


Demographics and host-kill parameters of *Diglyphus horticola* Khan against *Chromatomyia horticola* (Goureau)

S. Katoch, P. L. Sharma, S. C. Verma, R. S. Chandel, P. Sharma  and N. Sharma

Department of Entomology, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan, 173230 (HP), India

Research Paper

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Author for correspondence:

P. Sharma, Email: ps366502@gmail.com

Abstract

The pea leafminer, *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae) is a polyphagous and serious pest of peas. In India, this pest is attacked by many parasitoids and among them *Diglyphus horticola* Khan (Hymenoptera: Eulophidae) is an important one, however, demographics and pest-kill potential of this parasitoid has not been studied so far. This study presents the first report on its demographics and pest-kill potential on *C. horticola*. The parasitoid showed three modes of host-killing behaviour viz. host-feeding, parasitism and host-stinging. The parasitoid females killed more number of hosts by parasitism than host-feeding or host-stinging. The pre-adult survival, net reproductive rate, intrinsic rate of increase (r_m) and finite rate of increase (λ) were higher on the 5-days old host larvae than those reared on the 3-days old larvae. Demographics and pest-kill parameters of *D. horticola* were also better on 5-days old host larvae than on 3-days old host larvae. Based on the study, *D. horticola* appeared to be a promising biocontrol agent for the suppression of *C. horticola* in peas and could be promoted through conservation biological control. Further studies are required to standardize the mass production protocol and release rates to use the parasitoid by augmentation.

Introduction

The pea leafminer, *Chromatomyia horticola* (Goureau) (Diptera: Agromyzidae) is a polyphagous pest inflicting severe damage to different crops, especially peas, crucifers, onion and some ornamentals (Bhagat *et al.*, 1989; Ostrauskas *et al.*, 2005; Fathi, 2011). It is one of the most regular and serious pests of peas capable of causing up to 89.6% leaf infestation (Sharma *et al.*, 2014). Such a high leaf infestation could result in heavy yield losses, because more than 20% avoidable yield losses in peas have been reported when leaf infestation exceeds 40% (Mehta *et al.*, 1994). Larval feeding impairs photosynthesis ultimately reducing the flowering and pod bearing capacity of the plant (Wei *et al.*, 2000). Besides impairing photosynthesis, the leafminer transmits plant pathogens (Capinera, 2008). This pest has a wide host range, high fecundity, quick developmental rate and high survival rate (Mizukoshi and Togawa, 1999; Ye *et al.*, 2018). As a result, it presents a serious menace to pea cultivation. Generally, farmers rely on the application of systemic insecticides to control this pest. Nonetheless, indiscriminate use of chemical pesticides leads to many environmental consequences like pest resurgence, secondary pest outbreaks, insecticide resistance, elimination of beneficials from the ecosystem and pesticide residues. Hence, it is important to develop sustainable, cost effective and eco-friendly management tactics for this pest. Biological control based on the use of parasitoid can be a viable option (van Lenteren, 2012).

In nature, several parasitoids attack this pest and contribute significantly to reduce its population. *Diglyphus* (Eulophidae) is an economically important genus of solitary ectoparasitoids of agromyzid leafminers (Liu *et al.*, 2013), with a rich diversity and worldwide distribution (Hansson and Navone, 2017). However, the ecological adaptability, biocontrol potential and application practices of *Diglyphus* species have mainly been studied for *Diglyphus isaea* (Walker) and to a lesser extent for *Diglyphus begini* (Ashmead) and *Diglyphus intermedius* (Girault) (Liu *et al.*, 2013; Zhang *et al.*, 2014). There has been a very limited research on biology and bio-control potential of other species of the genus, even though they are dominant in occurrence. The female adult wasps of *Diglyphus* species show three types of host-killing behaviour, viz. reproductive parasitization (parasitism), non-reproductive host-killing by feeding (host-feeding), and host stinging without oviposition or feeding (host-stinging). These non-reproductive host-killing behaviours (host-feeding and host-stinging) significantly increase their biocontrol potential (Kaspi *et al.*, 2011; Zhang *et al.*, 2011; Liu *et al.*, 2015; Xuan *et al.*, 2018). For such species it is important to quantify the host-killing by each mode to evaluate the actual biocontrol potential of the species (Bernardo *et al.*, 2006; Liu

et al., 2015; Cheng *et al.*, 2017). *Diglyphus horticola* Khan is considered as one of the most common parasitoid of *C. horticola* infesting peas in India (Purwar *et al.*, 2003; Khan *et al.*, 2005; Bhat and Bhagat, 2010; Mahendran and Agnihotri, 2013; Kumar and Sharma, 2016). However, the information on its demographics and pest-kill potential is lacking. The present study was, therefore, carried out to generate information of the demographics parameters and pest-kill potential of *D. horticola* on *C. horticola*.

Materials and methods

Insect cultures

Chromatomyia horticola

The stock culture of *C. horticola* was held in the laboratory by collecting infested pea leaves in the field at the experimental farm of the Department of Entomology, Dr YS Parmar University of Horticulture and Forestry, Solan (HP) India (30.85°N; 77.16°E). The infested leaves were kept in plastic jars (1 litre capacity) for adult emergence. The newly emerged adults were shifted to insect rearing cages (45 × 45 × 45 cm) fitted with glass on three sides and nylon net (400 μm) on the front and provided with 30% honey solution (in cotton swabs) as food and potted pea plants as a substrate for oviposition. The exhausted plants were replaced with healthy ones periodically to ensure a continuous supply of insects for experiments. The insect colonies and the experiments were held at 25 ± 0.5°C, 70 ± 5% RH and 14L: 10D photoperiod.

Diglyphus horticola

The stock culture of *D. horticola* was initiated from parasitoid reared from the leafminer-infested pea leaves collected in the same field where *C. horticola* was collected. The collected leaves were kept in rearing jars (1 litre capacity) for the emergence of the parasitoid adults in the laboratory. The parasitoid was identified using keys given by Khan (1985) and Khan *et al.* (2005). A colony of the parasitoid wasps thus obtained was offered with *C. horticola* larvae for parasitism and 30% honey for feeding in the insect rearing cages. The environmental conditions used for maintaining the parasitoid culture were the same as described earlier. The parasitoid was reared for one generation before using in the experiments.

Developmental biology of *D. horticola*

Diglyphus spp, generally prefer to parasitize second and third instar host larvae. We, therefore, studied the developmental biology of *D. horticola* on 3- (≈ second instar) and 5-days old (≈ third instar) larvae of *C. horticola*. A colony of either 3- or 5-days old *C. horticola* larvae in pea leaves was exposed to a single pair of 24 h old adults of *D. horticola* in a plastic jar (1 litre) for parasitism for 24 h. Each set was replicated ten times. After 24 h, these larvae were observed under a stereo microscope to segregate the parasitized larvae from the host-fed, host-stung and healthy ones (Cheng *et al.*, 2017). The shrunk and mutilated larvae were considered as host-fed and those alive and feeding normally as healthy. The host-stung larvae were dead without any shrinkage or associated parasitoid egg or larva having dark area around stinging hole. A cohort of 20 parasitized larvae were separated and kept in glass tubes (150 × 25 mm) for adult emergence. Newly emerged adults were sexed (Khan, 1985; Khan *et al.*, 2005) and each pair was offered with honey droplets and host

larvae *ad libitum* for oviposition and/or feeding/stinging. After 24 h, the old batch of larvae was replaced with the new one and the process was continued until all the parasitoid died. Observations on the duration of egg to adult development, adult longevity, percentage of parasitism, host-feeding, host-stinging, sex ratio and fecundity were recorded.

