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# Comparative Study of Morphology and Developmental Biology of Two Agriculturally Important Whitefly Species *Bemisia tabaci* (Asia II 5) and *Trialeurodes vaporariorum* from North-Western Himalayan Region of India

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## HIGHLIGHTS

- A detailed description and illustration of stage-specific morphological characters of whiteflies *Bemisia tabaci* (Asia II 5) and *Trialeurodes vaporariorum*.
- Developmental biology of the whiteflies *Bemisia tabaci* (Asia II 5) and *Trialeurodes vaporariorum*.
- Preliminary information for field-level identification and the formulation of IPM strategies.
- First of its kind comparative study of morphology and biology of whiteflies in the Northwestern Himalayan region.

**Abstract:** *Bemisia tabaci* (Asia II 5) and *Trialeurodes vaporariorum* are the two devastating species of whiteflies infesting a wide range of vegetable crops in the North-Western Himalayan region. Therefore, the present investigation deals with a comparative study of the morphology and developmental biology of these two whitefly species. The total developmental period from egg to adult was 22.82 and 23.40 days for *B. tabaci* (Asia II 5) and *T. vaporariorum*, respectively, which did not differ significantly. The adult longevity, fecundity, and adult emergence in *T. vaporariorum* is quite higher than *B. tabaci* (Asia II 5) which were observed as 10.40 days, 118.00 eggs/female, 90.69 per cent and 6.80 days, 73.33 eggs/female, 86.59 per cent, respectively. Similarly, the egg hatching and the survival rate is higher in *T. vaporariorum* than in *B. tabaci* (Asia II 5) (95.11, 81.44 per cent and 91.68, 78.09 per cent). Morphological characters such as marginal setae, abdominal setae, vasiform orifice, lingula, and antennae, which were reliable characters for the identification of both whitefly species and diagnostics of the two whiteflies, were explicated in a comparative discussion.

**Keywords:** Biology; *Bemisia tabaci*; identification; morphology; *Trialeurodes vaporariorum*.

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## INTRODUCTION

Globally, the whitefly (Hemiptera: Aleyrodidae) is considered to be a serious pest of many agricultural crops, vegetables, ornamental plants and weeds [1-3]. It causes both direct and indirect damage by sucking the phloem sap from plants and excreting "honeydew" [4, 5], in addition to acting as a vector for plant viruses [6]. Approximately 161 genera containing 1,556 species of whitefly have been described since 2007 [7], of which only the genus *Bemisia* (Gennadius) and *Trialeurodes* (Westwood) are capable of transmitting plant viruses. In the past two decades, *B. tabaci* (Gennadius, 1889) has become a significant pest under field and polyhouse conditions and feeds over 900 host plants around the globe [8]. It alone acts as a vector for more than 300 plant viruses [9]. Plant viruses such as Begomovirus, Carlavirus, Crinivirus, Ipomovirus and Torradovirus are predominantly transmitted genera [10], thus leading to yield losses of around 20–100% and have exhibited resistance to more than 60 active ingredients [11]. Another important point to consider regarding *B. tabaci* is the cryptic species complex, where there are nearly 43 morphologically indistinguishable species which were deciphered by using different molecular tools [12-14]. Whereas, *T. vaporariorum* (Westwood, 1856) is another cosmopolitan whitefly that attacks more than 82 host plant species. It is a serious pest under greenhouse/glasshouse conditions [15] capable of transmitting only the genera Crinivirus and Torradovirus [16] and also showed resistance to a few insecticide classes (neonicotinoids, pyrethroids and ketoenols) [17].

The present investigation mainly focusses on two whitefly species, *B. tabaci* and *T. vaporariorum*, which infest tomato crops and are responsible for upto 100 per cent losses in the North-Western Himalayan region by transmission of ToCV (tomato leaf curl virus) and ToLCD (tomato leaf chlorosis disease) [18, 19]. Normally, *B. tabaci* is confused with *T. vaporariorum* [20], so, exact identification is a pre-requisite to incorporating the respective management tactics for both whiteflies. Another stumbling block is the lack of information on developmental biology with morphological data, which is necessary for specific vector management, as *B. tabaci* is a better vector for the virus than *T. vaporariorum* [21]. So, the present study investigates the biology and morphology along with the illustrations of each life stage of these two whitefly species collected from various locations of the Himalayan region, which will be very helpful for authentic and rapid identification of each life stage and the formulation of adequate pest management strategies for this pest. As a result, it confers new metrics for horticultural crop output in rural parts of the North-Western Himalayan region.

## MATERIALS AND METHODS

During 2018, populations of *B. tabaci* (Asia II5) and *T. vaporariorum* were collected from tomato (*Solanum lycopersicum*; Solanales: Solanaceae) plants at various sites of the Vegetable Research Centre in Pantnagar (29° 01' 53" N, 79° 22' 27" E, 232 m asl) and Padampuri (29° 38' 04" N, 79° 61' 94" E 1560 m asl) of North-Western Himalayan region. Random sampling was done in tomato fields at specified locations 10 m away from the roadside and 100 individuals were collected from each location and transferred into caged tomato seedlings. Both whitefly populations were reared on tomato host plants in the glass house at the Department of Entomology at GB Pant University of Agriculture and Technology, Uttarakhand, India, under the controlled conditions of temperature (25±2 °C), relative humidity (70±10 %) and photoperiods (16 L: 08 D). Identification of whiteflies was carried out by extraction of genomic DNA by using the Nucleopore® Insect DNA Extraction Mini kit (Genetix Biotech Asia Pvt. Ltd.). Further, genomic DNA was subjected to PCR amplification by using primers C1-J-2195 and TL2-N-3014 for *B. tabaci* [22] and primers TvapF and WFr for *T. vaporariorum* [23]. The population of whiteflies was maintained separately in insect-proof rearing cages in the glasshouse, and the genetic purity was scouted every month by molecular analysis. Following species confirmation, samples were drawn from these pure cultures for morphology and developmental biology studies.

Morphological studies were carried out for all stages of the whitefly species, *B. tabaci* and *T. vaporariorum*. Around 100 specimens were preserved in alcohol until further investigation. The alcohol preserved specimens were subjected to tissue softening by placing the stage specific specimen on 10% potassium hydroxide (KOH) for 12-24 hours till it became translucent. Then these specimens were treated with glacial acetic acid for 15 minutes and cleaned by using a camel brush. The cleaned specimen was stained with acid fuchsin (overnight), and then washed thoroughly by using sequential concentrations like 75, 90 and 99.9% ethyl alcohol (EtOH) in separate cavity blocks for 15 minutes to remove the extra stains.

After this, specimens were placed in clove oil for 30 minutes, until the cloudiness was reduced. Finally, the specimens were mounted in DPX on a glass slide and placed in a hot air oven for three weeks at 35°C. Dried slides were then observed under an Olympus (SZX12) microscope for morphological characters. Illustrations of all the developmental stages were done using an Olympus (SZX12) microscope with a drawing tube attached.

Developmental biology was studied during September-October 2019 with the help of a self-fabricated leaf cage proposed by Gill & Rataul [24]. Ten pairs of 24 hours old males and females of adult whitefly were collected from pure culture and placed in a leaf cage. After 24 hours of introduction, the presence of eggs was examined under the microscope and only fifteen eggs per leaf were left in each replication. Then, the plants were introduced into Rescholar insect growth chambers (RI-68-02) under controlled conditions (temperature =  $25 \pm 2$  °C, relative humidity =  $70 \pm 10\%$ , 4000 lx and a photoperiod of 16:8 h L:D). In the experiment, forty-five eggs from three leaves were used for accurate observation, avoiding overcrowding and being easy to handle. The insects were monitored daily under the microscope (Leica MC170 HD) to record the developmental changes until the nymphs attained the 4<sup>th</sup> instar. Biological parameters such as egg hatchability, developmental period of each instar, total developmental period, survival rate, adult emergence, longevity and fecundity were recorded.

### Statistical analysis

Data of egg hatchability, developmental period, survival rate, adult's emergence, longevity and fecundity of *B. tabaci* (Asia II 5) and *T. vaporariorum* were subjected for analysis of variance (ANOVA). When ANOVA showed a significant difference, the mean was determined using Student-Newman-Keuls test (SNK) at a 95% confidence level. All the statistical analysis was performed with SPSS software IBM (version 25.0).

