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Biological characteristics and parasitization potential of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) on the whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), a pest of greenhouse crops in north-western Indian Himalayas

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Abstract

Background The greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) constitutes key pests of greenhouses and field crops, which have developed pesticide resistance over the years. It has emerged as a difficult pest to manage owing to its indiscriminate exposure to higher dosages of insecticides. The use of natural enemies is environmentally safe alternative management tactic. Efficacy of *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) was determined by studying its biological characteristics on *T. vaporariorum*.

Results Parasitization by *E. formosa* was higher on fourth-instar nymphs of the greenhouse whitefly (GHWF) (37.2%), which was at par with that of third instar (36.4%), both differing significantly to the parasitization observed in second instar (19.6%). Total developmental duration of the parasitoid was longer on second instar (33.2 days) than that of fourth instar (29.9 days). Adult longevity was found significantly higher for the adults that emerged from fourth-instar nymphs of GHWF, which was at par to that of third instar and longevity of *E. formosa* adults was significantly higher in the absence of parasitoid host. The size of parasitoid that emerged from different instars of GHWF varied non-significantly. *T. vaporariorum* was reared on brinjal, cucumber, French bean, lettuce, tobacco, and tomato plants for mass production of *E. formosa*. Among them, French bean and tobacco were found to be the best host plants for mass production of *E. formosa* based on higher parasitization (37.2%). Total developmental period varied from 26.4 to 27.3 days on different host plants, the variations being non-significant. The maximum adult longevity was observed on cucumber (8.0 days) in the absence of host, and the adult size of parasitoid varied non-significantly. Results on host to parasitoid ratio of 5:1, 10:1, 20:1, 40:1 and 80:1 revealed that parasitization rate varied from 61.2 to 95.0% with maximum parasitization recorded in host–parasitoid proportion of 20:1 and minimum in 80:1. The total developmental duration varied non-significantly among all the proportions (15.1–15.4 days). Adult longevity was higher in the proportion of 40:1 (6.5 days), which was at par to the proportion of 5:1 (6.2 days), 20:1 (6.2 days), 80:1 (6.2 days) and 10:1 (6.1 days) in the absence of the host, respectively.

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Conclusion This study suggests that augmentative biological control of *T. vaporariorum* under polyhouse conditions with *E. formosa* appears to be an effective strategy for the management of this economic pest.

Keywords *Trialeurodes vaporariorum*, Biological control, *Encarsia formosa*, Parasitization

Background

The greenhouse whitefly (GHWF), *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae) is a serious pest in temperate regions under protected cultivations and in field crops where the summers are warm enough (Nasruddin et al. 2021). It is considered as a `New World` species, the origin of which is thought to be Mexico or the southwestern USA (Capinera 2008). GHWF is having distribution throughout Europe, parts of Africa, Asia, Australia, North America and South America (Hill 1987). In India, incidence of GHWF was first recorded at Thummantty in the Nilgiri Hills of Tamil Nadu on potato (Paul and David 1975) and subsequently, it has been reported on 102 host plants belonging to 36 plant families (Sood and David 2012). GHWF is multivoltine and has no diapause or dormant stages. Under protected environment, *T. vaporariorum* breeds throughout year and completes thirteen generations in a year (Sood et al. 2014). Additionally, the GHWF also transmits plant viruses and is known to be vectors of beet-pseudo yellow virus (BPYV), melon yellow virus (MYV) and four other plant viruses (CABI 2021). Presently, technology evolved for GHWF management is largely based upon the use of chemical pesticides (Singh 2017). The indiscriminate use of insecticides has led to the development of resistance in *T. vaporariorum* to several classes of insecticides. Besides this, the intensive use of insecticides has detrimental effects on non-target organisms and food safety, thereby necessitating the reliance on alternative eco-friendly measures (Kumari 2021).

Biological control agents have emerged as an alternative to conventional agriculture and is a potential tool for regenerating the ailing environment. *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae) was reported to parasitize at least 15 hosts on eight aleyrodid genera (Walia et al. 2021). The females of *E. formosa* are thelytokus, solitary endoparasitoids and destructive host feeder that uses individuals for host feeding and oviposition (Zang and Liu 2008). It is one of the commercialized parasitoids used for the control of *T. vaporariorum* and *Bemisia tabaci* (Gennadius) in many countries around the world (Singh et al. 2017; He et al. 2019). In India, only a few natural enemies have been found associated with GHWF. Kumar and Gupta (2006) recorded aphelinid endoparasitoids, viz., *Encarsia sophia* (Grault & Dodd) and *Eretmocerus* spp.; two coccinellids viz., *Coccinella septempunctata* Linn. and *Serangium montazerii* Fursch, and one chrysopid, *Chrysoperla zastrowi silemii* Stephens from Solan region of Himachal Pradesh. Reecha (2010) recorded parasitization rate of GHWF by three parasitoids, namely *Encarsia inaron* Walker, *E. sophia* and *Eretmocerus delhiensis* Mani to vary from 0.74 to 3.02%. Recently, Singh and Sood (2018) observed an endoparasitoid, *E. formosa* parasitizing GHWF nymphs at Palampur, which was the first report from India. Parasitization by *E. formosa* varied from 31.8 to 93.6% during different seasons in tomato crop grown in polyhouses where no or minimum insecticidal applications were made.

Prior to using a parasitoid in a biocontrol program, it is necessary to study its potential as a biocontrol agent, along with its biological attributes on its natural hosts under laboratory and polyhouse conditions to standardize the mass production and release protocols for augmentative biological control of the pest. Although *E. formosa* is a potential bio-agent for biological control strategies against several whiteflies species in different countries (Hoddle et al. 1998), this information is lacking in India. To fill this gap, a detailed study was conducted to study the efficacy of *E. formosa* by examining some important biological attributes to determine its potential as a successful biocontrol agent against *T. vaporariorum* infesting polyhouse crop in India.