Population growth parameters

Population growth parameters were studied by constructing fertility life tables. Data on age-stage specific survival and fecundity were analysed by using computer program TWSEX-MSChart (Chi, 2015; Hafeez *et al.*, 2019). The detailed procedure is as under:

X = Age of individuals in days (pivotal age)

l_x = Proportion of individuals still alive at age X (survival value)

m_x = Number of female off-springs produced per female at the age X (fecundity rate)

By using the above variables, the following population growth parameters were calculated:

$$\text{Gross reproductive rate (GRR)} = \sum_{x=0}^{\infty} m_x$$

$$\text{Net reproductive rate (R}_0\text{)} = \sum_{x=0}^{\infty} l_x m_x$$

$$\text{True intrinsic rate of increase (r}_m\text{)} = \sum_{x=1}^{\infty} e^{-rx} l_x m_x = 1$$

$$\text{True generation time (T)} = \text{Log}_e R_0 / r_m$$

$$\text{Finite rate of natural increase (}\lambda\text{)} = \text{antilog}_e r_m$$

$$\text{Doubling time (DT)} = \log_e 2 / r_m$$

$$\text{Age-stage life expectancy (e}_{xj}\text{)} = \sum_{i=x}^{\infty} \sum_{y=j}^k s_{iy}$$

$$\text{Age-stage reproductive value (v}_{xj}\text{)}$$

$$= \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{\infty} e^{-r(x+1)} \sum_{y=j}^k s_{iy} f_{iy}$$

Host-kill parameters

Host-kill parameters of *D. horticola* on *C. horticola* were calculated by using data on age specific survival and daily host-kill rate (parasitism, host-feeding or host-stinging) using the computer program CONSUME-MS Chart (Chi, 2020) by using following variables:

X = Age of individuals in days (pivotal age)

l_x = Proportion of individuals still alive at age X (survival value)

k_x = Number of hosts killed at the age X (kill rate)

By using the above variables, following host kill parameters were calculated:

The age-specific consumption (host-feeding/parasitism/host-stinging) rate (k_x) represents the number of hosts killed by the parasitoid individuals at age x and was calculated according to the following equation:

$$k_x = \frac{\sum_{j=1}^m s_{xj}c_{xj}}{\sum_{j=1}^m s_{xj}}$$

where c_{xj} is the age-stage-specific host-killing (host-feeding/parasitism/host-stinging) rate of individuals at age x and life stage j , whereas s_{xj} is the age-stage-specific survival rate of individuals at age x and stage j . When the age-specific survival rate (l_x), which is the number of individuals surviving to age x , was taken into consideration, the net age-specific host-killing (host-feeding/parasitism/host-stinging) rate (q_x) was calculated as follows:

$$q_x = l_x k_x$$

The cumulative host-killing (host-feeding/parasitism/host-stinging) rate (C_x) is defined as the number of hosts killed by a parasitoid from birth to age x and it was calculated as follows:

$$C_x = \sum_{i=0}^x q_i$$

$$P_O/F_O/S_O = \sum_{x=0}^{\infty} \sum_{j=1}^{\beta} s_{xj}c_{xj} = \sum_{x=0}^{\infty} l_x k_x$$

Net host-killing rate (host-feeding (F_O)/parasitism (P_O)/stinging (S_O)) = $\sum_{x=0}^{\infty} l_x k_x$

The net total host-killing rate (K_O) represents the total number of three host-killing events during one generation of the wasps. It was calculated as:

$$K_O = \sum_{x=0}^{\infty} l_x k_x = P_O + F_O + S_O$$

The stable host-killing (host-feeding/parasitism/host-stinging) rate (ψ) represents the proportion of individuals belonging to age x and stage j in a stable age-stage distribution (a_{xj})

$$\psi = \sum_{x=0}^{\infty} \sum_{j=0}^{\beta} a_{xj}c_{xj}$$

To assess the host-killing (host-feeding/parasitism/host-stinging) potential, the finite host-killing (host-feeding/parasitism/host-stinging) rate (ω) was calculated as follows:

$$\omega = \lambda \psi$$

The transformation rate from host population to parasitoid offspring (Q_P) is the mean number of hosts a parasitoid needs to kill to produce one offspring. It was calculated as:

$$Q_P = K_O/R_O$$

Table 1. Biological parameters of *D. horticola* on 3- and 5-days old *C. horticola* larvae reared on pea

Parameter	3-days old larvae Mean ± SE	5-days old larvae Mean ± SE
Egg (days)	1.5 ^a ± 0.1	1.4 ^a ± 0.1
Larva (days)	5.0 ^a ± 0.22	4.5 ^a ± 0.2
Pupa (days)	7.5 ^a ± 0.27	6.5 ^b ± 0.3
Egg to adult emergence (days)	14.0 ^a ± 0.5	12.4 ^b ± 0.5
Female longevity (days)	25.3 ^a ± 0.6	26.7 ^a ± 0.4
Male longevity (days)	10.5 ^b ± 0.4	12.1 ^a ± 0.4
Pre-oviposition period (days)	1.0 ^a ± 0.0	1.0 ^a ± 0.0
Oviposition period (days)	10.7 ^a ± 0.4	11.3 ^a ± 0.2
Post-oviposition period (days)	13.5 ^a ± 1.6	14.2 ^a ± 1.2
Fecundity (eggs/female)	36.8 ^b ± 0.4	63.2 ^a ± 1.5
Sex ratio (male: female)	1:1.1	1:1.3

Mean values in a row superscripted with the same letter do not differ significantly at $P = 0.05$.

The true kill rate (k_m) is the actual rate of host kill by a parasitoid per unit of time and was calculated by solving the equation,

$$\sum_{x=1}^{\infty} e^{-k_m x} l_x k_x = 1$$

Results

Developmental biology

Developmental biology of *D. horticola* was studied on three (≈ second instar) and five (≈ third instar) days old larvae of *C. horticola*. The pre-adult (especially pupal) development of the parasitoid was faster, adults lived longer and females were more fecund when reared on 5-days old host larvae compared to when reared on the 3-days old (table 1). Females lived longer than males irrespective of which host stage was parasitized. However, the pre-oviposition, oviposition and post-oviposition periods of the parasitoid did not differ significantly between the two host stages (table 1). Sex ratio was female-biased in each case, however, the proportion of females was higher on the 5-days old host larvae than on the 3-days old.

Population growth parameters

Population growth parameters of *D. horticola* were also better on the 5-days old host larvae than on the 3-days old. The pre-adult survival (figs 1 and 2), net reproductive rate, intrinsic rate of increase (r_m) and finite rate of increase (λ) were higher and the generation time was shorter in parasitoid reared on the 5-days old host larvae than those reared on the 3-days old (table 2). The parasitoid could double its population in 4.52 and 3.47 days when parasitism occurred in the 3-days and 5-days old host larvae, respectively. The parasitoid laid most of the eggs during its initial phase of the reproductive period (figs 1 and 2). The age-stage reproductive values (v_{xj}), which show the contribution of an individual at age 'x' and stage 'j' to the future population were estimated and presented in figs 3 and 4. Likewise, the age

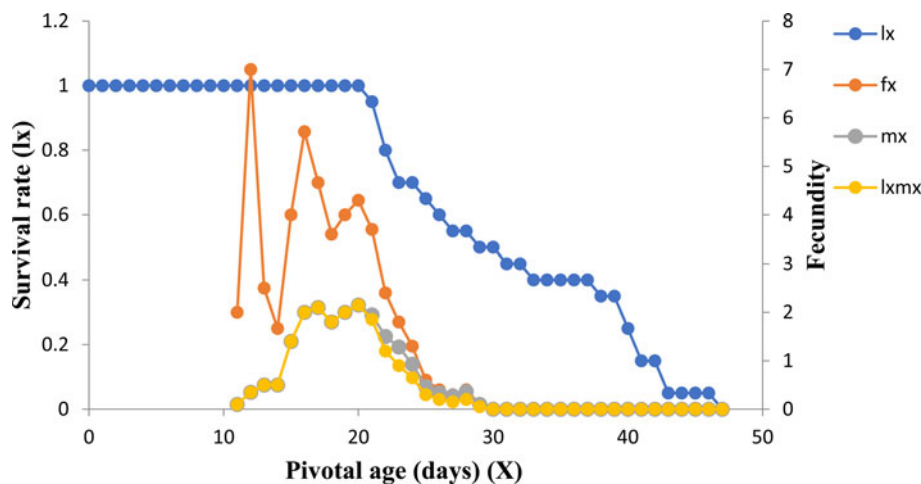


Figure 1. Age-specific survival rate (l_x), age-stage specific fecundity (f_x), age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) of *D. horticola* on 3-days old larvae of *C. horticola*

