse foods i.e. honey solution (10%, 30%, 70%), distilled water, and the aphid nymphs separately (Table 6). The adult longevity ranged from 8.75 to 17.75 days. Adult food

Table 1. Relative preference of *A. asychis*to different stages of *M. persicae*

Instars	Number of aphids parasitized	Parasitization (%)
1st instar	9.2^{ab}±0.4	46.0
2nd instar	10.1^{a}±0.6	51.0
3rd instar	8.7^{bc}±0.8	44.0
4th instar	6.6^{c}±0.5	33.0
CD (p=0.05)	1.5	

Mean values superscripted with same alphabet do not differ significantly at p=0.05

Table 2. Results of logistic regression analysis of the proportion of *M. persicae* parasitized by *A. asychis*

Coefficient	Estimates ± SE	t-value	P
Constant (p_0)	4.1962 ± 0.627317	6.69	<0.001
Linear (p_1)	-0.6713 ± 0.1262	-5.32	<0.001
Quadratic (p_2)	0.0333 ± 0.006568	5.07	<0.001
Cubic (p_3)	0.0002 ± 0.000133	-1.5	0.168

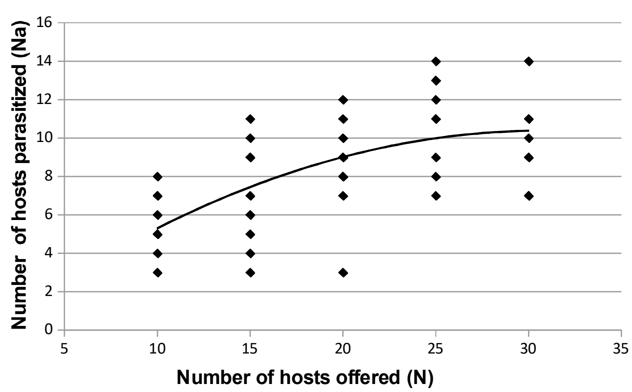

Fig. 1. Functional response of *A. asychis* to *M. persicae*: Number of host parasitized (Na) versus host density offered (N)

significantly influenced the adult longevity of the parasitoid (male: F= 27.544; df = 4, 15*; P < 0.001 and female:* F= 13.936; df = 4, 15*; P < 0.001*). The highest adult longevity for male was 16.5 days *and for female was* 17.75 days with 70 per cent honey solution which was, however, significantly at par with adults fed on the green peach aphid nymphs (male: 15.25 days and female: 15.50 days). The parasitoid adults fed on 10 or 30 per cent honey lived for 12 to 13.25 days with non-significant differences, while those allowed to feed on distilled water only survived for 8.75 (males) and 9.75 days (females) (Table 6).

In the present study, *A. asychis* parasitized all the immature stages of *M. persicae* with a preference for the second and first instar. The parasitoid preference for these early host stages could be due to their suitability for the parasitoid fitness and the lower abilities of the young aphids to defend against the parasitoid (Kouame and Mackauer 1991, Losey and Deno 1998, Liu 1985). Preference for the younger stages of *M. persicae* has also been reported in the parasitoids like *Aphidius colemani* Viereck (Hagvar and Hofsang 1991) and *Monotonus paulensis* (Ashmed) (Chau and Mackauer 2000). The selection of young hosts for oviposition by the parasitoid is considered as a desirable attribute to avoid excessive feeding by the host (Kouame and Makauer 1991). In accordance with the findings of Suck et al (2016) and Jia and Liu (2018), the present study also reported the preference of *A. asychis for the second* instar nymph of *M. persicae*.

In the present study, we further noticed that the total developmental period and the adult emergence were 16.23 days and 100 per cent, respectively. These parameters were slightly higher than reported by Ro and Long (1997) for this parasitoid-aphid combination on potato (developmental time: 14.5 days; emergence: 92.3%) indicating that the host plant influences the development and survival of the parasitoid. Earlier reports also confirmed that the host suitability for the parasitoid is effected by the host species, size, and age with specific reference to the growth period (Stary 1988, Hu et al 2002). Bernal and Gonzalez (1993) reported unsteady growth period of *A. asychis* on *Diuraphis noxia Kurdjumov.* Harley et al (1971) noticed the parasitoid development time diversified at variable temperatures. The total developmental period was 16 and 10 days at 23.9°C and 32.2°C, respectively. Highest female to male ratio of *A. asychis* to three species of sorghum aphids, greenbug, *Schizaphis graminum (Rondani)*, corn leaf aphid, *Rhopalosiphum maidis (Fitch),* and yellow sugarcane aphid, *Sipha flava (Forbes)* was 2:1 at the lowest temperature (23.9°C) and 1:1 at 32.2°C. Earlier investigations revealed that the percentage of female *A. asychis* adults may swing with host species (Jackson and Eikenbary 1971, Raney et al 1971). Bueno and Cleave (1997) reported that *Aphelinus perpallidus Gahan* had a developmental period of 18 to 22.5 days in both males and females. In this investigation, the fecundity of the parasitoid was 58.8 eggs female^{-1}, which fell within the range (33-159 eggs female^{-1}) as reported by Jackson and Eikenbary (1971). Byeon *et al* (2011) achieved highest daily fecundity (29.1 eggs for this parasitoid) on the same host. The sex ratio (F: M) of the parasitoid obtained in the current study (1:0.97) is also similar to that reported by Jackson and Eikenbary (1971).