Methods

Insect culture and host plants

The stock culture of *T. vaporariorum* was maintained at the constant temperature (25 ± 1 °C), relative humidity (70 ± 5%) and photoperiod (16:8: light: dark) in an insectary on potted French bean plants var. Anupam from whitefly infested tomato plants grown in the polyhouse. The GHWF culture was raised by exposing 10-day old potted plants of French beans for 48 h. to whitefly adults in oviposition cages (45 × 45 × 45 cm). Plants with GHWF eggs were placed in separate rearing cages for raising different developmental stages. The stock culture of *E. formosa* was initiated from mummified nymphs of *T. vaporariorum* collected from tomato plants grown in the polyhouses and were brought to the insectary and kept for adult emergence in Petri plates (5 × 1.5 cm). They were examined under binocular microscope and identified by comparing their morphological characteristics (based on physical appearance such as black head and thorax, yellow abdomen and transparent shining wings) with taxonomic keys (Singh and Sood 2018). The adults were transferred to potted French bean plants having different nymphal instars of GHWF with the help of aspirator. These plants were raised in soil-less media comprising coco peat and vermicomposting in the ratio of 1:1. Pots were watered daily via water sprinklers and fertilized with N, P_2O_5, K_2O: 19, 19, 19 once a week. These plants were kept in the insectary in the rearing cages (45 × 45 × 45 cm). The plants having GHWF immatures were exposed periodically to ensure continuous supply of the parasitoid.

Standardizing mass rearing of *E. formosa* on *T. vaporariorum*

Suitability of different immature stages of GHWF as host to E. formosa

Potted plants of French bean were used as host for rearing GHWF. All the leaves except one leaflet per plant was retained. These plants were exposed to greenhouse whitefly adults for oviposition in oviposition cages. After 24 h of exposure, plants were removed after dislodging the adults and were kept in screened rearing cages (45 × 45 × 45 cm) until they attained the desired nymphal instars (I, II, III and IV). About 50 individuals (nymphs) of greenhouse whitefly were maintained on each plant. When the desired stage of the whitefly nymphs was attained, two adults of *E. formosa* were introduced on plants inside the rearing cages. After 48 h, the adult parasitoids were removed using an aspirator and plants were continued to be placed in the cages at insectary until mummification of GHWF and adults' emergence of *E. formosa*. The experimentation was done in completely randomized design (CRD), replicating five times under laboratory conditions in the month of October–November 2019. During experimentation, the following observations were recorded.

Parasitization rate

The number of black mummified nymphs of GHWF and healthy nymphs was counted, and percent parasitization was worked out as per the following formula:

$$\textbf{Parasitization}\ (\%) = \frac{\text{Number of mummified nymphs of GHWF}}{\text{Number of intact nymphs+Number of mummified nymphs of GHWF}} \times \mathbf{100}.$$

Developmental duration

Observations on duration of different life stages of *E. formosa* were recorded as:

Egg–larval period Number of days elapsing between oviposition by the parasitoid to mummification of GHWF.

Pupal period Period elapsing between mummification of GHWF and adult emergence of *E. formosa*.

Developmental period of immature parasitoid Period elapsing between oviposition by the parasitoid and adult emergence of *E. formosa*.

Adult longevity Adult longevity of *E. formosa* was determined by providing excised leaves of French bean with and without nymphs of GHWF as host to the parasitoid in the Petri plates (5 × 1.5 cm) over agar–agar gel bed (1.5%) with the abaxial leaf surface facing upward. These were designated as in the presence of host (+) and in the absence of host (−), respectively. One-day-old *E. formosa* adults ($n = 10$) were confined individually in Petri plates covered with cling wrap (plastic food wrap), perforated using paper pin to avoid moisture deposition. Observations on surviving adults were made until the death of all individuals. Change in adult longevity of *E. formosa* in the presence/absence of host was worked out using the following formula:

$$\text{Change in adult longevity } (\%) = \frac{\text{Adult longevity in the absence of host} - \text{Adult longevity in presence of host}}{\text{Adult longevity in the absence of host}} \times 100.$$

Adult size

Body length and head breadth of the adult parasitoid females emerging from different nymphal instars of GHWF parasitized by *E. formosa* were measured under a stereo zoom binocular microscope using 'Nikon Imaging Software'. These observations were made on ten adults from each parasitized host instar.

Growth index

Observations recorded on parasitization rate and total developmental duration of the parasitoid were utilized to determine growth index as one of the parameters for ascertaining preferred host stage for parasitization by *E. formosa* as per method outlined by Sharma et al. (1982) with some modifications:

$$\text{Growth index} = \text{Parasitization } (\%)/\text{Total developmental duration (days)}$$

Table 1 Raising of different host plants of greenhouse whitefly under polyhouse

Host plant	Cultivar	Spacing (plants × rows)	Date of sowing/ transplanting
Brinjal	Punjab Bahar	60 × 45 cm	September 2019*
Cucumber	Kian	30 × 60 cm	February 2020*
French bean	Anupam	45 × 10 cm	February 2020**
Tobacco	Prabha	80 × 80 cm	September 2019*
Lettuce	Iceberg	12 × 4 cm	September 2019*
Tomato	Palam tomato hybrid-1	70 × 30 cm	September 2019*

*Transplanting

**Sowing

Effect of host plant of GHWF on growth and development of *E. formosa*

Six host plants namely, brinjal (*Solanum melongena* L.), cucumber (*Cucumis sativus* L.), French bean (*Phaseolus vulgaris* L.), lettuce (*Lactuca sativa* L.), tobacco (*Nicotiana tabacum* L.) and tomato (*Solanum lycopersicum* L.) were evaluated for suitability to be used as hosts for mass production of *E. formosa* on *T. vaporariorum*. Thirty plants of each crop were raised under polyhouse as per the package of practices of respective crops in pesticide free environment (Table 1) (Anonymous 2017).

These plants were provided with appropriate host stage of the whitefly based on outcome of present studies to *E. formosa* adults. Micro-cages (diameter: 2.5 cm; height: 2 cm) were manufactured from polycarbonate tubes. On the sides, four holes were drilled; three of them were covered with muslin cloth for ventilation and one was kept uncovered for releasing whitefly and parasitoid adults into the micro-cages. One end of the tube was covered with cling wrap. Open end of the micro-cage was placed on ventral leaf surface. A cardboard of (2 × 2 cm) cushioned with fine layer of sponge sheet was placed on the upper leaf surface to provide support for mounting the micro-cage. These cages were mounted on leaves of the host plant with the help of tying clips. Fifteen pairs of GHWF adults were released in micro-cage for oviposition through the uncovered opening using aspirator so as to obtain the cohort of individuals of desired stage. After releasing whitefly adults, the opening was plugged with cotton swab outlined with muslin cloth. Adults were allowed to oviposit for 24 h. Thereafter, they were sucked out and eggs were allowed to hatch and reach the desirable stage. About 50 third-instar nymphs were retained inside a micro-cage. Two-day-old adults of *E. formosa* ($n=2$) were introduced in the micro-cage with the help of aspirator and were allowed to parasitize the whitefly nymphs for 48 h. Thereafter, the parasitoid adults were removed and the nymphs were reared until mummification. The mummified nymphs along with leaves were brought to the laboratory and kept on agar–agar gel bed (1.5%) in Petri plates (diameter: 5 cm) for adult emergence. The experiment was replicated five times under polyhouse conditions in the month of March to April 2020, and the obtained data were analyzed with CRD. Observations were recorded on parasitization rate, duration of different developmental stages and adult longevity as elaborated under sub-head suitability of different immature stages of GHWF as host to *E. formosa*.