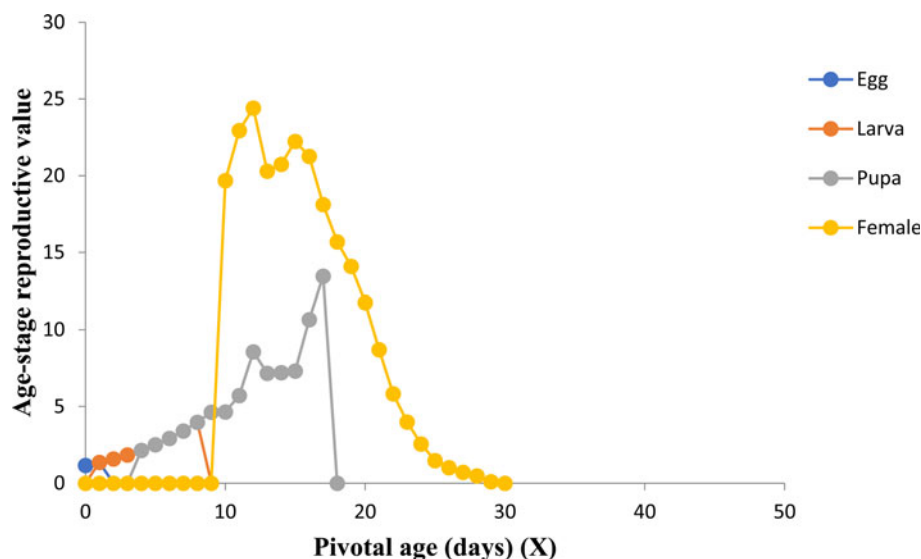


Figure 2. Age-stage specific reproductive value (v_{xj}) of *D. horticola* on 3-days old larvae of *C. horticola*

Table 2. Population growth parameters of *D. horticola* on 3- and 5-days old larvae of *C. horticola*

Parameter	3-days old larvae (Mean ± SE)	5-days old larvae (Mean ± SE)
GRR (females/female)	20.09 ^a ± 4.00	32.62 ^a ± 7.03
R_0 (females/female)	18.40 ^a ± 4.12	31.60 ^a ± 7.09
r_m (females/female/day)	0.1533 ^b ± 0.0146	0.1998 ^a ± 0.0157
T (days)	19.00 ^a ± 0.83	17.28 ^a ± 0.57
λ (females/day)	1.166 ^b ± 0.017	1.221 ^a ± 0.019
DT (days)	4.52 ^a ± 0.10	3.47 ^a ± 0.04

Mean values in a row superscripted with the same letter do not differ significantly at $P = 0.05$.

specific life expectancy (e_x) which represents the time period individuals of age (x) are expected to live, i.e. the remaining longevity of the individuals, was also estimated (figs 5 and 6). The survivorship curves (s_{xj}) for stages overlap indicating that individuals of

the cohort used in the study had different development rates (figs 7 and 8).

Host-kill parameters

The parasitoid started host killing by all modes (host-feeding, parasitism and host-stinging) within 24 h after emergence. In each case the parasitoid females killed more number of hosts by parasitism than host-feeding or host-stinging (figs 9 and 10). Since there was no host killing by the egg, larval, or pupal stages of the parasitoid, there was a blank stage before the females emerged. The daily host-feeding rate of *D. horticola* on 3-days and 5-days old larvae was the maximum on the 21st and 18th day, respectively (table 3) (figs 11 and 12). The age-specific parasitism rate curves of *D. horticola* increased gradually until 20th day on 3-days old larvae and 17th day on 5-days old larvae, and then dropped with age (figs 13 and 14). The host-parasitism parameters viz., P_0 , k_m , ψ and ω on 3-days old host larvae were 16.1 ± 3.6 , 0.145 ± 0.014 , 0.144 ± 0.036 and 0.167 ± 0.044 , respectively while these parameters on 5-days old host larvae were 27.0 ± 6.1 , 0.189 ± 0.015 , 0.185 ± 0.045 and 0.226 ± 0.059 , respectively

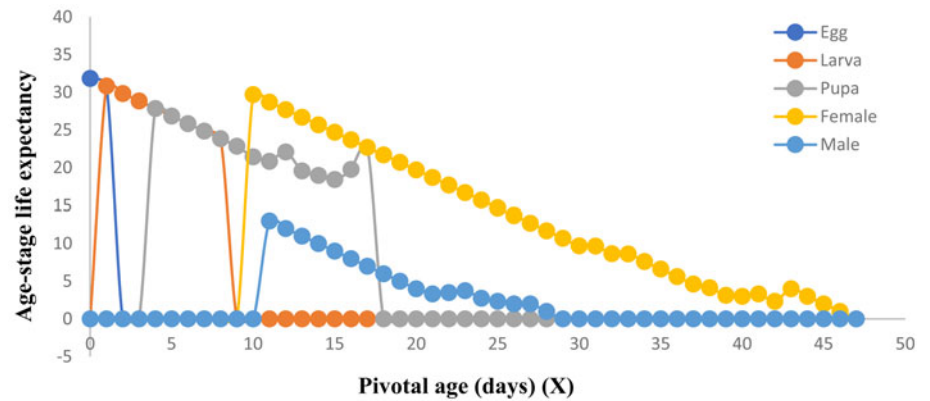


Figure 3. Age-stage life expectancy (e_{xi}) of *D. horticola* on 3-days old larvae of *C. horticola*

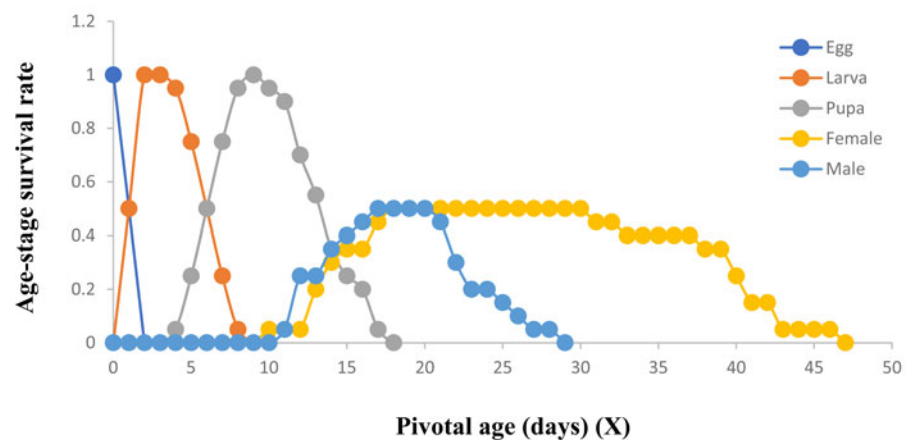


Figure 4. Age-stage survival rate (s_{xi}) of *D. horticola* on 3-days old larvae of *C. horticola*