This study yields a Type- II functional response of the parasitoid to the second instar nymphs of *M. persicae*. Type-II functional response seems prevalent in aphids parasitoidsand previously been reported in *Aphelinus thomsoni Graham against Drepanosiphum platanoidis*ese (Schrank) (Collins *et al* 1981), *Aphelinusal bipodus Hayat and Fatima, Aphidius colemani* Viereck *and, Aphidius matricariae* Haliday against *M. persicae* (Sampaio et al 2001, Tahriri et al 2007) and *A. asychis for M. persicae* (Byeon et al 2011). A type II response indicates that there is a reduction in the rate of parasitization with accretion in the prey density (Brown and Rothery 1993).

In the study, the attack rate (a) (0.034 ± 0.004 h^{-1}) and handling time (T_h) (1.32 ± 0.23 h) indicates that *A. asychis* is a suitable candidate for the control of the *M. persicae*. The handling time of *A. asychis* (present study) was slightly lower than *A. colemani* and *A. matricariae* on the same host and

Table 3. Functional response of *A. asychis* to *M. persicae*

Host density offered (N)	Number of hosts parasitized (Na) (Mean ± SE)	Proportion of hosts parasitized (Na/N) (Mean ± SE)	1/Na	1/NT (T=24h)
10	5.6^{d} ± 0.5	0.56 ± 0.48	0.19	0.004
15	7^{cd} ± 0.8	0.47 ± 0.06	0.17	0.003
20	8.5^{bc} ± 0.8	0.43 ± 0.04	0.14	0.002
25	11.1^{a} ± 0.7	0.44 ± 0.03	0.09	0.002
30	9.9^{ab} ± 0.8	0.33 ± 0.03	0.11	0.001
CD (p=0.05)	2.1			

Mean values superscripted with same alphabet do not differ significantly at p=0.05

Table 4. Functional response parameters of *A. asychis* on *M. persicae*

Parameter	Estimate ± SE
Attack rate (a)	00.034 ± 0.004
Handling time (T_h)	01.32 ± 0.23
Effectiveness of parasitoid (a/ T_h)	00.03
Maximum parasitisation rate (K) = T/ T_h	18.25
Coefficient of determination (R^2)	00.77

Table 5. Developmental biology of *A. asychis* on *M. persicae*

Parameter	Mean ± SE
Pre-oviposition period	1.00±0.00
Oviposition period	6.60±0.40
Oviposition to mummification period	6.70±0.38
Mummification to adult emergence period	9.53±0.29
Total developmental period	16.23±0.45
Post- oviposition period	1.60±0.40
Fecundity	58.80±10.44
Sex ratio (F:M)	1:0.97

Table 6. Adult longivity of *A. asychis* on different foods

Food	Longivity (Mean ± SE) (Days)	
	Male	Female
Honey solution (10%)	$9.50^{c}\pm1.19$	$12.00^{cd}\pm0.577$
Honey solution (30%)	$13.00^{b}\pm0.408$	$13.25^{bc}\pm0.629$
Honey solution (70%)	$16.50^{a}\pm0.29$	$17.75^{a}\pm0.48$
Distilled water	$8.75^{c}\pm0.48$	$9.75^{d}\pm0.48$
Green peach aphid	$15.25^{a}\pm0.48$	$15.50^{ab}\pm1.5$
CD (p=0.05)	1.98	2.52

Mean values in a column superscripted with same alphabet do not differ significantly at p=0.05

further observed that temperature had a significant influence on the handling time of these parasitoids (Zamani et al 2006).

Carbohydrate concentration in the adult diet seems to be important for their longevity as the adults feeding on 70 per cent honey survived longer than those feeding on 30 or 10 percent honey, host nymphs, and distilled water. Honey is a rich source of nutrients as it includes levulose (fructose) and dextrose (glucose) and provides food to the parasitoid. The adult longevity of the parasitoid recorded in the present study (17.75 days) was slightly less than reported by Jackson and Eikenbary (1971) (19.7 days) and Ro and Long (1997) (20 days) on the green peach aphid, *M. persicae*.

CONCLUSION

A. asychis parasitizes all the nymphal stages of *M. persicae* and prefered the second nymphal instar and followed a Type-II functional response. Based on the biological parameters, it can be concluded that the parasitoid has a potential for augmentative and conservation biological control of the pest. It is also pertinent to mention that the second instar nymphs of the aphid may be used for mass production of the parasitoid.

ACKNOWLEDGMENTS

Thanks to the Professor and Head, Department of Entomology, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh, India for providing the facilities for the study. Authors also acknowledge the efforts of Dr Neeraj Sankhyan, Professor of English, for editing the manuscript for English language.

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Received 05 June, 2022; Accepted 22 September, 2022