Determining appropriate host-to-parasitoid ratio for mass production of *E. formosa*

Five host–parasitoid proportions, namely 5, 10, 20, 40 and 80: 1, using third-instar nymphs of GHWF raised on French bean plants were evaluated during September to October 2020. Two-week-old potted plants of French bean raised in soil-less media (comprising coco peat and vermicompost in the ratio of 1:1) were used for the studies. Only one leaf was retained on the plant, and all others were pinched off. Such plants were kept in the oviposition cage having large number of adult whiteflies. Adults were allowed to oviposit for 24 h. Thereafter, the plants were removed and kept in insectary at the temperature of 25 ± 1 °C, 70 ± 5% RH and a L16:D8 photoperiod on screened rearing cages for obtaining third-instar nymphs. The experimentation was done in a completely randomized design (CRD), replicating five times.

For determining effective host–parasitoid ratio, 5, 10, 20, 40 and 80 third-instar GHWF nymphs were maintained on individual plants and one freshly emerged adult was introduced to each plant. Adults of *E. formosa* were allowed to parasitize GHWF nymphs until their death. Eight to ten such sets were maintained for each proportion. Presence of adult parasitoids was ascertained up to 5 days, and any sets having lesser exposure duration were discarded. Observations were recorded on the parasitization rate, developmental duration and body measurements of adults. For data analysis, five plants having at least 5-day exposure of parasitoid were selected.

Recording of environmental parameters

Environmental parameters, viz. minimum and maximum temperature and relative humidity, were recorded daily with the help of a digital thermo-hygrometer placed in the insectary and the polyhouse. Details are being presented in Table 2.

Statistical analysis

The data obtained from different treatments were subjected to statistical analysis by using completely randomized design (CRD) using Wasp 2.0 developed by ICAR-Central Coastal Agricultural Research Institute,

Table 2 Environmental parameters recorded during the study period

S. no.	Particular	Experimentation period	Temperature (°C) (mean)		Relative humidity (%) (mean)	
			Min	Max	Min	Max
1	Determining preferred stage of *T. vaporariorum* as host of *E. formosa* (insectary)	October–November, 2019	13.0	19.7	48.2	69.8
2	Determining suitable host plant of *T. vaporariorum* for mass production of *E. formosa* (polyhouse)	March–April 2020	14.7	23.5	65	75
3	Determining suitable host plant of *T. vaporariorum* for mass production of *E. formosa* (insectary)	September–October, 2020	18.1	28.4	76	82

Min-Minimum; Max-Maximum

Table 3 Utilization of different nymphal instars of *T. vaporariorum* by *E. formosa* for parisitization

Whitefly instar	Parisitization (%) by *E. formosa*		Parasitoid emergence from mummified nymphs (%)
	Mean ± SE	Range	
I	0.0 ± 0.0[c]	–	–*
II	19.6 ± 5.3[b]	14–26	100.0[a]
III	36.4 ± 6.7[a]	28–44	100.0[a]
IV	37.2 ± 2.5[a]	34–40	100.0[a]
$LSD_{(P=0.05)}$	5.72		NS
$F_{3, 19}$	84.37		–
P value	< 0.001		–

*No parasitization was observed and was not included in analysis

Mean values within the columns bearing the same letters are not significantly different-LSD ($P = 0.05$)

Goa, India. The significance of treatments was tested by least significant difference (LSD) at $P = 0.05$ level of significance for comparison among the treatments. Two-factor analysis of variance (ANOVA) was applied in adult longevity studies, and arcsine transformations were applied on the data obtained from present studies.

Results

Standardizing mass rearing of *E. formosa* on *T. vaporariorum*

Suitability of different immature stages of GHWF as host to E. formosa

Parasitization rate *Encarsia formosa* was able to successfully parasitize II, III and IV nymphal instars of *T. vaporariorum* (Table 3). No parasitization was evident in the first instar nymphs of the whitefly. Data obtained on parasitization by the parasitoid on different host stages depict the parasitization was higher in fourth-instar nymphs of GHWF (37.2%), being at par to third instar (36.4%), both differing significantly (LSD = 5.72, $F_{3, 19} = 84.37$, $P < 0.001$) to the parasitization observed in second instar (19.6%). It was also observed that no mortality occurred in pupal stage of the parasitoid as evident from cent percent adult emergence from all the parasitized instars of the whitefly.

Developmental duration The parasitoid was observed to complete its development successfully and reach the adult stage on II to IV nymphal instars of the whitefly (Table 4). Total developmental duration from egg to adult emergence ranged from 28 to 36 days, being significantly higher when parasitization occurred on second-instar nymphs

Table 4 Biological parameters of *E. formosa* on different nymphal instars of *T. vaporariorum*

Whitefly instar	Developmental duration (days) of the parasitoid stage					
	Egg–larval		Pupal		Egg to emergence	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
II	14.0 ± 0.6[a]	12–16	19.3 ± 0.5[a]	19–20	33.2 ± 0.8[a]	31–36
III	10.9 ± 0.4[b]	10–15	19.8 ± 0.4[a]	18–19	30.7 ± 0.3[b]	29–33
IV	10.3 ± 0.4[c]	9–15	19.6 ± 0.7[a]	18–19	29.9 ± 0.4[c]	28–33
$LSD_{(P=0.05)}$	0.54		NS		0.65	
$F_{2, 14}$	124.08		1.60		68.44	
P value	< 0.001		0.237		< 0.001	

Mean values within the columns bearing the same letters are not significantly different-LSD ($P = 0.05$)

(33.2 days) as compared to third and fourth instars, which resulted in total duration of 30.7 and 29.9 days, respectively (LSD = 0.65, $F_{2, 14}$ = 68.44, P < 0.001). Cumulative duration of egg and larval period determined on the basis of time elapsing between oviposition to mummification was 14.0, 10.9 and 10.3 days for II, III and IV parasitized instars of GHWF (LSD = 0.54, $F_{2, 14}$ = 124.08, P < 0.001), respectively. The mean duration of pupal period varied from 19.3 to 19.8 days on different instars, the variations being non-significant (LSD = NS, $F_{2, 14}$ = 1.60, P = 0.237) (Table 4).