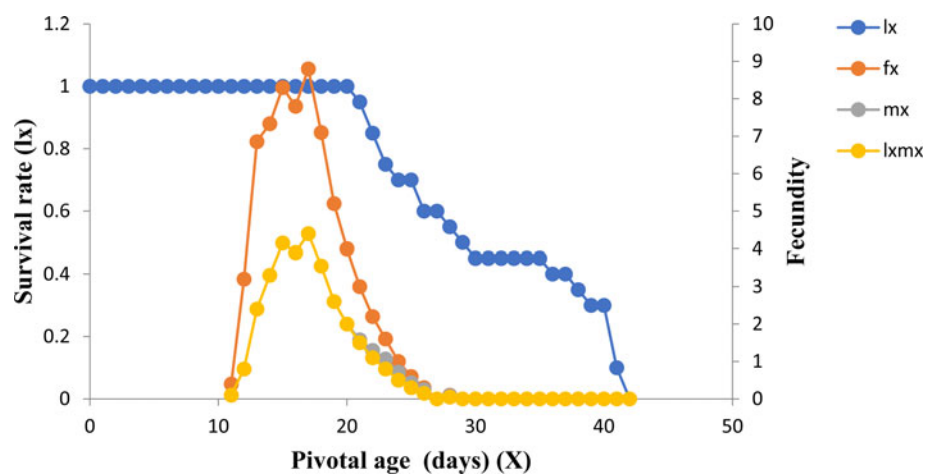


Figure 5. Age-specific survival rate (l_x), age-stage specific fecundity (f_x), age-specific fecundity (m_x) and age-specific maternity ($l_x m_x$) of *D. horticola* on 5-days old larvae of *C. horticola*

(table 4). The daily host-stinging events are represented in figs 15 and 16, and the host-stinging parameters are given in table 5. The host-stinging parameters namely S_0 , k_m , ψ , and ω were 4.05 ± 1.05 , 0.075 ± 0.015 , 0.042 ± 0.012 and 0.049 ± 0.015 , on 3-days old host larvae and 3.95 ± 0.98 , 0.077 ± 0.015 , 0.028 ± 0.007 and 0.034 ± 0.009 on 5-days old host larvae, respectively (table 5). The total host-killing rate was estimated by pooling the parasitism, host-feeding and host-stinging events. The k_x and q_x values peaked at the age of 17 days irrespective of which host age larvae

were used (figs 17 and 18). The net host-killing rate (K_0) of the wasp on 3- and 5-days old larvae of *C. horticola* was 29.8 ± 6.7 and 42.9 ± 9.6 , respectively. The stable host kill rate (ψ) and finite host killing rate (ω) were 0.269 and 0.314, respectively, on 3-days old host larvae. These parameters on 5-days old host larvae were 0.2879 and 0.3516, respectively. The transformation rate (Q_p) of *D. horticola* on 3- and 5-days old host larvae was 0.52 and 0.38 (feeding data) and 1.62 and 1.36 (total killing data), respectively (table 6).

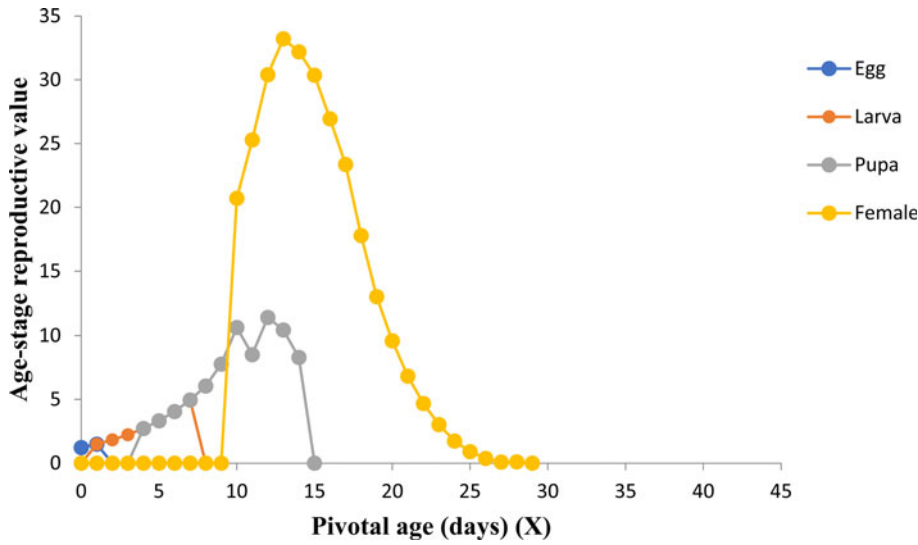


Figure 6. Age-stage specific reproductive value (v_{xj}) of *D. horticola* on 5-days old larvae of *C. horticola*

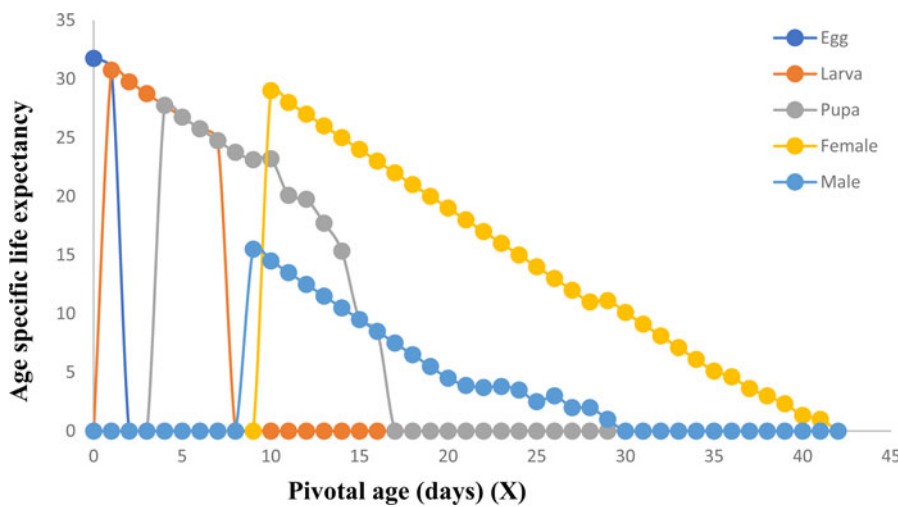


Figure 7. Age-stage life expectancy (e_{xj}) of *D. horticola* on 5-days old larvae of *C. horticola*

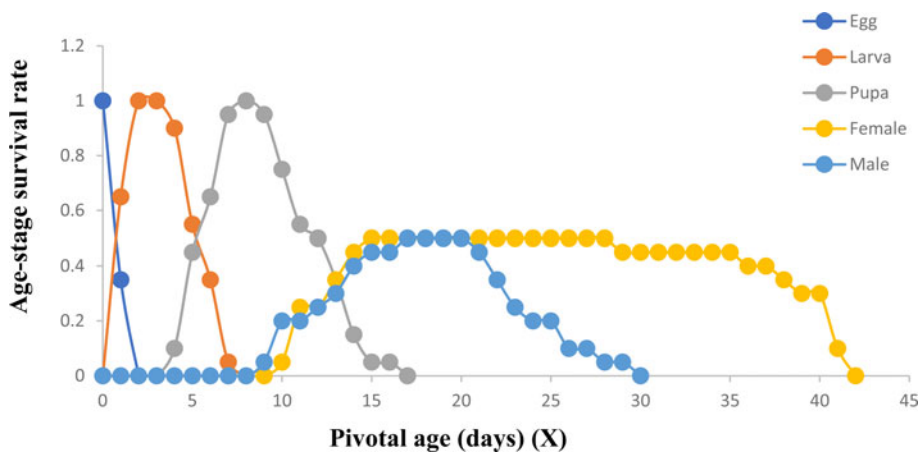


Figure 8. Age-stage survival rate (s_{xj}) of *D. horticola* on 5-days old larvae of *C. horticola*