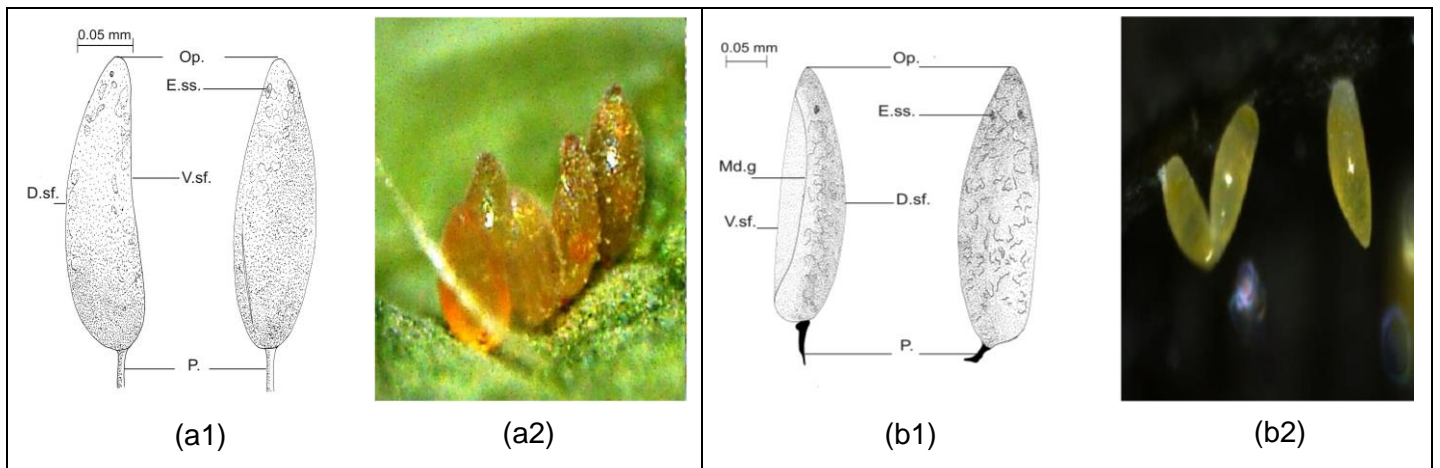
## RESULTS

### Comparative study of stage-specific morphology of *B. tabaci* (Asia II 5) and *T. vaporariorum*

**Eggs:** Comparative morphological study of *Bemisia tabaci* (Asia II 5) (Figures 1 a1 & 1 a2) and *Trialeurodes vaporariorum* (Figures 1b1 & 1b2) revealed that females of both species laid freshly yellow-coloured eggs under the surface of leaves, which were covered with white waxy powder. The elliptical (apically pointed with a broadly rounded base) shape of the eggs was inserted upright into the leaf tissue by a short stalk (pedicel). The eggs could easily be differentiated based on their size, colour, arrangement patterns, and nature of the eggshell after hatching.

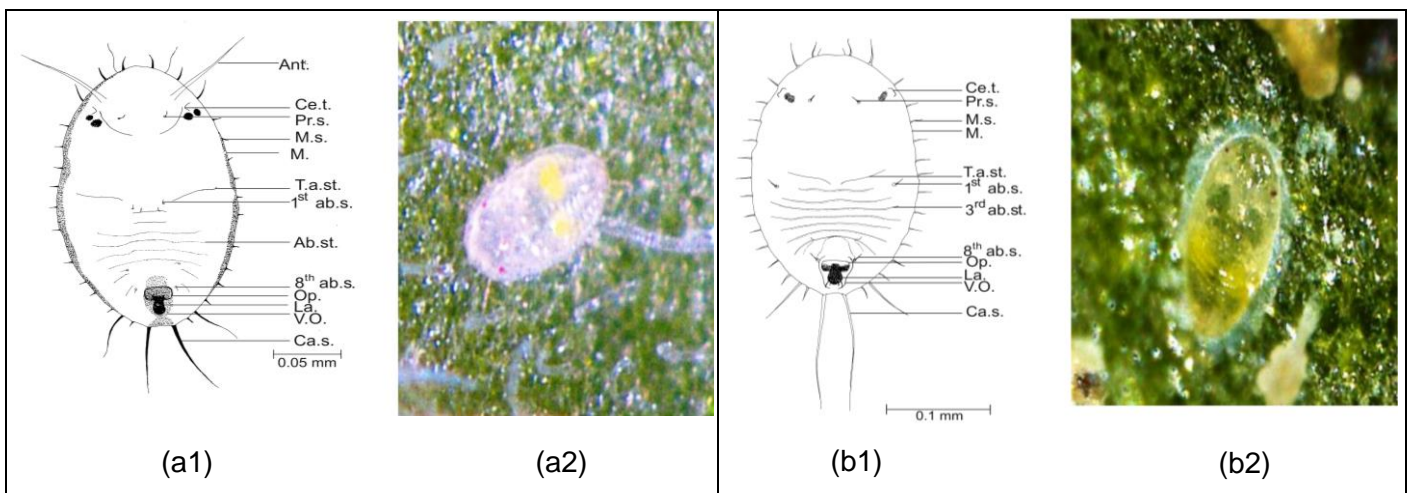
*Trialeurodes vaporariorum* laid yellow-coloured eggs (length= 0.22 mm; breadth= 0.09 mm; n=30) in neat circle or semicircle which turned dark brown or almost black before hatching while, in *B. tabaci* (Asia II 5) the eggs (length= 0.20 mm; breadth= 0.10 mm; n=30) were creamy yellow which changed into light golden brown and were laid singly in a scattered manner. Prior to hatching, the eggs of *T. vaporariorum* became kidney-shaped where the convex and concave side of the egg represented the dorsal and ventral surface of the emerging nymph, respectively. The eggshell became flattened laterally after the emergence of the nymph.

**First nymphal instar:** The first instar nymphs of both the species had functional legs and antennae, which crawled over the leaf surface until settled at one site for feeding and remain there during the subsequent moults. The first instar of both species was elliptical shaped and surrounded by narrow white margin. They are almost identical and can't be differentiated in vivo except egg colour which was pale-yellow in *T. vaporariorum* and dark yellow in case of *B. tabaci* (Asia II 5). The first instar nymph had paired reddish eye-spots in the anterolateral region of the cephalothorax and two distinct yellow fat-bodies in the abdomen. The dorsal surface had a thin and membranous integument, covered with a layer of transparent wax. Slight differences were observed in the mounted specimens of the two species, where, *T. vaporariorum* had 17 pairs of well marginal setae while in *B. tabaci* (Asia II 5) there were 16 pairs.



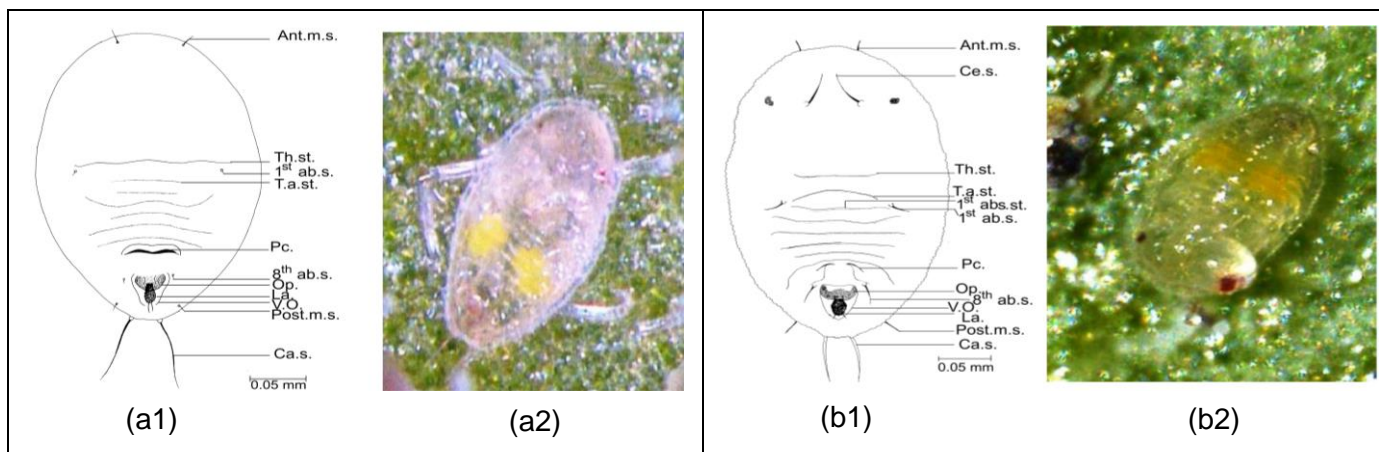
**Figure 1.** Morphology of egg (lateral and dorsal view) a1 & a2- *Bemisia tabaci* (Asia II 5), b1 & b2- *Trialeurodes vaporariorum*. Op.- apex; E.ss.- Eyes spots; V.sf.- Ventral surface; D.sf.- Dorsal surface; Md. g- Median groove; P.- Peduncle.

Similarly, cephalic tubercles were strongly developed and sub-rectangular shaped in *T. vaporariorum*; while they were weakly developed and sub-elliptical shaped, along with a pair of cephalic setae in *B. tabaci* (Asia II 5). A total of eight sutures (including thoracoabdominal suture) were present in the abdominal area of both the species along with two abdominal setae. The vasiform orifice of *T. vaporariorum* was broadly subcordate in shape and opened posteriorly, while in *B. tabaci* (Asia II 5) it was almost quadrate in shape and closed posteriorly. The lingula was half-covered by the operculum in both species, however, in *T. vaporariorum* the distal end of the lingula was armed with spines. The size of the nymph varied slightly in the two species with *T. vaporariorum* (Figures 2 b1 & 2 b2) measuring 0.27 mm in length and 0.16 mm in breadth (n=30), and *B. tabaci* (Asia II 5) (Figures 2 a1 & 2 a2) nymph measuring 0.25 mm in length and 0.15 mm in breadth (n=30).