Adult longevity Observations recorded on longevity of adults of *E. formosa* emerging from different parasitized nymphal instars of GHWF in the presence and absence of parasitoid host are being presented in Table 5. It was evident that the adult longevity of the parasitoid varied significantly among individuals emerging from different parasitized nymphal instars. Adult longevity being significantly higher for the adults emerging from fourth-instar GHWF (7.2 days), being at par to third instar (6.8 days) (LSD = 0.89, $F_{5, 59}$ = 5.34, P < 0.001). Adults emerging from the second-instar nymphs exhibited significantly lowest longevity (6.4 days). It was also observed that the longevity of *E. formosa* adults was significantly higher in the absence of parasitoid host (Table 5).

The interaction studies revealed that the adults emerging from parasitized nymphs in fourth instar and not provided with host resulted in significantly maximum adult longevity of 7.9 days (Table 5), being at par to parasitoid adults emerging from parasitization occurring in third instar (7.3 days) under similar conditions (LSD = 0.0.52, $F_{1, 59}$ = 19.65, P < 0.001). It was followed by the adults emerging from second instar (6.9 days) (absence of host) and fourth instar (6.5 days) (presence of host). Adult longevity was significantly minimum (5.9 days) when the adults emerged from second-instar nymphs were provided with the host to parasitoid adults.

Table 5 Adult longevity of *E. formosa* emerging from different parasitized nymphal instars of *T. vaporariorum* in the presence and absence of parasitoid host

Whitefly instar	Adult longevity (mean ± SE) of *E. formosa* in days		
	Host present (+)	Host absent (−)	Mean
II	5.9 ± 0.9[a]	6.9 ± 1.6[bc]	6.4[b]
III	6.3 ± 0.7[a]	7.3 ± 0.8[cd]	6.8[ab]
IV	6.5 ± 0.6[abc]	7.9 ± 0.9[d]	7.2[a]
Mean	6.2[b]	7.4[a]	

Interactions—LSD at 5%, nymphal instar (A) 0.63, $F_{2, 59}$ = 3.26, P value = 0.453

Host ± (B) 0.52, $F_{1, 59}$ = 19.65, P value < 0.001

Nymphal instar × Host ± 0.89, $F_{5, 59}$ = 5.34, P value < 0.001

Table 6 Size of *E. formosa* adults emerging from different parasitized nymphal instars of *T. vaporariorum*

Parasitoid adults emerged from parasitized whitefly instar	Body length (mm) Mean ± SE	Head breadth (mm) Mean ± SE
II	0.674 ± 0.018	0.271 ± 0.001
III	0.672 ± 0.014	0.267 ± 0.009
IV	0.680 ± 0.015	0.277 ± 0.004
$LSD_{(P=0.05)}$	NS	NS
$F_{2, 29}$	0.46	3.31
P value	0.636	0.051

Adult size Body length and head breadth of *E. formosa* adult females emerging from different parasitized instars of GHWF ranged between 0.672 and 0.680 mm (LSD = NS, $F_{2, 29}$ = 0.46, P = 0.636) and 0.267 to 0.277 mm (LSD = NS, $F_{2, 29}$ = 3.31, P = 0.051), respectively (Table 6). However, the variations in size were found to be non-significant.

Growth index The value of growth index was maximum for *E. formosa* parasitizing fourth-instar nymphs of GHWF (1.3) and was closely, followed by parasitization occurring on third instar (1.2). However, parasitization of second-instar nymphs resulted in minimum growth index value (0.6) (Fig. 1).

Effect of host plant of GHWF on growth and development of E. formosa

Parasitization rate Parasitization of third-instar nymphs of whitefly by *E. formosa* on different host plants varied

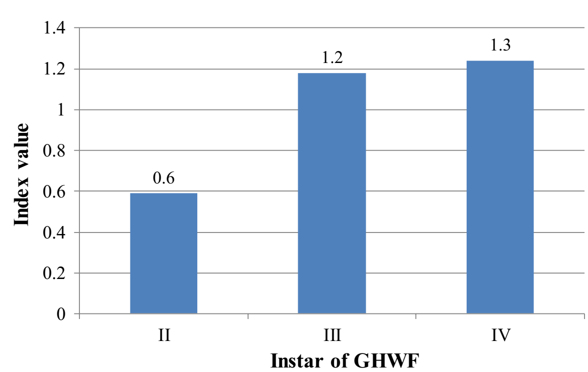

Fig. 1 Growth index of *E. formosa* on different nymphal instars of *T. vaporariorum*

Table 7 Parasitization of third-instar nymphs of *T. vaporariorum* by *E. formosa* on different host plants

Whitefly host plant	Parasitization (%)		Parasitoid emergence from mummified whitefly nymphs (%)
	Mean ± SE	Range	
Brinjal	30.0 ± 3.2[b]	26–34	100.0[a]
Cucumber	31.6 ± 7.2[ab]	20–38	100.0[a]
French bean	37.2 ± 2.3[a]	34–40	100.0[a]
Lettuce	0.0 ± 0.0[c]	0	–
Tobacco	37.2 ± 4.5[a]	32–44	100.0[a]
Tomato	30.8 ± 4.0[ab]	24–32	100.0[a]
$LSD_{(P=0.05)}$	5.34		NS
$F_{5, 29}$	58.43		–
P value	0.001		–

Mean values within the columns bearing the same letter are not significantly different-LSD ($P = 0.05$)

Table 9 Adult longevity of *E. formosa* emerged from parasitized nymphs of *T. vaporariorum* on different host plants in the presence and absence of parasitoid host

Whitefly host plant	Adult longevity (Mean ± SE) of *E. formosa* in days		
	Host present (+)	Host absent (−)	Mean
Brinjal	5.8 ± 0.8	7.3 ± 0.8	6.5
Cucumber	5.6 ± 0.6	8.0 ± 0.6	6.8
French bean	6.2 ± 0.7	7.1 ± 0.9	6.7
Tobacco	5.8 ± 0.8	7.7 ± 0.8	6.8
Tomato	5.6 ± 0.2	7.1 ± 0.7	6.4
Mean	5.8	7.4	