Discussion

This study presents the first report on its demographics and pest-kill potential. Like many eulophids, *D. horticola* was synovigenic

with low fecundity at emergence, and killed the host larvae by three modes viz., parasitism, host-feeding and host-stinging. Similar results were reported for *Hemiptarsenus varicornis*

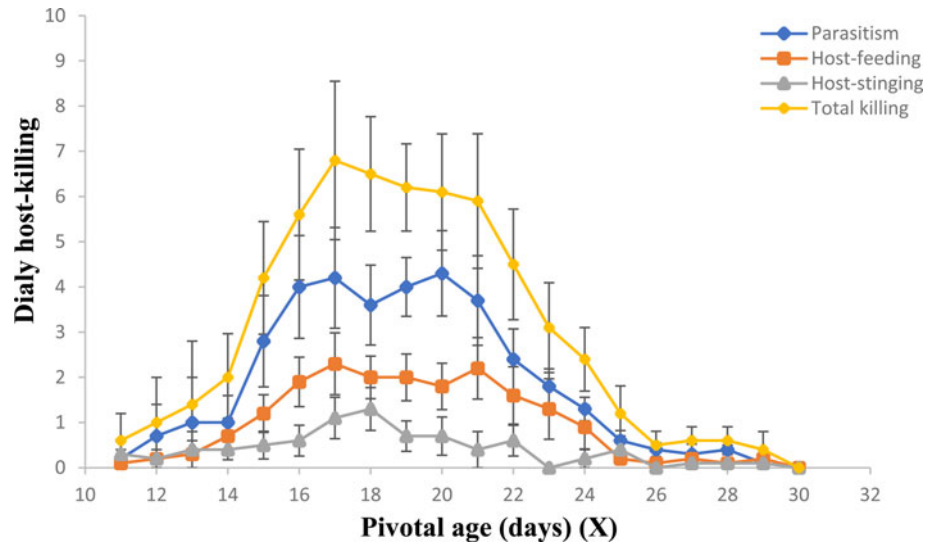


Figure 9. Daily host killing of 3-days old larvae of *C. horticola* by *D. horticola* through different modes

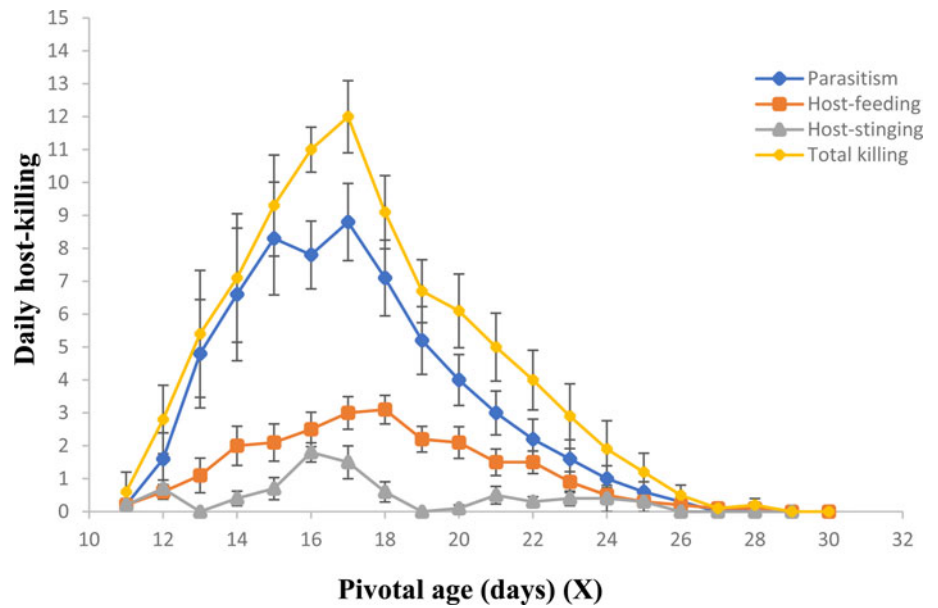


Figure 10. Daily host killing of 5-days old larvae of *C. horticola* by *D. horticola* through different modes

Table 3. Host-feeding parameters of *D. horticola* on 3- and 5-days old larvae of *C. horticola*

Sr. no.	Parameter	Estimate ± SE	
		3-days old larvae	5-days old larvae
1.	Net host-feeding rate (F_0) (hosts/parasitoid)	9.65 ^a ± 2.21	12.00 ^a ± 2.72
2.	Intrinsic host-feeding rate (k_m) (hosts/parasitoid/day)	0.116 ^a ± 0.013	0.136 ^a ± 0.014
3.	Stable host-feeding rate (ψ)	0.0839 ^a ± 0.0199	0.0745 ^a ± 0.0182
4.	Finite host-feeding rate (ω)	0.0978 ^a ± 0.0245	0.0910 ^a ± 0.0235
5.	Transformation rate (Q_p)	0.52 ^a ± 0.03	0.38 ^b ± 0.02

Mean values in a row superscripted with the same letter do not differ significantly at $P=0.05$.

(Girault) parasitizing *Liriomyza trifolii* (Burgess) (Cheng *et al.*, 2017) and *D. isaea* (Walker) parasitizing *Liriomyza sativae* Blanchard (Zhang *et al.*, 2011). In fact, previous studies indicate; that most host-feeding parasitoid are synovigenic. For example, *D.*

isaea, *H. varicornis*, *Neochrysocharis formosa* (Westwood) feed on host body fluid and lay anhydrous eggs (Liu *et al.*, 2013; Zhang *et al.*, 2014; Liu *et al.*, 2015; Ye *et al.*, 2018). The pre-adult development time of *D. horticola* on *C. horticola* (current study) was

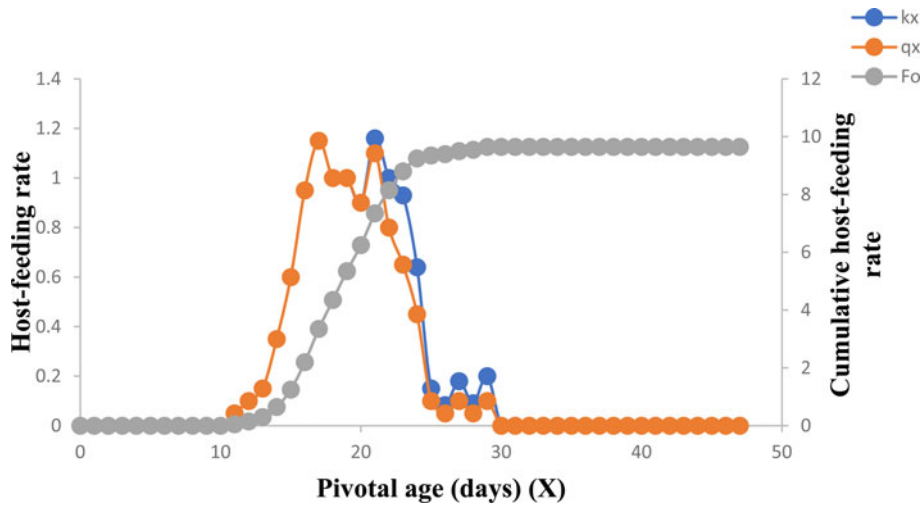


Figure 11. Age-specific host-feeding rate (k_x), net age specific host-feeding rate (q_x) and cumulative host-feeding rate (F_0) of *D. horticola* on 3-days old larvae of *C. horticola*