**Figure 2.** First instar nymphs of a1 & a2- *Bemisia tabaci* (Asia II 5), b1 & b2- *Trialeurodes vaporariorum*. Ant.- Antenna; Ce.t.- Cephalic tubercles; Pr.s.- Prothoracic setae; M.s.- Marginal setae; M.-Margin; T.a.st.- Thoraco-abdominal suture; 1<sup>st</sup> ab. s.- First abdominal segment; Ab.st.- abdominal suture; 8<sup>th</sup> ab.s.- Abdominal setae on 8<sup>th</sup> segment; Op.-Operculum; La.-Lingula; V.O.- Vasiform orifice; Ca.s.- Caudal setae. 3<sup>rd</sup> ab. st.- Third abdominal suture.

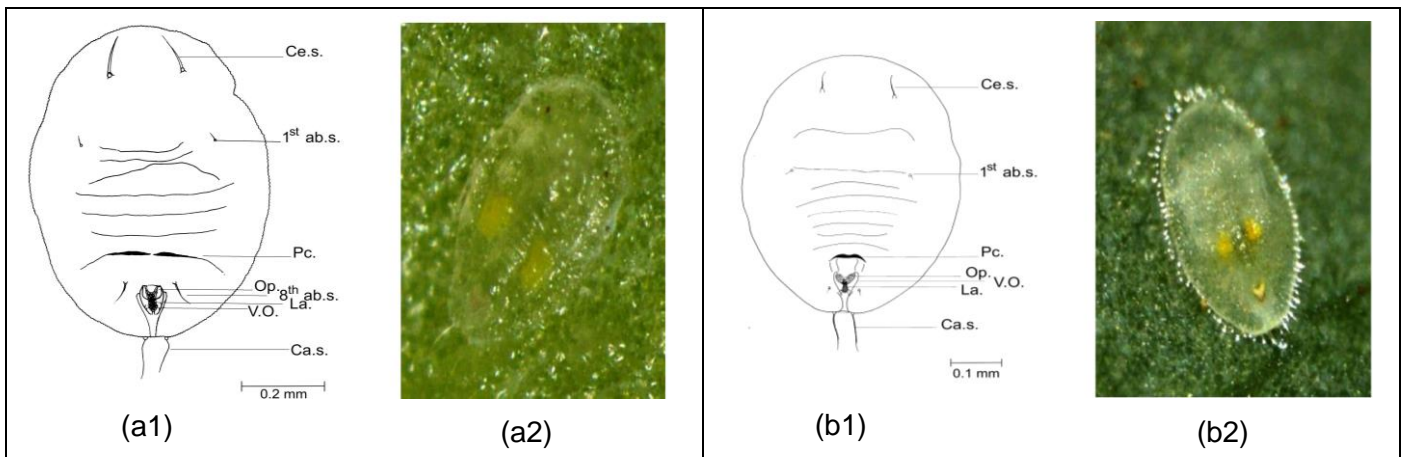
**Second nymphal instar:** As compared to first instar the functional legs and antennae was small in size while, marginal setae degenerated in second nymphal instar. The second instar nymphs of *T. vaporariorum* were oval with a distinctly crenulated margin, while in *B. tabaci* (Asia II 5), the crenulated margin was not distinct. In both the species, anterior and posterior marginal setae were small and less noticeable but the caudal setae were well developed and prominent. In case of *T. vaporariorum*, two pairs of the dorsal setae were prominently present on the 8<sup>th</sup> abdominal segment and cephalic segment, while in *B. tabaci* (Asia II 5), 8<sup>th</sup> abdominal setae were minute and cephalic setae were completely absent.



**Figure 3.** Second instar nymph of (a1 & a2) *Bemisia tabaci* (Asia II 5) and (b1 & b2) *Trialeurodes vaporariorum*. Ant.m.s.- Anterior marginal seta; Th.st.-Thoracic suture; 1<sup>st</sup> abs.s.- seta on first abdominal segment; T.a.st.- Thoraco-abdominal suture (transverse moulting suture); Pc.- Pockets; 8<sup>th</sup> ab.s.- Seta on eighth abdominal segment; Op.- Operculum; La.- Lingula; V.O.- Vasiform Orifice; Post.m.s.- Posterior marginal seta; Ca.s.- Caudal seta. Ce.s.- Cephalic seta; 1<sup>st</sup> abs.st. – first abdominal suture.

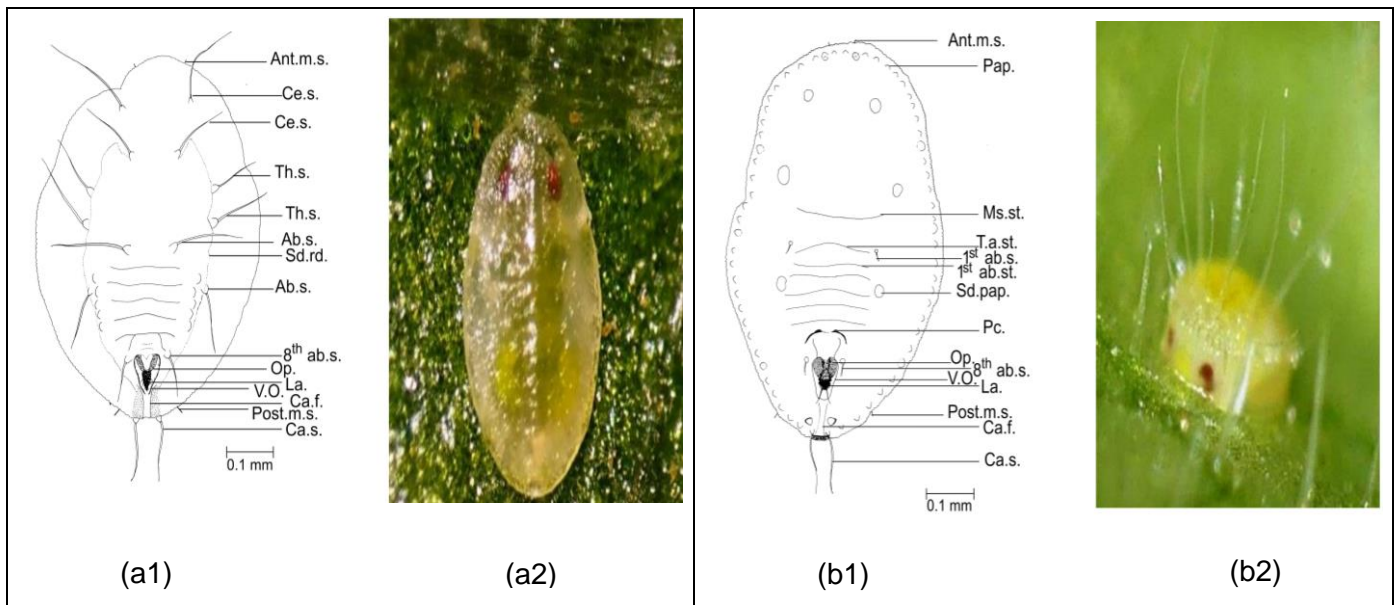
The vasiform orifice was subcordate with notched posterior end and the size of lingula was the same as that of the orifice, with a pair of lateral lobes in *T. vaporariorum*, while in *B. tabaci* (Asia II 5), orifice was a triangular shape with posteriorly open and lingula was swollen distally and pointed. Both species had distinct sutures on the eighth abdominal segments along with two sutures observed on the cephalothorax that confine the meso and metathorax. The living nymphs were distinguished only by their size *i.e.*, *B. tabaci* (Asia II 5) nymphs (Figures. 3 a1 & 3 a2) (length= 0.35 mm; breadth= 0.22 mm; n=30) were slightly smaller than those of *T. vaporariorum* (Figures 3 b1 & 3 b2) (length= 0.38 mm; breadth= 0.25mm; n=30).

**Third nymphal instar:** Third instar of both species was considerably flattened, larger and more transparent than the second instar and it was not distinguishable *in vivo*. In the case of *T. vaporariorum*, characters like distinct marginal crenulations and marginal, caudal, dorsal and subdorsal setae were similar to the second instar. However, they can easily be distinguished from the second instar based on their larger size, rearward bend in the antennae, the vasiform orifice and its associated structures, which resemble with the pupal instar (vasiform orifice is located farther away from the caudal margin, lingual with two pairs of lateral lobes and faintly indicated caudal furrow are present in the third instar). In case of *B. tabaci* (Asia II 5), third instar showed more similarity with the pupal stage instead of second nymphal instar, mainly in presence of three pairs of dorsal setae, vasiform orifice and a faintly visible caudal furrow and can be distinguished from the pupal instar based on antennae which is similar to the second instar. The third instar of the two species showed resemblance in the presence and position of the dorsal setae, while, differs in various aspects including non-uniformly crenulated margins, triangular shape of vasiform orifice and swollen lingula without lobes in *B. tabaci* (Asia II 5). Also, the average measurements of mounted specimens of the third instar in *T. vaporariorum* (Figures 4 b1 & 4 b2) were 0.62 mm in length and 0.38 mm in breadth (n=30), while in *B. tabaci* (Asia II 5), (Figures 4 a1 & 4 a2) was 0.50 mm in length and 0.34 mm in breadth(n=30).