Interactions—LSD at 5%, host plant (A) NS, $F_{4, 99} = 0.58$

Host ± (B) 0.42, $F_{1, 99} = 61.68$, *P* value < 0.001

Host plant × Host ± 0.93, $F_{9, 99} = 7.68$, *P* value < 0.001

from 0 to 44% (Table 7). Parasitization of GHWF nymphs was maximum on French bean and tobacco plants (37.2%) (LSD = 5.34, $F_{5, 29} = 58.43$, $P = 0.001$), being significantly higher to other host plants. It was followed by cucumber (31.6%), tomato (30.8%) and brinjal (30.0%), which in turn were at par to each other. However, on lettuce, no parasitization by *E. formosa* was observed. It was also evident that pupal stage of the parasitoid experienced no mortality on evaluated host plants.

Developmental duration Duration of developmental stages of *E. formosa* recorded on different host plants of the whitefly is being presented in Table 8. Total duration of egg and larval period ranged from 8 to 13 days, with the mean duration of 9.3 to 10.0 days, whereas the pupal period ranged from 16 to 19 days. Total developmental period (egg to adult emergence) varied from 24 to 31 days with the mean duration of 26.4 to 27.3 days on different whitefly host plants, the variation being non-significant (LSD = NS, $F_{4, 24} = 2.60$, $P = 0.615$) for all the developmental stages (Table 8).

Adult longevity Observations recorded on longevity of *E. formosa* adults emerging from third-instar nymphs of the whitefly reared on different host plants in the presence and absence of parasitoid host are being presented in Table 9. It was evident that the host plant of the whitefly did not influence the adult longevity of the parasitoid significantly. However, absence or presence of host of the parasitoid affected it significantly. The longevity varied from 5.6 to 6.2 days in the presence of host and 7.1 to 8.0 days in the absence of host of the parasitoid. Interaction effect of host plant and ± of the host of parasitoid revealed the adult longevity to be maximum on cucumber (8.0 days) in the absence of host, which was at par to that observed on tobacco (7.7 days), brinjal (7.3 days), French

Table 8 Biological parameters of *E. formosa* on third-instar nymphs of *T. vaporariorum* feeding on different host plants

Whitefly host plant	Developmental duration (days) of the parasitoid stage					
	Egg–larval		Pupal		Egg to adult emergence	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Brinjal	10.0 ± 0.4	9–13	17.3 ± 0.7	16–17	27.3 ± 0.5	25–30
Cucumber	9.3 ± 0.6	8–12	17.1 ± 0.8	16–17	26.4 ± 0.9	24–29
French bean	9.3 ± 0.5	8–12	17.1 ± 1.1	16–17	26.4 ± 1.1	25–29
Tobacco	9.7 ± 0.5	8–12	17.6 ± 0.8	16–19	27.2 ± 0.5	25–31
Tomato	9.6 ± 0.6	9–13	17.5 ± 0.5	16–17	27.0 ± 0.8	25–29
$LSD_{(P=0.05)}$	NS		NS		NS	
$F_{4, 24}$	2.44		0.65		2.60	
P value	0.745		0.632		0.615	

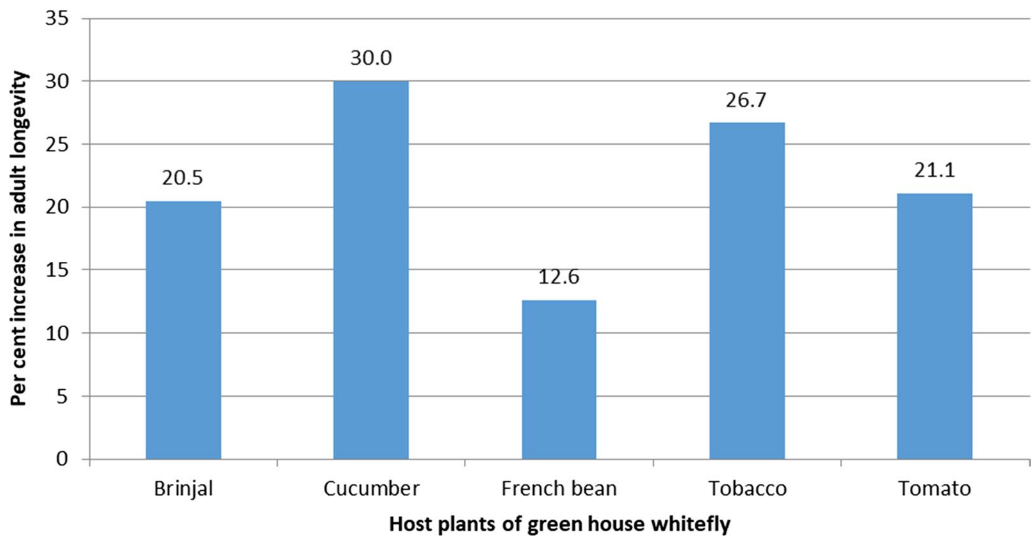

Fig. 2 Increase in longevity of *E. formosa* adults emerging from the whitefly nymphs on different host plants as compared to those provided with parasitoid host

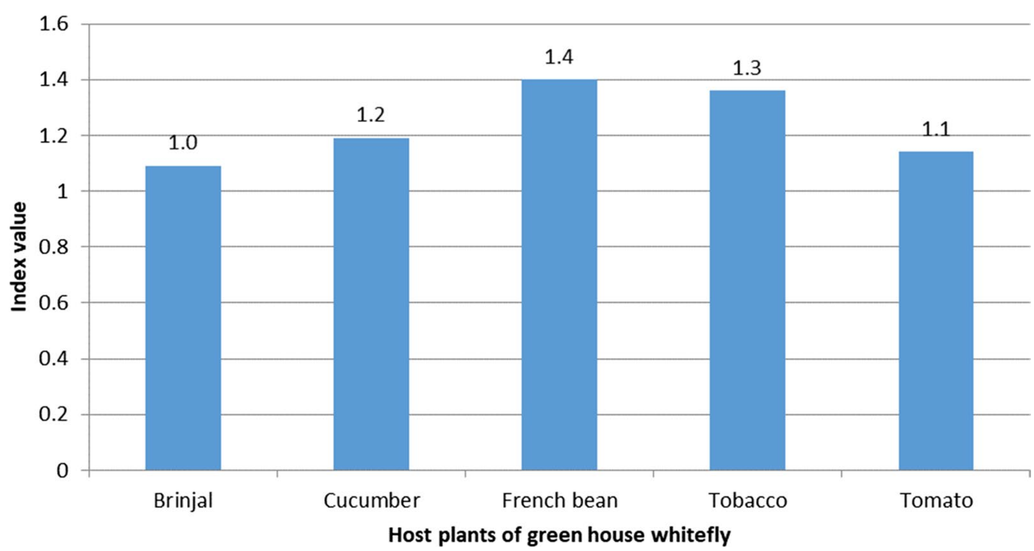

Fig. 3 Growth index of *E. formosa* parasitizing third-instar nymphs of *T. vaporariorum* on different host plants