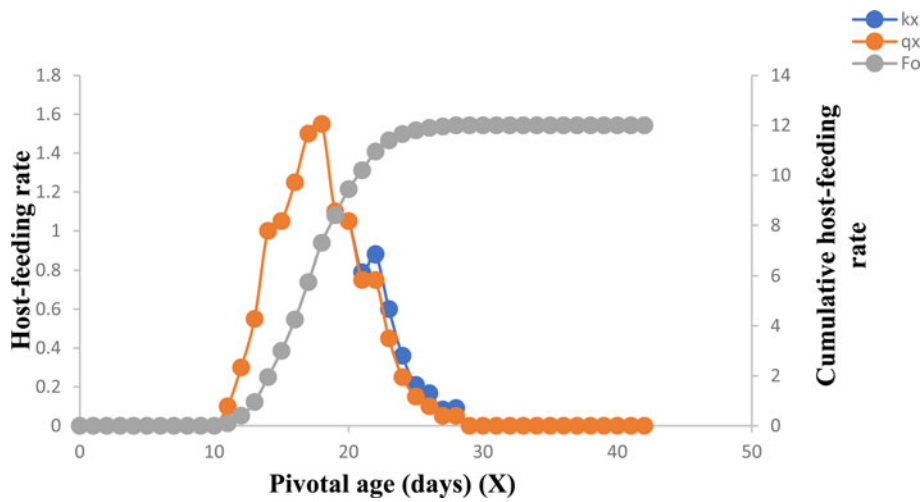


Figure 12. Age-specific host-feeding rate (k_x), net age specific host-feeding rate (q_x) and cumulative host-feeding rate (F_0) of *D. horticola* on 5-days old larvae of *C. horticola*

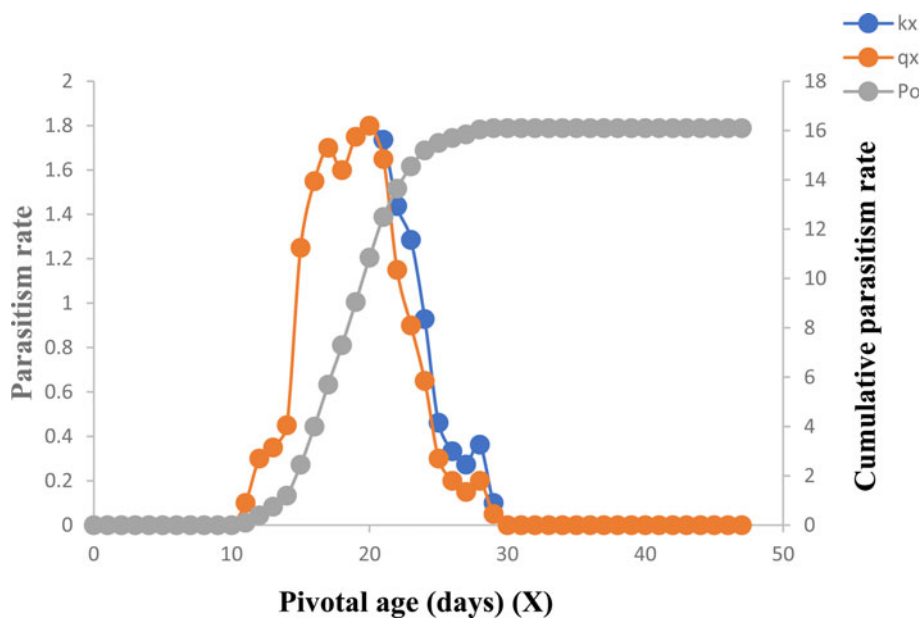


Figure 13. Age-specific parasitism rate (k_x), net age specific parasitism rate (q_x) and cumulative parasitism rate (P_0) of *D. horticola* on 3-days old larvae of *C. horticola*

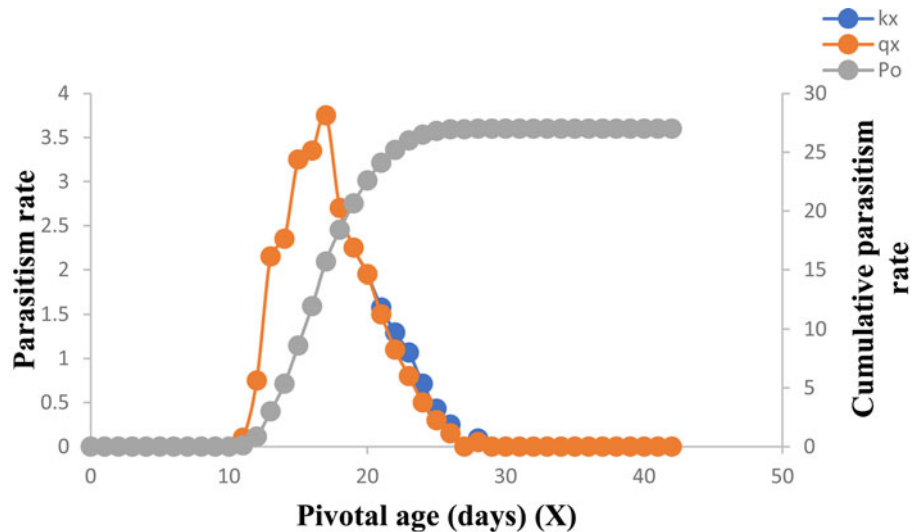


Figure 14. Age-specific parasitism rate (k_x), net age specific parasitism rate (q_x) and cumulative parasitism rate (P_0) of *D. horticola* on 5-days old larvae of *C. horticola*

Table 4. Host-parasitism parameters of *D. horticola* on 3- and 5-days old larvae of *C. horticola*

Sr. no.	Parameter	Estimate \pm SE	
		3-days old larvae	5-days old larvae
1.	Net parasitism rate (P_0)	16.1 ^a \pm 3.6	27.0 ^a \pm 6.1
2.	Intrinsic rate of host parasitism (k_m) (hosts/parasitoid/day)	0.145 ^b \pm 0.014	0.189 ^a \pm 0.015
3.	Stable host parasitism rate (ψ)	0.144 ^a \pm 0.036	0.185 ^a \pm 0.045
4.	Finite host parasitism rate (ω)	0.167 ^a \pm 0.044	0.226 ^a \pm 0.059

Mean values in a row superscripted with the same letter do not differ significantly at $P=0.05$.

nearly the same as reported for *D. isaea* parasitizing *C. horticola*, but longer than that of *D. isaea* parasitizing *Liriomyza huidobrensis* (Blanchard), *L. trifolii* and *L. sativae* (Bazzocchi et al., 2003; Haghani et al., 2007) at similar temperature conditions. Earlier, Cheah (1988) also reported slower development of *D. isaea* on *Chromatomyia syngenesiae* (Hardy). Larval period of *D. horticola* (present study) was shorter than that of *D. isaea*, while the pupal period was nearly the same (Ibrahim and Madge, 1979; Minkenberg and van Lenteren, 1986). The development time of different immature stages of *D. horticola* recorded in the present study was also similar to that of *Diglyphus intermedius* (Girault) and *D. wani* parasitizing *L. sativae* at similar conditions (Patel and Schuster, 1983; Ye et al., 2018). The development time of *D. isaea* on *L. sativae* (Zhang et al., 2014) was shorter than recorded for *D. horticola* on *C. horticola* in the current study; probably due to higher rearing temperature used by the earlier workers. The parasitoid longevity was, nevertheless, higher than that of *Diglyphus wani* Liu (Ye et al., 2018) and lower than that recorded for *D. isaea* (Zhang et al., 2011, 2014) parasitizing *L. sativae*. The fecundity of the parasitoid reported in the current study was quite less than obtained for *D. isaea* on *L. sativae* (Zhang et al., 2011, 2014), but nearly similar to that reported for *D. wani* on *L. sativae* (Ye et al., 2018). Adult longevity and fecundity were higher when the parasitoid was reared on older host larvae compared to the younger ones. In idiobionts, size of the host larvae at parasitism is crucial not only for the successful development but also for the fitness of the developed parasitoid adults. The older larvae, which were bigger in size, probably

provided better nutrition to the parasitoid larvae which ultimately enhanced the parasitoid's longevity and fecundity. Similar results have earlier been reported for *H. varicornis* parasitizing *L. trifolii* (Cheng et al., 2017) and *D. isaea* parasitizing *L. sativae* (Zhang et al., 2011).