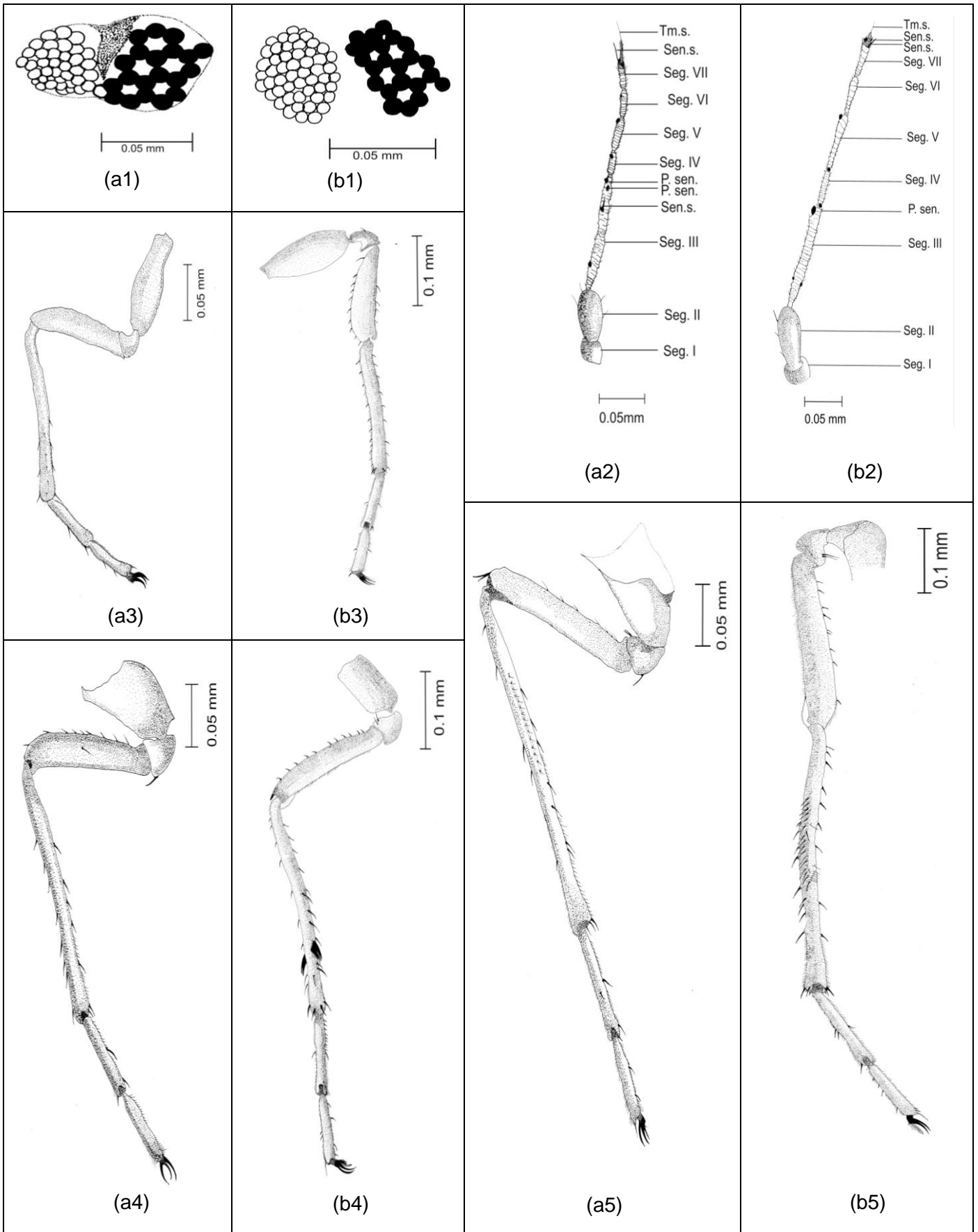
**Figure 4.** Third instar nymphs of (a1 & a2) *Bemisia tabaci* (Asia II 5) and (b1 & b2) *Trialeurodes vaporariorum*. Ce.s.- Cephalic setae; 1<sup>st</sup> ab.s.- Seta on first abdominal segment; Pc.- Pocket; Op.- Operculum; 8<sup>th</sup> ab.s.- Seta on eighth abdominal segment; La.-Lingula; V.O.- Vasiform orifice; Ca.s.- Caudal setae.

**Fourth nymphal instar (pupal stage):** The pupal instar was identified most easily out of all the immature stages as it was dissimilar from the previous instars. Pupae of both the species were distinguished *in vivo*. In *T. vaporariorum*, the living pupa was pale, opaque and yellowish-white in appearance, raised from the surface of the leaf and surrounded by a thin palisade of transparent-whitish wax, and the dorsal surface bears glassy wax rods, while in case of *B. tabaci* (Asia II 5), the pupa was distinctly yellow, and the palisade of wax and dorsal glassy wax rods were absent. However, tufts of white wax were frequently present in thoracic tracheal pores. Average measurements of microscopic mounts differed in both the species with *T. vaporariorum* (Figures 5 b1 & b2) measuring 0.71 mm in length, 0.43 mm in breadth (n=30) and in *B. tabaci* (Asia II 5), (Figures 5 a1 & a2) 0.69 mm in length; and 0.50 mm in breadth (n=30). In mounted specimens, *T. vaporariorum* pupa was oval-shaped, posteriorly rounded with a uniformly crenulated margin, distinct crenulations on the margin of thoracic and caudal pores. While *B. tabaci* (Asia II 5) pupa was oval or elliptical shaped, posteriorly pointed with irregular crenulation and indistinct tracheal pores on the margin. In *T. vaporariorum*, about 64 well-developed papillae were present in a single row on the sub margin, among them 4-5 were relatively larger. Four pairs of large well-developed papillae were also present on the sub dorsum. However, in *B. tabaci* (Asia II 5), submarginal papillae were completely absent and indicated as small inconspicuous “micro-setae”. The paired dorsal setae of *T. vaporariorum* were found in the 1<sup>st</sup> and 8<sup>th</sup> abdominal segment, while in *B. tabaci* (Asia II 5) dorsal setae varied in each specimen and one to seven pairs of well-developed dorsal setae were present. In *T. vaporariorum*, the pupal instar was differentiated from previous instars by the presence of moulting sutures, larger size, elongate vasiform orifice, presence of caudal furrow and three pairs of lateral lobes in the lingula. While in *B. tabaci* (Asia II 5), vasiform orifice was triangular and the lingula was pointed as in the preceding instar. Also, a well-developed caudal furrow was present.