bean (7.1 days) and tomato (7.1 days) plants in the absence of parasitoid host (LSD = 0.93, $F_{9,\ 99} = 7.68$, $P < 0.001$), respectively. Minimum longevity of adult parasitoids (5.6 days) was evident on cucumber and tomato plants in the presence of parasitoid host (Table 9). It was worked out that the longevity of *E. formosa* adults in the absence of host emerging from different host plants increased up to 30% in cucumber, followed by tobacco (26.7%) (Fig. 2). However, longevity was influenced to be minimum on French bean (12.6%).

Growth index Based on parasitization rate recorded on different host plants and developmental duration of *E. formosa* on these host plants the growth index worked out revealed it to be maximum on French bean (1.4) being closely, followed by tobacco (1.3) suggesting them to be most suitable for multiplication of *E. formosa* (Fig. 3). Cucumber and tomato resulted in moderate values of 1.2 and 1.1. Value was minimum for brinjal (1.0) depicting it to be least suitable.

Table 10 Parasitization by *E. formosa* in different host–parasitoid proportions

Host-to-parasitoid ratio*	Parasitization (%)		Parasitoid emergence from mummified nymphs (%)
	Mean ± SE	Range	
5:1	80.0 ± 14.4[b]	60.0–100.0	100.0[a]
10:1	84.0 ± 11.6[ab]	70.0–100.0	100.0[a]
20:1	95.0 ± 3.6[a]	90.0–100.0	100.0[a]
40:1	90.0 ± 4.8[ab]	85.0–97.5	100.0[a]
80:1	61.2 ± 3.2[c]	56.3–65.0	100.0[a]
$LSD_{(P=0.05)}$	11.41		NS
$F_{4, 24}$	11.28		–
P value	< 0.001		–

*III instar nymphs of *T. vaporariorum* raised on French bean plants

Mean values within the columns bearing the same letters are not significantly different-$LSD_{(P=0.05)}$

Table 11 Biological parameters of *E. formosa* on different nymphal instars of *T. vaporariorum* in different host–parasitoid proportion

Host–parasitoid ratio*	Developmental duration (days) (mean ± SE) of the parasitoid stage		
	Egg–larval	Pupal	Egg–adult
5:1	7.6 ± 0.7	7.5 ± 0.9	15.1 ± 0.8
10:1	7.6 ± 0.6	7.8 ± 0.7	15.3 ± 0.1
20:1	7.7 ± 0.6	7.8 ± 0.8	15.4 ± 0.5
40:1	7.7 ± 0.6	7.5 ± 0.7	15.2 ± 0.3
80:1	7.6 ± 0.1	7.7 ± 0.2	15.3 ± 0.2
$LSD_{(P=0.05)}$	NS	NS	NS
$F_{4, 24}$	0.08	0.18	0.54
P value	0.988	0.947	0.707

*IIIrd instar of *T. vaporariorum* on French bean host plant

Determining appropriate host to parasitoid ratio for mass production of E. formosa

Parasitization rate The results of the host (whitefly nymphs) and parasitoid (*E. formosa* adults) ratio of 5:1, 10:1, 20:1, 40:1 and 80:1 evaluated are being presented in Table 10. It was evident that the parasitization varied from 61.2 to 95.0% with a maximum of (95.0%) occurring in host parasitoid proportion of 20:1, which being at par to 40:1 (90.0%) (LSD = 11.41, $F_{4, 24}$ = 11.28, P < 0.001). Parasitization observed in the proportion of 10:1 (84.0%) was at par to 5:1 (80.0%). Parasitization was at its minimum (61.2%) in host–parasitoid ratio of 80:1 (Table 10).

Developmental duration Developmental duration (egg to adult stage) of *E. formosa* studied in different host–parasitoid proportions revealed it to vary from 15.1 to 15.4 days (LSD = NS, $F_{4, 24}$ = 0.54, P = 0.707) in different host–parasitoid proportions (Table 11). The mean

Table 12 Adult longevity of *E. formosa* emerged from different host–parasitoid proportions in the presence and absence of parasitoid host

Host–parasitoid ratio*	Adult longevity (mean ± SE) of *E. formosa* in days		
	Host present (+)	Host absent (−)	Mean
5:1	4.9 ± 0.7	6.2 ± 1.1	5.5
10:1	5.0 ± 0.6	6.1 ± 1.0	5.5
20:1	5.0 ± 0.5	6.2 ± 0.9	5.6
40:1	5.3 ± 0.5	6.5 ± 1.2	5.9
80:1	5.3 ± 0.7	6.2 ± 0.7	5.8
Mean	5.1	6.2	

*Adults emerged from whitefly nymphs (III-instar) on French bean host plant

Interactions—LSD at 5%, host–parasitoid ratio (A) NS, $F_{4, 99}$ = 0.35

Host ± (B) 0.46, $F_{1, 99}$ = 24.30, P value < 0.001

Host–parasitoid ratio × host ± 1.03, $F_{9, 99}$ = 2.89, P value = 0.004

duration of egg–larval period varied from 7.6 to 7.7 days (LSD = NS, $F_{4, 24}$ = 0.08, P = 0.988) and the pupal period from 7.5 to 7.8 days (LSD = NS, $F_{4, 24}$ = 0.18, P = 0.947) in different host–parasitoid proportions. However, the variation in duration of all developmental stages of *E. formosa* was non-significant.