Biocontrol potential of any biocontrol agent could be assessed by its ability to grow in numbers. Any population is expected to grow if its intrinsic rate of increase (r_m) > 0 and net reproductive rate (R_0) > 1 (Southwood and Henderson, 2000; Chen et al., 2017). In the current study, these parameters for *D. horticola* were estimated as 0.1553–0.1998 and 18.4–31.6, respectively. Since the r_m and R_0 values were quite higher than 0 and 1, respectively, *D. horticola* would have a high potential to increase its population size under field conditions. Demographics parameters of *D. horticola* (current study) were better than reported for *N. formosa* on *Tuta absoluta* (Meyrick) (Guleria et al., 2020), but lower than that of *D. wani* on *L. sativae* (Ye et al., 2018). Higher population growth potential of *D. wani* could be due to its thelytoky (Ye et al., 2018).

Although intrinsic rate of increase is the measure of the species' biotic potential, it may not always be suitable to measure the parasitoids' pest-kill potential. This parameter elucidates the pest-kill potential of a solitary parasitoid where the oviposition events are equal to the pest-kill events, but not for parasitoids like *D. horticola*, which kills the host larvae by non-reproductive mode in addition to parasitism. Instead, Chi (2015) and van Lenteren et al. (2019) suggested estimating the pest-killing rate (k_m), stable host-killing rate (ψ) and finite host-killing rate (ω). We, therefore, estimated these

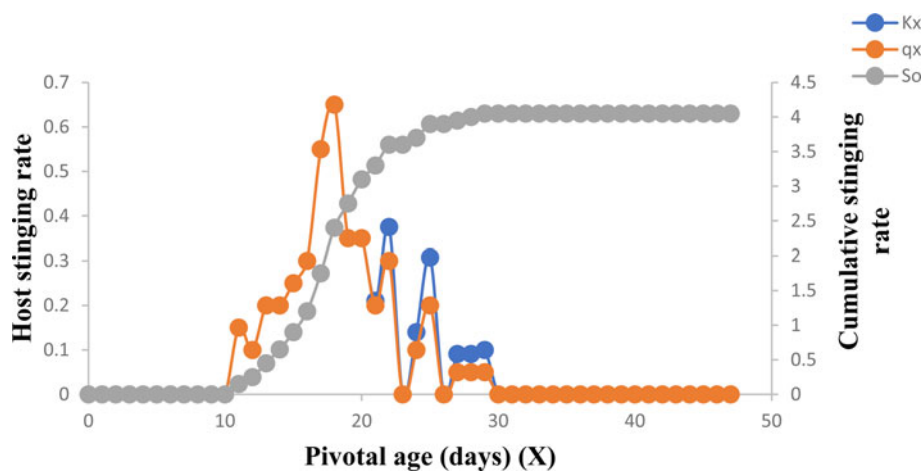


Figure 15. Age-specific host-stinging rate (k_x), net age specific host-stinging rate (q_x) and cumulative host-stinging rate (S_o) of *D. horticola* on 3-days old larvae of *C. horticola*

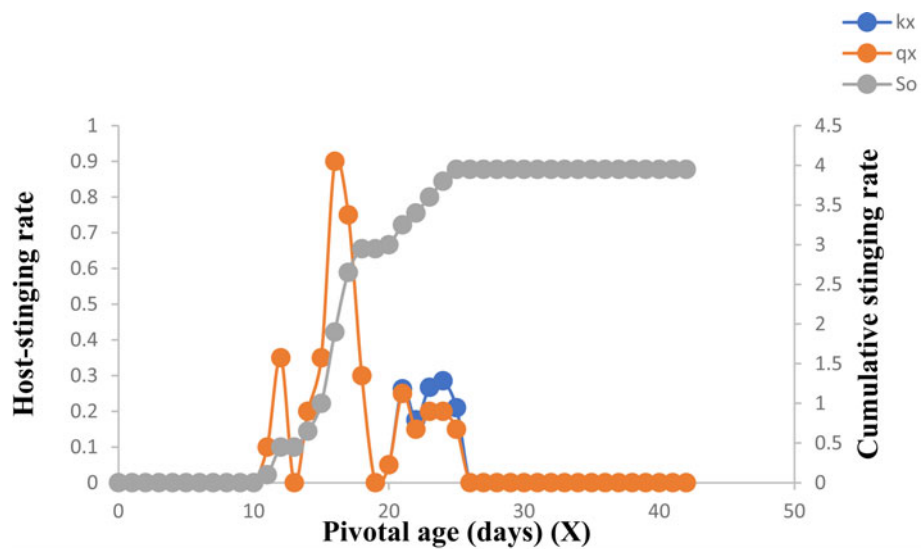


Figure 16. Age-specific host-stinging rate (k_x), net age specific host-stinging rate (q_x) and cumulative host-stinging rate (S_o) of *D. horticola* on 5-days old larvae of *C. horticola*

Table 5. Host-stinging parameters of *D. horticola* on 3- and 5-days old larvae of *C. horticola*

Sr. no.	Parameter	Estimate ± SE	
		3-days old larvae	5-days old larvae
1.	Net host-stinging rate (S_o) (hosts/parasitoid)	4.05 ^a ± 1.05	3.95 ^a ± 0.98
2.	True intrinsic rate of host-stinging (k_m) (hosts/parasitoid/day)	0.075 ^a ± 0.015	0.077 ^a ± 0.015
3.	Stable age host-stinging rate (ψ)	0.042 ^a ± 0.012	0.0280 ^a ± 0.007
4.	Finite host-stinging rate (ω)	0.049 ^a ± 0.015	0.034 ^a ± 0.009

Mean values in a row superscripted with the same letter do not differ significantly at $P = 0.05$.

parameters of *D. horticola* parasitizing 3- and 5-days old larvae of *C. horticola*. These parameters were higher on 5-days old host larvae than on 3-days old. The parasitoid host-killing rate was much higher than its intrinsic rate of increase, confirming that the total biocontrol potential of the parasitoid is much higher than measured in terms of parasitism alone. Killing their hosts by means of host-feeding and/or host-stinging, in addition to parasitism, is prevalent in many hymenopteran parasitoid, especially eulophids

(Wang *et al.*, 2012; Liu *et al.*, 2015; Benelli *et al.*, 2017; Cheng *et al.*, 2017; Bodino *et al.*, 2018; Ye *et al.*, 2018; Guleria *et al.*, 2020). *D. horticola* killed a higher number of host larvae by oviposition than by host-feeding or host-stinging, which is in agreement with the results reported for *N. tutae*, *N. cosmopterix* and *N. formosa* parasitizing *T. absoluta* (Bodino *et al.*, 2018; Guleria *et al.*, 2020), but in contrast to what is reported for *H. varicornis* parasitizing *L. trifolii* (Cheng *et al.*, 2017) and *D. wani* parasitizing *L.*