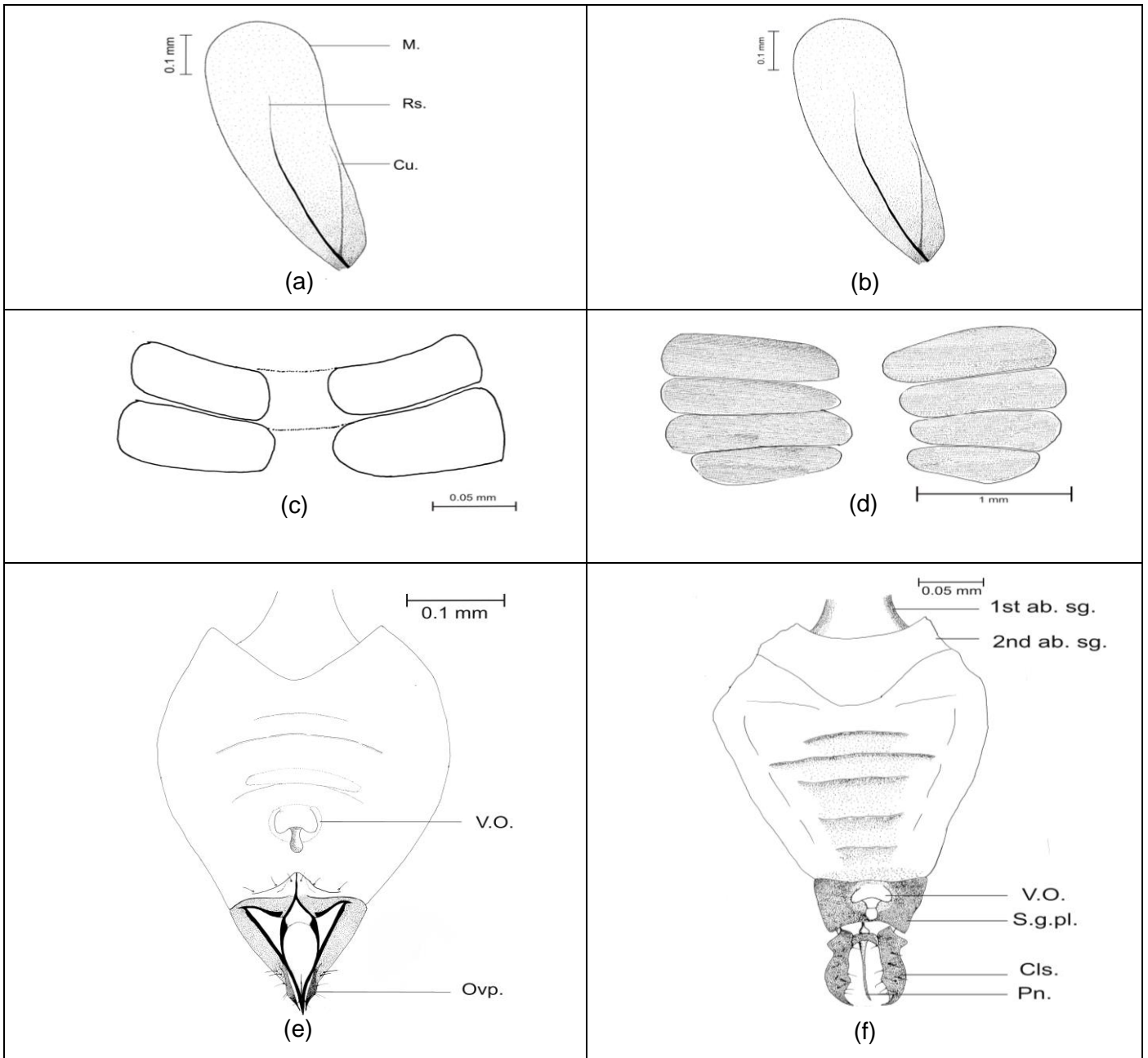
**Figure 5.** Fourth instar nymphs of (a1 & a2) *Bemisia tabaci* (Asia II 5) and (b1& b2) *Trialeurodes vaporariorum*. Ant.m.s.- Anterior marginal setae; Pap.- Papilla; Ce.s.- Cephalic setae; Th.s.- Thoracic setae; Ab.s.- Abdominal setae; Sd.rd.- Subdorsal ridge; 1<sup>st</sup> ab.st.- Suture on first abdominal segment; 1<sup>st</sup> ab.s.- Seta on first abdominal segment; 8<sup>th</sup> ab.s.- Seta on eighth abdominal segment; Op.- Operculum; La.- Lingula; V.O.- Vasiform orifice; Ca.f.- Caudal furrow; Post.m.s.- Posterior marginal setae; Ca.s.- Caudal setae; Ms.st.- Mesothoracic setae; T.a.st.- Thoraco-abdominal suture (transverse moulting suture); Sd.pap.- Sub dorsal papilla; Pc.- Pockets.

**Adult stage:** The adults of both the species were similar in appearance with a pale yellow colour and presence of two pairs of white immaculate wings, which were thinly dusted with a white waxy powder. In *T. vaporariorum* anterior margin of forewing was curved while in *B. tabaci* (Asia II 5) it was straight. In resting position, the wings of *B. tabaci* (Asia II 5) looked to be narrow and more pointed towards posteriorly as compared to *T. vaporariorum*. In the case of *B. tabaci* (Asia II 5), adults were somewhat smaller than *T. vaporariorum* with a darker yellow colour (Figures 9a & 9b). In mounted specimens of *B. tabaci* (Asia II 5) adults, the compound eyes were divided and each eye had two groups of lenses with a single lens forming a “bridge” between the two groups, while in *T. vaporariorum* adults, the eyes were divided but there was no lens forming a bridge between the two groups of facets (Figures 6 a1 and 6 b1). The antennae in *B. tabaci* (Asia II 5) comprised of seven segments with pit sensoria on 3<sup>rd</sup>, 5<sup>th</sup>, 6<sup>th</sup> and 7<sup>th</sup> segment and stout sensory setae on the 3<sup>rd</sup> and 7<sup>th</sup> segment. The antennae in *T. vaporariorum* were similar to those of *B. tabaci* (Asia II 5), but without pit sensorium on 6<sup>th</sup> segment and stout sensory setae on the 3<sup>rd</sup> segment (Figures 6 a2 and 6 b2). The tibiae of the mesothoracic legs is important key for the identification to differentiate between both species. The mesothoracic tibiae of *B. tabaci* (Asia II 5) were surrounded with randomly arranged stout spines, while in *T. vaporariorum*, the stout spines were typically arranged in two lateral “tufts” (Figures 6 a 3-5 and 6 b3-5). No remarkable variation were observed in prothoracic and metathoracic leg of both species. The ventral surface of abdomen in both the species had two pairs of wax plates on 3<sup>rd</sup> and 4<sup>th</sup> segment in females and four pairs of wax plates (from 3<sup>rd</sup> to 6<sup>th</sup> segment) in males (Figures 7 c-d and 8 c-d). The surface of these wax plates was distinctly reticulate in case of *B. tabaci* (Asia II 5), while in *T. vaporariorum*, the structures were striated. The supragenital plate in *B. tabaci* (Asia II 5) was weakly developed in the female but modified into a tube-like collar in males which projects from the tip of the abdomen along with the male genitalia distally. While in *T. vaporariorum*, the supra-genital plate was well developed and clearly defined in the female and modified into a tube-like collar in males which was strongly sclerotized and darkly pigmented. The abdomen in case of *T. vaporariorum* males also differed in presence of several rows of distinct pores on the dorsal surface. Vasiform orifice in immature stages is sub-circular shape with elongated narrow lingula. In adult, the shape and size of vasiform orifice (located on the supra-genital plate) were similar in both the species.