Adult longevity Observations recorded on longevity of *E. formosa* adults emerging from different host parasitoid proportions in the presence and absence of host are presented in (Table 12). It was evident that the adult longevity differed significantly with the presence and absence of hosts, being more in the absence of parasitoid host. It varied from 4.9 to 5.3 days in the presence of host of *E. formosa* and was prolonged to 6.1 to 6.5 days in the absence of host. Longevity was higher in the proportion 40:1 (6.5 days), which was at par to the proportion 5:1 (6.2 days), 20:1 (6.2 days), 80:1 (6.2 days) and 10:1 (6.1 days) (LSD = 0.46, $F_{1, 99}$ = 24.30, P < 0.001), respectively, in the absence of host, whereas longevity of 5.3 days recorded in the proportion of 40:1 and 80:1 in the presence of host was at par to the proportion 5:1 and 10:1 (absence of host). Adult longevity was at its minimum in the proportion 5:1 (4.9 days) in the presence of host. Increase in adult longevity of *E. formosa* in the absence of host emerging from different host–parasitoid proportion is presented in (Fig. 4) where maximum (21%) increase in adult longevity was observed in the proportion 5:1, followed by 20:1 (19.3%), 40:1 (18.5%) and 10:1 proportion (18.0%), respectively. Increase in longevity was minimum in 80:1 proportion with (15.0%).

Growth index Growth index derived on the basis of parasitization rate and the developmental duration of *E. formosa* in different host–parasitoid proportions presented

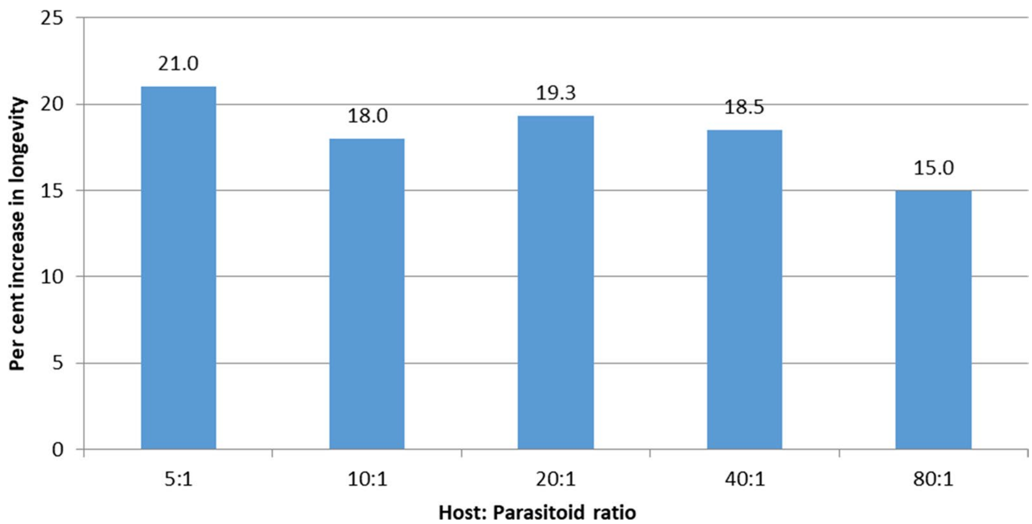

Fig. 4 Increase in adult longevity of *E. formosa* in the absence of parasitoid host emerging from different host–parasitoid proportion

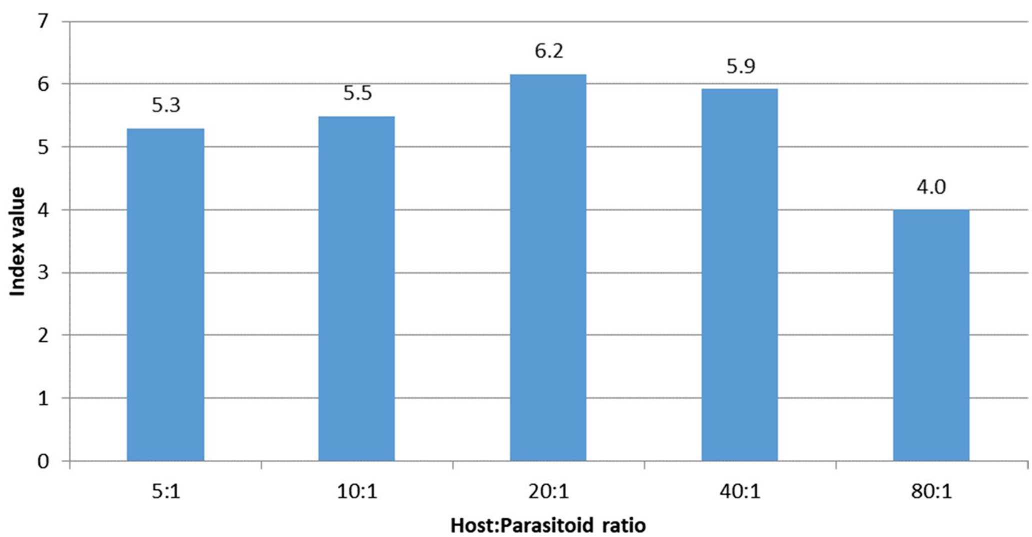

Fig. 5 Growth index of *E. formosa* parasitizing *T. vaporariorum* in different host–parasitoid proportions

in Fig. 5. It was evident that the maximum index value corresponded to 20:1 proportion with index value of 6.2, followed by 40:1 proportion (5.9). At narrow proportion of 5:1 and 10:1, the index value declined to 5.3 and 5.5, respectively. The value was minimum in 80:1 proportion with index value of 4.0.

Discussion

Based on the findings on standardizing mass production of *E. formosa* on *T. vaporariorum*, it was evident that the parasitization rate was significantly influenced by the GHWF stage, its host plant as well as host-to-parasitoid ratio. However, developmental duration, adult longevity and adult size were not influenced by these parameters. Parasitization by *E. formosa* was more when third- and fourth-instar nymphs of the whitefly were provided to *E. formosa* and duration of developmental period being less. Thus, third and fourth instars of the GHWF to be more suitable for parasitization and mass production of *E. formosa*. The present outcome derives support from the findings of Qiu et al. (2004) who stated that third, fourth and transitional sub-stages of whiteflies were most suitable for parasitization resulting in the highest percentage of *E. formosa* emergence and greatest parasitoid survival. Also, Zang and Liu (2008) observed the *E. formosa* reared on *T. vaporariorum* and *B. tabaci* to prefer third instar to oviposit, when nymphs of all four instars were offered at the same time.