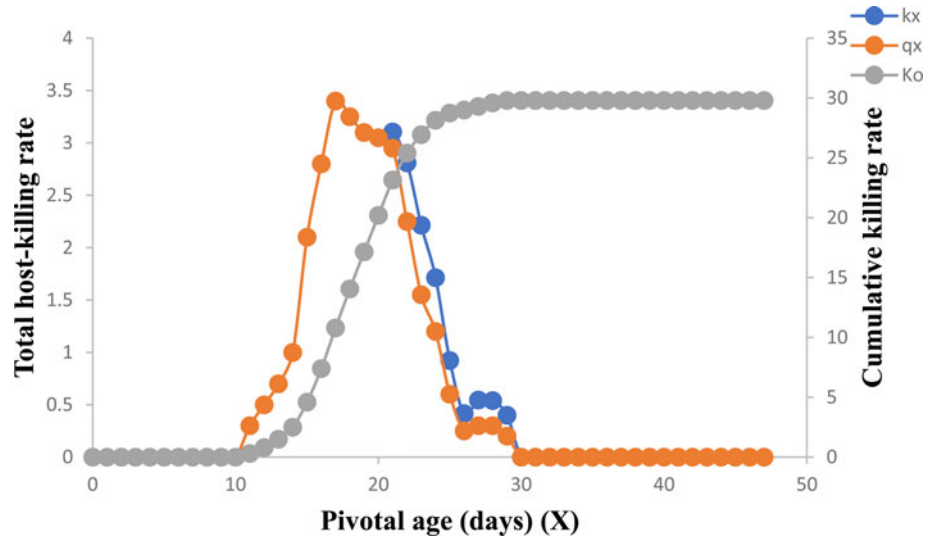


Figure 17. Age-specific total host-killing rate (k_x), net age specific total host-killing rate (q_x) and cumulative total host-killing rate (K_o) of *D. horticola* on 3-days old larvae of *C. horticola*

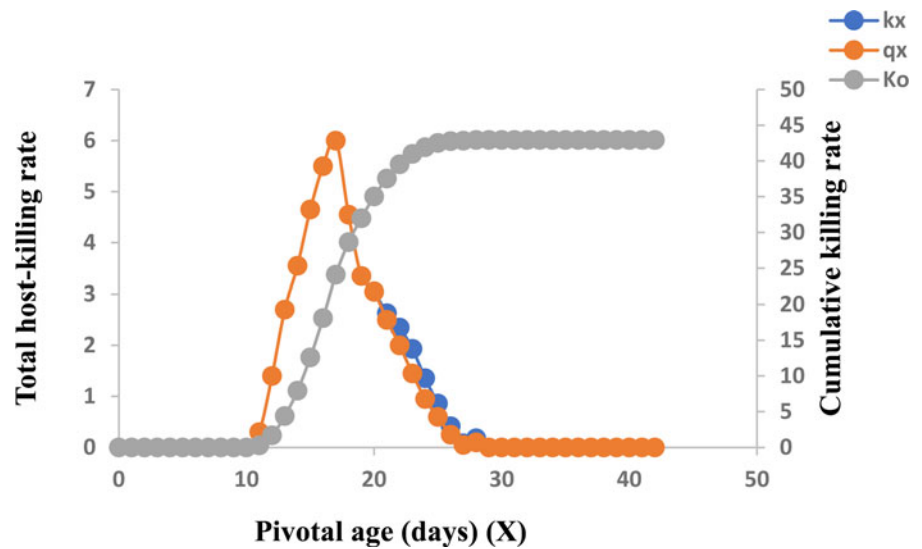


Figure 18. Age-specific total host-killing rate (k_x), net age specific total host-killing rate (q_x) and cumulative total host-killing rate (K_o) of *D. horticola* on 5-days old larvae of *C. horticola*

Table 6. Host-killing parameters of *D. horticola* on 3- and 5-days old larvae of *C. horticola*

Sr. no.	Parameter	Estimate ± SE	
		3-days old larvae	5-days old larvae
1.	Net total killing rate (K_o) (hosts/parasitoid)	29.8 ^a ± 6.7	42.9 ^a ± 9.6
2.	Intrinsic rate of host kill (k_m) (hosts/parasitoid/day)	0.1793 ^a ± 0.0150	0.2146 ^a ± 0.0160
3.	Stable host kill rate (ψ)	0.2694 ^a ± 0.0667	0.2879 ^a ± 0.0698
4.	Finite host killing rate (ω)	0.3140 ^a ± 8.2117	0.3516 ^a ± 9.0404
5.	Transformation rate (Q_p)	1.62 ^a ± 0.05	1.36 ^b ± 0.02

Mean values in a row superscripted with the same letter do not differ significantly at $P=0.05$.

sativae (Ye *et al.*, 2018). Variations in the percentages of host-feeding and oviposition events are also linked with the parasitoid trade-off between the present and the subsequent reproduction (Desouhant *et al.*, 2005). The significance of host feeding is that the

parasitoid takes lipids and proteins, which are essential for continuous ovigenesis, from host tissue and haemolymph (Zhang *et al.*, 2014; Liu *et al.*, 2015; Benelli *et al.*, 2017; Cheng *et al.*, 2017). The percentage of host-feeding and oviposition events may,

nevertheless, differ with parasitoid-host interaction (Zhang *et al.*, 2014; Liu *et al.*, 2015; Cheng *et al.*, 2017; Ye *et al.*, 2018). *D. horticola* showed a lower daily reproductive ability than *D. isaea*, *D. wani* and *D. intermedius* (Heinz and Parrella, 1989; Patel *et al.*, 2003; Zhang *et al.*, 2014; Ye *et al.*, 2018). Consequently, the finite host-killing rate of *D. horticola* estimated in the present study was lower than reported for these parasitoid. The host-killing events also depend on the density of leafminer larvae on individual leaflets (Patel *et al.*, 2003). Although host feeding enhances longevity and fecundity across parasitoid species, conversion of the consumed hosts into progeny may vary from species to species. *D. horticola* required 0.52 3-day old or 0.38 5-day old host larvae to host-feed to produce one egg which was lower than required by *D. wani* foraging on *L. sativae* (Ye *et al.*, 2018).

In addition to host-feeding, *D. horticola* killed the hosts by stinging without parasitism or feeding. This mode of host killing is also common in eulophids (Cheng *et al.*, 2017; Ye *et al.*, 2018), but the significance largely remains unknown and its impact on the host insect population has largely been ignored. Nevertheless, there are reports that indicate that such aborted stinging, in addition to direct mortality, reduces the pest fecundity or fertility (Abram *et al.*, 2019). Daily host stinging by the parasitoid fluctuated between 0.65 and 4.05 hosts per parasitoid. The hosts killed by *D. horticola* by stinging in the current study were higher than reported for *D. wani* parasitizing agromyzid leafminers (Ye *et al.*, 2018). Earlier, Cheng *et al.* (2017) reported a constant daily host-stinging (3–8 events; maximum 28.7%) of *L. trifolii* by *H. varicornis*. Host stinging is believed to benefit the parasitoid in two ways: one by protecting the host plant leaf from desiccation by reducing the pest density on it (Patel *et al.*, 2003) and second by paralyzing the larvae to reserve food in case the nearby larvae is not sufficient to support the parasitoid development (Bernardo *et al.*, 2006). Host nutrients and parasitoid abilities to use these nutrients may vary from species to species and lead to the variations in host-kill potential of different parasitoid (Ye *et al.*, 2018). In general, the host-feeding parasitoids have greater pest control potential as compared to non-host-feeding parasitoid (Zhang *et al.*, 2014; Liu *et al.*, 2015; Cheng *et al.*, 2017). In the current study, we found that the k_m values of *D. horticola* (0.1793–0.2146) were higher than the r_m (0.1517–0.1757) values of *C. horticola* on peas under similar conditions (Bhat, 2017), indicating that the parasitoid would cause a higher kill rate than the pest's growth rate and hence would be able to reduce the pest population in the field. Furthermore, the assessment of *D. horticola* on the basis of its parasitization potential alone would undermine its biocontrol potential against the pest.

Conclusion

Biological and population parameters of *D. horticola* were better on 5-days old host larvae than on 3-days old. In addition to parasitism, the parasitoid killed the host larvae through the host-feeding and host-stinging. Thus, *D. horticola* is a promising biocontrol agent for the suppression of *C. horticola* in peas and could be promoted through conservation biological control. However, further studies are required to standardize its mass production and utilization through augmentative biological control.

Data

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