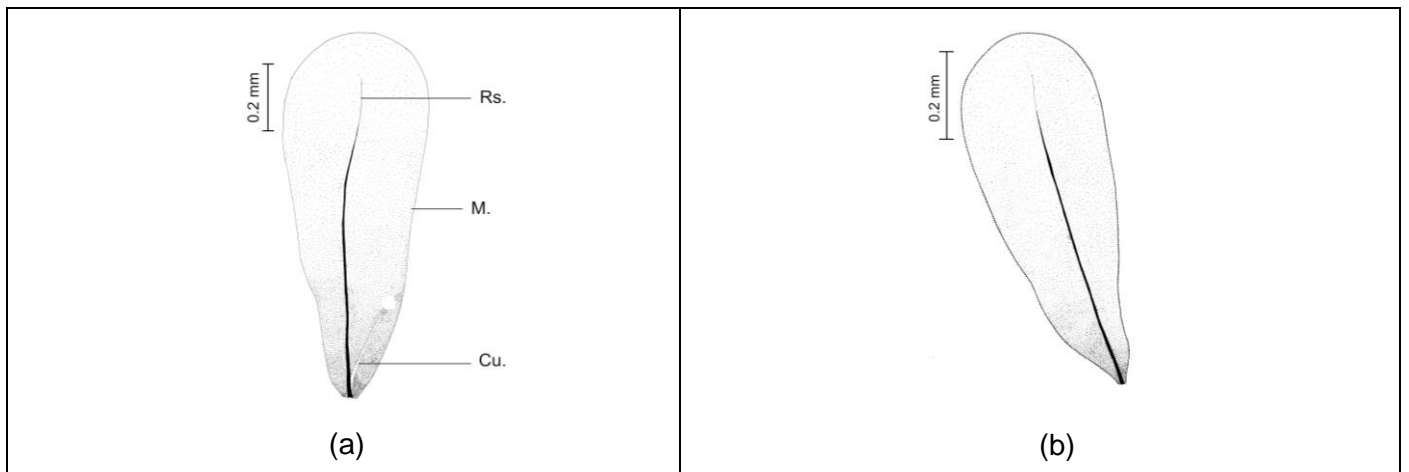


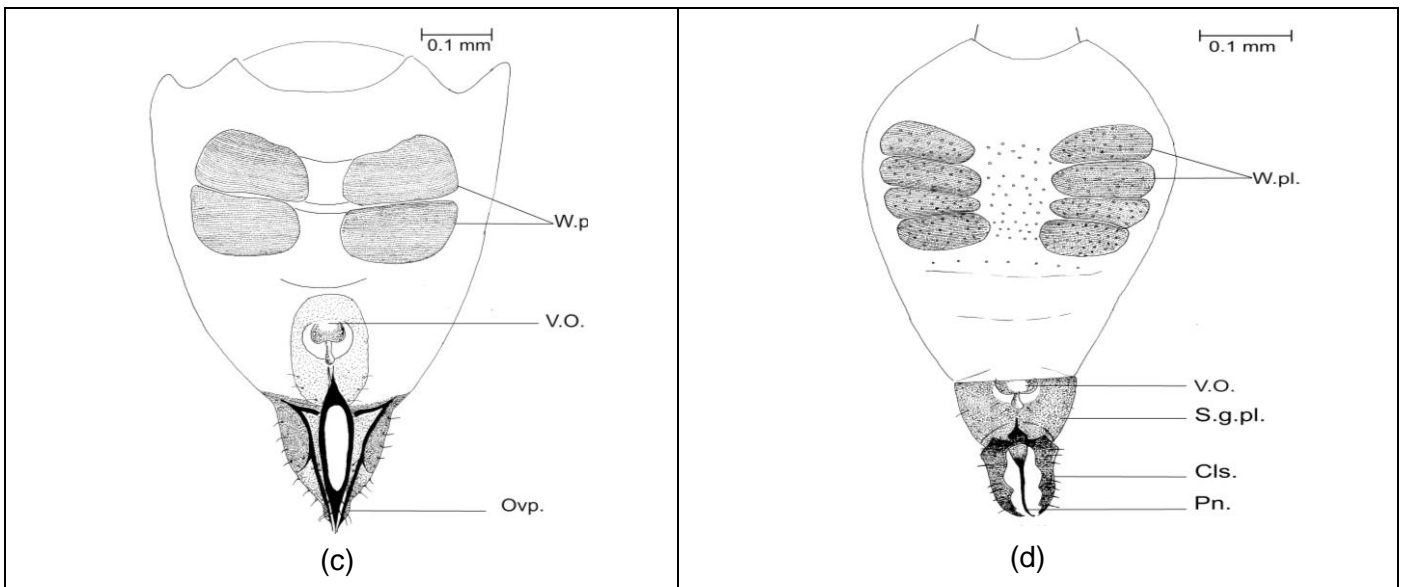
**Figure 6.** a- *Bemisia tabaci* (Asia II 5) b- *Trialeurodes vaporariorum*. 1- Eye; 2- Antenna; 3- Foreleg; 4- Midleg; 5- Hindleg; Tm.s.- Terminal seta; Sen.s.- Sensory seta; Seg.- Segment; P.sen.- Pit sensorium.





**Figure 7.** *Bemisia tabaci* (Asia II 5). a- forewing; b- hindwing; c- waxplate (female); d- waxplate (male); e- abdomen (female); f- abdomen (male). M.- Margin; Rs.- Radius; Cu.- Cubitus; ab. Sg.- First abdominal segment; V.O.- Vasiform Orifice; s.g.pl.- Supra genital plate; Cls.- Claspers; Pn.- Penis; Ovp.- Ovipositor





**Figure 8.** *Trialeurodes vaporariorum*. a- forewing; b- hindwing; c- abdomen (female); d- abdomen (male) M.- Margin; Rs.- Radius; Cu.- Cubitus; V.O.- Vasiform Orifice; s.g.pl.- Supra genital plate; Cls.- Claspers; Pn.- Penis; Ovp.- Ovipositor; W.pl.- Wax plate.



**Figure 9.** Adult a- *Bemisia tabaci* (Asia II 5) b- *Trialeurodes vaporariorum*.

### Developmental biology of *B. tabaci* (Asia II 5) and *T. vaporariorum*

The ANOVA showed that there was no significant difference between total development duration of *B. tabaci* (Asia II 5) and *T. vaporariorum* ( $F= 1.14$ ;  $p= 0.34$ ) (Table 1). There was a significant difference between developmental periods of 1<sup>st</sup> instar ( $F= 10.86$ ;  $p= 0.03$ ), while no significant difference was found in other developmental stages (Table 1). Similarly, adult longevity differed significantly ( $F= 32.40$ ;  $p= 0.00$ ) between the *B. tabaci* (Asia II 5) ( $6.80\pm 0.37$  days) and *T. vaporariorum* ( $10.40\pm 0.50$  days) (Table 2). Female laid an average of  $73.33\pm 11.78$  and  $118.00\pm 9.73$  eggs during their lifetime in *B. tabaci* (Asia II 5) and *T. vaporariorum*, respectively, which were significantly different ( $F= 8.53$ ,  $p= 0.04$ ) (Table 2). However significant difference were observed between the egg hatchability of *B. tabaci* (Asia II 5) and *T. vaporariorum* ( $F= 4.171$ ;  $p= 0.048$ ). The egg hatching percent in *T. vaporariorum* (95.11 per cent) was higher than that of *B. tabaci* (Asia II 5) (91.68 per cent). However, no significant difference were found between adult emergence and survival rate of *B. tabaci* (Asia II 5) and *T. vaporariorum* ( $F= 3.716$  and  $0.649$ ;  $p= 0.061$  and  $0.601$ ) (Table 3).

**Table 1.** Preimaginal development duration in days (average  $\pm$  standard error)<sup>a</sup>, of *Bemisia tabaci* (Asia II 5) and *Trialeurodes vaporariorum* on Tomato at 25  $\pm$ 2°C.

Whitefly species	Development duration (days)					
	Egg	1 <sup>st</sup> instar	2 <sup>nd</sup> instar	3 <sup>rd</sup> instar	4 <sup>th</sup> instar	Egg to Adult
<i>Bemisia tabaci</i>	7.10 $\pm$ 0.15 <sup>c</sup>	3.73 $\pm$ 0.33 <sup>a</sup>	3.27 $\pm$ 0.12 <sup>a</sup>	3.47 $\pm$ 0.09 <sup>a</sup>	5.25 $\pm$ 0.20 <sup>b</sup>	22.82 $\pm$ 0.32 <sup>d</sup>
<i>Trialeurodes vaporariorum</i>	7.07 $\pm$ 0.09 <sup>c</sup>	5.03 $\pm$ 0.22 <sup>b</sup>	3.43 $\pm$ 0.19 <sup>a</sup>	2.93 $\pm$ 0.09 <sup>a</sup>	4.93 $\pm$ 0.09 <sup>b</sup>	23.40 $\pm$ 0.46 <sup>d</sup>
<b>F value</b>	0.04	10.86	0.57	18.29	1.84	1.14
<b>P value</b>	0.86	0.03	0.49	0.10	0.25	0.34

<sup>a</sup>Original data; means followed by the same letter in the row do not differ by Student-Newman-Keuls test (SNK) ( $P \leq 0.05$ ).

**Table 2.** Adult longevity and life time fecundity in days (average $\pm$ standard error) of *B. tabaci* (Asia II 5) and *Trialeurodes vaporariorum* on tomato at 25  $\pm$ 2°C

Whitefly species	n	Mean longevity (days $\pm$ SE)	Range	Lifetime fecundity $\pm$ SE	Range
<i>Bemisia tabaci</i>	20	6.80 $\pm$ 0.37	6-8	73.33 $\pm$ 11.78	54-85
<i>Trialeurodes vaporariorum</i>	20	10.40 $\pm$ 0.50	8-12	118.00 $\pm$ 09.73	95-134
<b>F value</b>	-	32.40	-	8.534	-
<b>P value</b>	-	0.000	-	0.043	-

**Table 3.** Egg hatching, adult emergence and survival rate for *B. tabaci* (Asia II 5) and *T. vaporariorum* on tomato plants at 25  $\pm$ 2°C (shown as a percentage)

Whitefly species	N	Egg hatching	Adult emergence	Survival rate egg to adult
<i>Bemisia tabaci</i>	20	91.68	86.59	78.09
<i>Trialeurodes vaporariorum</i>	20	95.11	90.69	81.44
<b>F value</b>	-	4.171	3.716	0.639
<b>P value</b>	-	0.048	0.061	0.601

## DISCUSSION

The present investigation provides first extensive data on the morphology, identification and developmental biology of *B. tabaci* (Asia II 5) and *T. vaporariorum* from North-Western Himalayan region of India. *B. tabaci* (Asia II 5) and *T. vaporariorum* are known as major insect pests of vegetable crops and they act as vectors of several viral diseases. Different stages of whiteflies are often intercepted in quarantine and encountered in field conditions which require immediate decision making to hold the pest from causing further damage. Comparative studies on the morphology of various life stages and biology of both the investigated species may provide information that can be useful for their quick identification. The necessity of identification of the harmful whitefly species *B. tabaci* and *T. vaporariorum* that comes with imported plant materials was emphasized in [25].

Though elaborate description and illustrations of the developmental stages of *B. tabaci* and *T. vaporariorum* based on dorsal surface, colour and size had been given in [26], some of the important characters such as the antennae, tracheal folds and caudal furrow were neglected in the study. These characters were utilized for description by [27], but the study was limited only to *B. tabaci*. Electron microscopic study of the morphology of eggs, larvae and puparium of *T. vaporariorum* was done by [28] but their study did not present the morphology of all instars in detail. In the present study, efforts have been made to include all possible characters such as the antennae, dorsal setae, body size, colour, tracheal fold and marginal setae for description which were neglected in the previous studies. The results of the morphological studies revealed that the size of immature stages was larger in *T. vaporariorum* as compared to *B. tabaci* (Asia II 5). A considerable variation in the size of the immature stages of these two whitefly species was also mentioned in [29]. Several morphological characters for identification of *B. tabaci* and *T. vaporariorum* were used in [30], but the most reliable characters such as the number of marginal setae of first instars and presence of dorsal setae (cephalic and abdominal) in the second instar were inadequately described. The present study has elaborately described these characters. The number of marginal setae of first instars was found to be 16 and 17 in *B. tabaci* (Asia II 5) and *T. vaporariorum*,

respectively. In second instar, both cephalic and abdominal setae were present in *T. vaporariorum* while absent or poorly developed in *B. tabaci* (Asia II 5).