Contrary to the present findings where no parasitization was observed in first instar nymphs, Hu et al. (2002) observed *E. formosa* depositing eggs on first instar nymphs of *T. vaporariorum* too. However, no parasitization by *E. formosa* in first instar nymphs was observed by Hoddle et al. (1998), which is supportive to our findings. It is attributed to the movement of first instar nymphs of GHWF, and owing to small size, *E. formosa* avoids to utilize first instar nymphs for parasitization.

Duration of different developmental stages of *E. formosa* was recorded on different instars of *T. vaporariorum*. Present findings of having longer duration in second instar are in conformity to those observed by Hu et al. (2002). They observed *E. formosa* to complete its development in 14.4 days on fourth instar, 14.2 days on third instar and 18.4 days on second instar. However, the duration was longer in our studies. It was also evident that total developmental period reduced by 7.5 and 9.9% when the parasitoid utilized third- and fourth-instar nymphs of GHWF than the second-instar nymphs. The observations are in conformity to those recorded by Hu et al. (2003) who observed the development of *E. formosa* to be slower when parasitization took place in first and second instar of *B. tabaci* than in third and fourth instars.

Host plant of GHWF did not affect the duration of different developmental stages, except the parasitization rate. In present studies, the order of preference of host plant for parasitization of GHWF nymphs was French bean > tobacco > cucumber > tomato > brinjal. Findings of van Lenteren et al. (1977) depicting greenhouse whitefly control with *E. formosa* to be good on tomato, poor on cucumber and intermediate on eggplant are partially supportive to our results where parasitization was more on French bean and tobacco and minimum on brinjal. Also, van Lenteren (1995) recorded parasitoid to have lesser parasitization rate on eggplant, which is in line to the present findings. Further, more parasitization observed on French bean and tobacco can also be attributed to the waxy leaf surface of the leaves, which aids parasitoid to search host more efficiently, whereas more hairiness and rough surface of cucumber and brinjal is not favored by GHWF for oviposition as well as by the parasitoid. Also, it has been observed by van Lenteren (1995) that walking speed of *E. formosa* was three times faster on hairless mutant of cucumber than on the hairy cucumber.

The foregone text revealed that the host–parasitoid proportion of 20:1 and 40:1 is the most appropriate for multiplication of *E. formosa* on third instar of GHWF nymphs. It was evident that parasitization was low when the host–parasitoid ratio was either narrow (5:1 and 10:1) or wide (80:1). It increased at moderate proportions evaluated (20:1 and 40:1). Our observations depicting 20:1 proportion to be most suitable derive support from the findings of Yano (1989) who reported that when introduced densities of hosts were 1, 4 and 16 per plant and the introduced densities of parasitoids were 2, 8 and 32 per plant on each introduction, respectively. The density of 4 hosts per plant and 8 parasitoids per plant per introduction gave best results. The other two cases give more unstable population changes and observed that the reduction in parasitization efficiency with an increase in parasitoid density promoted the stability of the system.

Here also, no influence on other parameters like duration, adult longevity and adult size was evident. The duration of different developmental stages of *E. formosa* was influenced by the prevailing temperature conditions. Total developmental period varied from 29.9 to 33.2, 26.4 to 27.3 and 15.1 to 15.4 in three different studies, and the variations are being attributed to prevailing temperature regimes. The mean minimum and maximum temperature were 13.0 and 19.7, 14.7 and 23.5, 14.7 and 23.5, 18.1 and 28.4℃ during three generations studied.

It also derives support from the findings of Woets and van Lenteren (1976) on tomato, cucumber, eggplant and sweet pepper and Arakawa (1982) on tobacco, who reported that development of immature of *E. formosa* in fourth instar, when host was *T. vaporariorum* required 15 days at 22.5–25 °C, which revealed the same developmental duration for different host plants, whereas development of the Dutch strain of *E. formosa* from eggs to adults on *T. vaporariorum* was reported to last from 11.9 to 15 days at 27 °C on tomato plants (Stenseth 1971) and 14.8 on bean plants (Peric 1999). Hoddle et al. (1998) had also reported that at 21 °C, when third instar of *T. vaporariorum* was provided as host, the developmental duration of the parasitoid was 25 days.

Adult longevity of *E. formosa* was observed to increase in the absence of parasitoid host in comparison with the presence of the host, which is in conformity to the findings of Drobnjakovic et al. (2016) who recorded longevity was considerably shorter 1.26–1.40 times in the presence of host. Increase in adult longevity in the absence of host is attributed to the behavioral characteristics of *E. formosa* to resorb their follicles in the absence of suitable host as observed by van Lenteren et al. (1987). In our findings, the maximum longevity was recorded when the parasitoid emerging from nymphs reared on cucumber plants, which was in conformity to findings of van Lenteren et al. (1987) who also recorded the wasps that emerged from hosts on cucumber lived significantly longer than those from host on tomato or tobacco.

Head breadth and body length of *E. formosa* females observed in the present studies varied from 0.267 to 0.277 and 0.674 to 0.680, respectively, which is in close proximity to that recoded by Singh and Sood (2018) who observed that the body length ranged between 0.6 and 0.7 mm. Utilization of different nymphal instar of GHWF was found not to affect the size of adults emerged, which also derives support from the findings of Hu et al. (2003). They observed non-significant difference in larval, pupal length or in adult head width when any of the instars of *T. vaporariorum* was parasitized by *E. formosa*.

Conclusions

The results concluded that *E. formosa* completed successfully its development on all nymphal instars of *T. vaporariorum*, except the first nymphal instar and may improve the utilization of *E. formosa* for biocontrol of *T. vaporariorum*. The study suggested that the older host stages of the whitefly would be the optimal target for *E. formosa* in field or greenhouse releases. The high adult emergence of the parasitoid, *E. formosa*, irrespective of the mummified host stage, holds promise for a potential use in augmentative biological control programs. These results are valuable in generating information that *E. formosa* can be included in biocontrol programs designed for controlling the whitefly population on crops grown under protected conditions.

Author contributions

D conducted the experiments, collected and analyzed the data, wrote the manuscript, and collected literature. DSG analyzed the data, collected literature, and wrote the manuscript. AKS designed and supervised the experiments, provided technical guidance, and critically revised the manuscript for intellectual content. All authors read and approved the final version of the manuscript.

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All data and materials are mentioned in the manuscript.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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