Phenotypic plasticity is found in *B. tabaci* [31-35] and it occurs due to the leaf surface topography and population density. Difference in the dorsal setal arrangement in the present investigation may be attributed to the development on different host surfaces. These variations are significant and needs to be taken into consideration during the identification process. Phenotypic variations of *B. tabaci* and *B. afer* was studied by [36] and corroborated that the variations were complex in the latter species.

In whitefly taxonomy, more emphasis is given to the morphology of puparium and the adult morphology were often ignored due to less morphological variation [37]. The present study has peeped into it and observed that both male and female adults of *T. vaporariorum* and *B. tabaci* (Asia II 5) can be distinguished by size, wing appearance, eyes and antennae. *T. vaporariorum* is larger than *B. tabaci* (Asia II 5) and the wing is held back flatly in a triangular manner in *T. vaporariorum* at the resting position while it is kept in acute angle with linear manner in *B. tabaci* (Asia II 5). These results confirm with the description given by [38]. Mitochondrial DNA markers and morphological characters such as the number of ommatidia connecting the upper and lower compound eyes, position, shape and size of the sensorial cone on the antenna were taken for comparing six whitefly species including *B. tabaci* and *T. vaporariorum* by [39]. The upper and lower compound eyes in *B. tabaci* were connected by one ommatidia, whereas the upper and lower compound eyes were completely disconnected from each other. Same morphological features were revealed in the present study as well. Similarly, 6<sup>th</sup> pit sensorium of the antennae was present in *B. tabaci* (Asia II 5) while absent in *T. vaporariorum* these results were also supported by [40]. Although several studies were conducted individually on *B. tabaci* or *T. vaporariorum*, only a few [26] were conducted as comparative studies. Comparative morphology of *B. tabaci* and *T. vaporariorum* was studied by [41], but adult morphology was not described and illustrated and the study did not cover the biology of both whiteflies. Therefore, the present study has combined both morphological and biological aspects.

The developmental period for *B. tabaci* (Asia II 5) and *T. vaporariorum* was much similar to [42] and [43], working on the tomato plants at 25 °C. Development time of 23.6 days for *B. tabaci* on cotton at 25 °C was reported by [44], a value which is nearly equal to the present study at the same temperature. On the other hand, developmental time of *T. vaporariorum* on tomato was slightly longer than the duration of 25 days at 25 °C reported by [45]. For both whiteflies, the duration of the egg stage was not affected by the host plants. As the egg stage of the whiteflies does not take nutrients from the host, it was not affected by the host characteristics [46]. This assertion is in contradiction with the results of [47], which ascertained the pedicel of egg absorbs the solute and water from leaf surface. The longevity found for *B. tabaci* on tomato plants was not in agreement with the findings of [48]. Cultivar/genotype used for the investigation could have influenced the longevity as also reported by [49]. On the other hand, the longer longevity of *T. vaporariorum* in comparison to *B. tabaci* found on tomato plants was in line with the findings of [50]. *B. tabaci* and *T. vaporariorum* have different preferences for tomato that results in differences on their biotic potential. The total fecundity obtained for *B. tabaci* (Asia II 5) (73.33 eggs/female) reared on tomato showed low values when compared to 114 eggs/female at 25°C reported by [51]. In case of *T. vaporariorum*, total fecundity (118 eggs/female) reared on tomato was much similar to the finding of [52] (121.6 eggs/female). The fecundity was usually very variable and depends, among other factors, on age and temperature and also on the host plant. Similarly, egg hatching and adult emergence were found to be maximum in *T. vaporariorum*, as compared to *B. tabaci* (Asia II 5). This finding is similar with [53] and the variations are due to the fact that the insects prefer to lay their eggs on hosts that provide the best conditions for their offspring's nutrition and growth. *T. vaporariorum* had the higher survival rate (81.44%) in comparison to *B. tabaci* (Asia II 5) (78.09%), which was also confirmed by [54]. Survival rates are determined by antibiosis associated with lower quality foliage and the presence of anti-nutritional compounds or harmful secondary metabolites [55, 56].

The present study provides a comprehensive understanding of the biology of two whitefly species which are notorious as they damage the crops directly by feeding and indirectly by transmitting devastating plant viruses. Further, this study gains its importance as it gives a comprehensive knowledge of the biology and morphology of two whitefly species and fills the knowledge gap that has existed for decades. However, future studies are much needed on taking this information further to nullify the effect caused by the whiteflies on agriculture.

## CONCLUSION

From the present study, it can be concluded that marginal setae, abdominal setae, vasiform orifice, lingula and antennae were more reliable characteristics for the identification and differentiation of both species during the developmental stage, while body colour, size, shape and wing span are the morphological characteristics for adult identification in field conditions. The tomato crop was found more suitable for the growth and development of *T. vaporariorum* as compared to *B. tabaci* (Asia II 5). As a result, this research could aid in the early detection and monitoring of viral disease vectors in the North-western Himalayan region.

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## REFERENCES

1. Polston JE, Anderson PK. The emergence of whitefly-transmitted gemini viruses in tomato in the western hemisphere. *Plant Dis.* 1997;81:1358-69.
2. Varma A, Malathi VG. Emerging geminivirus problems: a serious threat to crop production. *Ann. Appl. Biol.* 2003;142:145-64.
3. Hu J, De Barro P, Zhao H, Wang J, Nardi F, Liu SS. An extensive field survey combined with a phylogenetic analysis reveals rapid and widespread invasion of two alien whiteflies in China. *PLoS One.* 2011; 6:e16061.
4. Colvin J, Omongo CA, Govindappa MR, Stevenson PC, Maruthi MN, Gibson G, et al. Host-plant viral infection effects on arthropod-vector population growth, development and behaviour: management and epidemiological implications. *Adv. Virus Res.* 2006;67:419-52.
5. Prijovic M, Marčić D, Drobnjaković T, Međo I, Perić P. Life history traits and population growth of greenhouse whitefly on different tomato genotypes. *Pesticid. Phytomed.* 2013;28:239-45.
6. Navas-Castillo J, Fiallo-Olivé E, Sánchez-Campos S. Emerging virus diseases transmitted by whiteflies. *Annu. Rev. Phytopathol.* 2003;49:219-48.
7. Martin JH, Mound LA. An annotated checklist of the world's whiteflies (Insecta: Hemiptera: Aleyrodidae). *Lista de las moscas blancas del mundo (Insecta: Hemiptera: Aleyrodidae).* *Zootaxa.* 2007;1492:1-84.
8. Polston JE, De Barro P, Boykin LM. Transmission specificities of plant viruses with the newly identified species of the *Bemisia tabaci* species complex. *Pest Manag. Sci.* 2014;70:1547-52.
9. Jones DR. Plant viruses transmitted by whiteflies. *Eur. J. Plant Pathol.* 2003;109:195-219.
10. Gilbertson RL, Batuman O, Webster CG, Adkins S. Role of the insect supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. *Annu. Rev. Virol.* 2015;2:67-93.
11. Roditakis E, Roditakis NE, Tsagkarakou A. Insecticide resistance in *Bemisia tabaci* (Homoptera: Aleyrodidae) populations from Crete. *Pest Mgmt. Sci.* 2005;61:577-82.
12. Firdaus S, Vosman B, Hidayati N, Jaya Supena ED, Visser RGF, Van Heusden AW. The *Bemisia tabaci* species complex: additions from different parts of the world. *Insect Sci.* 2013;20:723-33.
13. Boykin LM, De Barro PJ. A practical guide to identifying members of the *Bemisia tabaci* species complex: And other morphologically identical species. *Front. Ecol. Evol.* 2014;2:45.
14. Kanakala S, Ghanim M. Advances in the genomics of the whitefly *Bemisia tabaci*: An insect pest and a virus vector. In: Raman C, Goldsmith MR, Agunbiade TA, editors. *Short Views on Insect Genomics and Proteomics.* Basel, Switzerland: Springer; 2015. p.19-40.
15. Brown JK. The *Bemisia tabaci* complex: genetic and phenotypic variability drives begomovirus spread and virus diversification. *Plant Dis. APSNet*; 2007 [cited 2021 Jul 9]. Available from: <http://www.apsnet.org/online/feature/btabaci>
16. Van der Vlugt, RAA, Verbeek M, Dullemans AM, Wintermantel WM, Cuellar WJ, Fox A, Thompson, JR. *Torradoviruses.* *Ann. Rev. Phytopath.* 2015;53:485-512.
17. Longhurst C, Babcock JM, Denholm I, Gorman K, Thomas, JD, Sparks TC. Cross-resistance relationships of the sulfoximine insecticide sulfoxaflor with neonicotinoids and other insecticides in the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum*. *Pest Manag. Sci.* 2013;69:809-13.
18. Saikia AK, Muniyappa V. Epidemiology and control of tomato leaf curl virus in Southern India. *Tropical Agric.* 1989;66:350-4.
19. Kalloo G. Leaf curl virus of tomato and chili in India. *Proceedings of the Phase I Final Workshop of the South Asian Vegetable Research Network*; 1996 Jan 23-28; Shanhua, Tainan, Taiwan: AVRDC Publication, 1997.

20. Avidov Z. Bionomics of the tobacco whitefly (*Bemisia tabaci*, Gennad.) in Israel. *Kiavim*. 1969;1(1):25-41.
21. Wintermantel WM, Wisler GC. Vector specificity, host range, and genetic diversity of tomato chlorosis virus. *Plant Dis*. 2006;90:814-9.
22. Simon C, Frati F, Beckenbach A, Crespi B, Liu, H, Flook, P. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomol. Soc. Ame*. 1994;87:651-701.
23. Scott IAW, Workman PJ, Drayton GM, Burnip GM. First record of *Bemisia tabaci* biotype Q in New Zealand. *New Zealand Plant Prot*. 2007;60:264-70.
24. Gill CK, Rataul HS. Leaf cage for studies on cotton whitefly *Bemisia tabaci* (Gennadius). *Indian J. Entomol*. 1994;56(2):201-3.
25. European and Mediterranean Plant Protection Organization. Diagnostic protocols for regulated pests -*Bemisia tabaci*; 2004;34:281-8.
26. Hill BG. A morphological comparison between two species of whitefly, *Trialeurodes vaporariorum* (Westw.) And *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) which occur on tobacco in the Transvaal. *Phytophytactica*. 1969;1:127-46.
27. Chaubey R, Naveen NC, Ramamurthy VV. An illustrated taxonomic diagnosis of *Bemisia tabaci* (Gennadius) with additional notes on its life stages. *Indian J. Entomol*. 2010;72(4):321-5.
28. Oana P, Pop D, Cuc G, Oros S, Oltean I, Bunescu H, et al. Studies regarding the whitefly external morphology of the egg and larvae (*Trialeurodes vaporariorum* Westw.). *Bull. USAMV-CN*. 2007;63:249-53.
29. Chaubey R, Andrew RJ, Naveen NC, Rajagopal R, Ahmad B, Ramamurthy VV Morphometric Analysis of Three Putative Species of *Bemisia tabaci* (Hemiptera: Aleyrodidae) Species Complex from India. *Ann. Entomol. Soc. Am*. 2015;108(4):600-12.
30. Malumphy, Christopher, Walsh, Katherine, Suarez, Belen, Collins, Dominique W, Boonham, N. Morphological and molecular identification of all developmental stages of four whitefly species (Hemiptera: Aleyrodidae) commonly intercepted in quarantine, *Zootaxa*. 2009;29:18-23.
31. Azab AK, Megahed MM, El-Mirsawi HD. Effect of degree of pubescence of host-plant on the number and distribution of dorsal spines in pupa of *Bemisia tabaci*. *Bull. Soc. Entomol*. 1969;53:353-7.
32. David BV, Ananthakrishnan TN. Host correlated variation in *Trialeurodes rara* Singh and *Bemisia tabaci* (Gennadius). *Current Science*. 1976;45:223-5.
33. Harakly FA. Variation in pupae of *Bemisia tabaci* bred on different hosts. *Bull. Soc. Entomol*. 1973;57:407-412.
34. Mohanty AK, Basu AN. Effect of host plants and seasonal factors on intraspecific variations in pupal morphology of the whitefly vector, *Bemisia tabaci*. *J. Entomol. Res*. 1986;10:19-26.
35. Neal JW, Bentz, JA. Evidence for the stage inducing phenotypic plasticity in pupae of the polyphagous whiteflies *Trialeurodes vaporariorum* and *Bemisia argentifolii* (Homoptera: Aleyrodidae) and the raison d'etre. *Ann. Entomol. Soc. Am*. 1999;92(6):774-87.
36. Martin NA, editor. Whitefly: biology, identification and life cycle. *Crop and Food Research Broadsheet*. 1999;90:1-8.
37. Mound, LA, Halsey SH. Whitefly of the world. A systematic catalogue of the Aleyrodidae (Homoptera) with host plant and natural enemy data. Chichester, London, United Kingdom: British museum (natural History) and Willey; 1978. 340 p.
38. Ribak G, Dafni E, Gerling D. Whiteflies stabilize their take-off with closed wings *J. Exp. Biol*. 2016;219(11): 1639-48.
39. Calvert LA, Cuervo M, Arroyave JA, Louis M, Constantino, Bellotti A, et al. Morphological and Mitochondrial DNA Marker Analyses of Whiteflies (Homoptera: Aleyrodidae) Colonizing Cassava and Beans in Colombia. *Ann. Entomol. Soc. Am*. 2001;94(4):512-519.
40. Baig MM, Dubey AK, Ramamurthy VV. Biology and morphology of life stages of three species of whiteflies (Hemiptera: Aleyrodidae) from India. *Pan-Pacific Entomologist*. 2015;91(2):168-83.
41. Rodríguez-López MJ, Garzo E, Bonani JP, Fereres A, Fernández MR, Moriones E. Whitefly resistance traits derived from the wild tomato *Solanum pimpinellifolium* affect the preference and feeding behavior of *Bemisia tabaci* and reduce the spread of Tomato yellow leaf curl virus. *Phytopathology*. 2011;101:1191-201.
42. Ahn KS, Lee KY, Choi MH, Kim JW, Kim GH. Effect of temperature and host plant on development and reproduction of the sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae). *Korean J. Appl. Entomol*. 2001;40:203-9.
43. Bonato O, Lurette A, Vidal C, Fargues J. Modelling temperature-dependent bionomics of *Bemisia tabaci* (Q-biotype). *Physiol. Entomol*. 2007;32:50-5.

44. Bethke JA, Paine TD, Nuessly GS. Comparative biology, morphometrics, and development of two populations of *Bemisia tabaci* on cotton and poinsettia. *Ann. Entomol. Soc. Am.* 1991;84:407-11.
45. Movasaghi S, Zarabi M, Asemay N, Salehi B. Comparative bio-ecological studies of greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood) (Hom.: Aleyrodidae) on cucumber and tomato. *Hexapoda.* 2009;17: 76-9.
46. Sa´nchez A, Geraud-Pouey F, Esparza D. Biología de la mosca blanca del tabaco, *Bemisia tabaci* (Homoptera: Aleyrodidae) y potencial para desarrollar sus poblaciones sobre cinco especies de plantas hospederas. *Rev Fac Agron.* 1997;14(2):193-206.
47. Lida K, Kitamura T, Honda K. Comparison of egg-hatching rate, survival rate and development time of the immature stage between biotypes B and Q of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) on various agricultural crops. *Appl. Entomol. Zool.* 2009;44:267-73.
48. Coudriet DL, Prabhaker N, Kishaba AN, Meyerdirk DE. Variation in developmental rate on different hosts and overwintering of sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae). *Environ. Entomol.* 1985;14:516-9.
49. Boica AL, Campos ZR, Lourencao AL, Campos AR. Adult attractiveness and oviposition preference of *Bemisia tabaci* (Genn.) (Homoptera: Aleyrodidae) B biotype in cotton genotypes. *Sci. Agric.* 2007;64:147-51.
50. Van de Merendok S, Van Lenteren JC. Determination of mortality of greenhouse whitefly *Trialeurodes vaporariorum* (Westwood) (Homoptera: Aleyrodidae) eggs, larvae, and pupae on four host-plant species: eggplant (*Solanum melongena* L.), Cucumber (*Cucumis sativus* L.), Tomato (*Lycopersicon esculentum* L.), and Paprika (*Capsicum annuum* L.). *Meded. Fac. Landbouww. Rijksuniv.* 1978;43:421-9.
51. Yang TC, Chi H. Life tables and development of *Bemisia argentifolii* (Homoptera: Aleyrodidae) at different temperatures. *J. Econ. Entomol.* 2006;3:691-8.
52. Lorenzo ME, Grille G, Basso C, Bonato O. Host preferences and biotic potential of *Trialeurodes vaporariorum* and *Bemisia tabaci* (Hemiptera: Aleyrodidae) in tomato and pepper. *Arth. Plant Int.* 2016;10:293-301.
53. Nombela G, Beitia F, Muñiz M. Variation in tomato host response to *Bemisia tabaci* (Hemiptera: Aleyrodidae) in relation to acyl sugar content and presence of the nematode and potato aphid resistance gene Mi. *Bull. Entomol. Res.* 2000;90:161-7
54. Enkegaard A. The poinsettia strain of the cotton whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae), biological and demographic parameters on poinsettia (*Euphorbia pulcherima*) in relation to temperature. *Bull. Entomol. Res.* 1993;83:535-46.
55. Liu TX, Oetting RD. Oviposition preference of *Bemisia tabaci* (Gennadius) on eight species of greenhouse grown plants. *J. Agric. Entomol.* 1994;11:177-9.
56. Byrne DN, Bellows TS. Whitefly biology. *Annu. Rev. Entomol.* 1991;36:431-57.



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