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## Lifecycle, Biology, and Descriptions of Greenhouse Biological Control Agent, Nesidiocoris tenuis (Reuter, 1895) (Hemiptera: Miridae)

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The plant bug *Nesidiocoris tenuis* (Reuter, 1895) is used as a biological control agent for various crops such as tomato and paprika under greenhouse conditions. In this study, all nymphal stages and male and female genitalia of *N. tenuis* are described and illustrated. Additionally, brief biological information through rearing, including life cycle and feeding habits, is also provided along with photographs.

Key words: Biological control agent, Description, Life cycle, Miridae, Nesidiocoris tenuis

#### INTRODUCTION

Nesidiocoris tenuis (Reuter, 1895), belonging to the plant bug family Miridae (Hemiptera: Heteroptera: Miridae: Bryocorinae: Dicyphini), is widely distributed in the Palaearctic and Mediterranean regions (Kerzhner and Josifov, 1999; Sanchez et al., 2008). This polyphagous mirid is commonly found on various plants in a variety of natural ecosystems (Alomar and Albajes, 1996; Perdikis and Lykouressis, 2004; Sanchez et al., 2004), and has a wide range of host plants, including some greenhouse crops (Sanchez et al., 2008). Therefore, previous reports classified this species as an insect pest, as it can damage crops by directly feeding on host plants such as tomato and pepper (Schuh and Slater, 1995; Yasunaga, 2000; Wheeler, 2001; Arnó et al., 2009; Wheeler, 2000a). Conversely, in many countries, this species has recently been used as a biological control agent in agro-ecosystems, in order to control major greenhouses insect pests such as whiteflies [Bemisia tabaci (Gennadius, 1889), Trialeurodes vaporariorum (Westwood, 1856)], aphids [Macrosiphum euphorbiae (Thomas, 1878)], thrips [Frankliniella occidentalis (Pergande, 1895)], and moths [Spodoptera exigua (Hübner, 1808)], as this species is zoophytophagous, with predatory-preference feeding habits (Wheeler, 2001; Sanchez et al., 2008; Wheeler, 2000b; Bueno and van Lenteren, 2012; Urbaneja et al., 2012; Lins Jr et al., 2014). However, this tiny bug (mainly found in its nymph form) is often difficult to identify, leading to problems with the development and maintenance of an effective quarantine system for use during the import and export of agricultural products and nurseries (Yasunaga, 2000). N. tenuis is considered to have been introduced accidentally to multiple regions via agri-

#### MATERIALS AND METHODS

To observe morphological characters, photographs and illustrations of examined specimens were taken by a Leica M165C microscope. Measurements were taken using an equipped software program with the same microscope. All measurements are given in millimeters (mm). For observation of the male genitalia, genital segment of each specimen was detached, and then soaked and boiled in 10% KOH solution at 70°C at 10-20 minutes until it became transparent. After being placed distilled water, genitalia and parameres were dissected and examined. Terminology follows mainly those of Yasunaga (1991), Braimah et al. (1982) and Yasunaga and Schwartz (2007). To describe morphological characters of each nymphal stage and to observe biological characteristics, nymphs and adults were bred with host plant (Lycopersicon esculentum, Capsicum annuun var. angulosum; Solanaceae) by feeding aphids (Aphis gossypii Glover, 1877) as prey in a cage (width: 400 mm; length: 400 mm; height: 550 mm) at 26°C and a photoperiod of 16:8 (L:D) hours in an insect breeding room. Then, photographs of life cycle and biology were taken by a Nikon D300 camera.

## RESULTS

**Taxonomy**Nesidiocoris tenuis (Reuter, 1895)
Cyrtopeltis tenuis Reuter, 1895: 139.
Nesidiocoris tenuis Kerzhner, 1988: 67.

cultural trade (Yasunaga, 2000), because this bug will hide in narrow spaces and oviposits deep in plant tissues. To prevent accidental introduction, it is necessary to identify individuals accurately (including eggs and nymphs) properly, using morphological characters and biological information. Therefore, in this study all nymphal stages are described, and genitalic structures of the male and female adults are illustrated for the first time. Additionally, biological information such as life cycle, oviposition traces, and feeding habits is also provided, along with relevant photographs.

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## Morphology DESCRIPTION

#### First instar (Fig. 1A)

Body generally oval, length 1.41–1.43. COLORATION: generally greenish brown. Head: yellowish green; compound eyes dark brown; tylus slightly brown; antennae generally pale brown, middle part of first antennal segment dark brown, base of second and third antennal segment dark brown; rostrum generally brown, 1/2 apical part of fourth rostral segment dark brown. Thorax: almost yellowish green, both sides slightly yellow; legs almost pale brown, third tarsal segment dark brown. **Abdomen**: almost yellowish green. SURFACE AND VESTITURE: glossy, head and pronotum covered with sparse pubescences; tibiae with two rows of erect setae, abdomen covered with dense pubescences. STRUCTURE: Head: relatively bigger than pronotum; ocelli absent; vertex width longer than first antennal segment; antennae somewhat long and slender, proportion of first to fourth antennal segments 0.1:0.1:0.2:0.2; rostrum reaching to hindcoxae, proportion of first to fourth rostral segments 0.1:0.1:0.1:0.2. Thorax: pronotum almost rectangle with slightly rounded lateral margins; scutellum absent; wing pads not developed; legs generally slender. **Abdomen**: rounded, apical segment of abdomen slightly protuberant.

**MEASUREMENTS**: (n=3): Body length, tylus–apex of abdomen: 1.41-1.43; head length, excluding collar: 0.32-0.33; head width, including compound eyes: 0.28-0.29; vertex width: 0.19-0.20; 1st antennal segment length: 0.11-0.12; 2<sup>nd</sup> antennal segment length: 0.16-0.18; 3<sup>rd</sup> antennal segment length: 0.22-0.23; 4th antennal segment length: 0.25-0.26; total antennal length: 0.74-0.79; 1st rostral segment length: 0.12-0.13; 2nd rostral segment length: 0.14–0.15; 3<sup>rd</sup> rostral segment length: 0.14–0.15; 4th rostral segment length: 0.20-0.21; total rostral segment length: 0.60-0.64; anterior pronotal margin width (straight): 0.23-0.24; mesal pronotal length: 0.18-0.29; basal pronotal margin width (straight): 0.29-0.30; foreleg (femur: tibia: tarsus): 0.37-0.38: 0.34-0.35: 0.23-0.24; midleg (femur: tibia: tarsus): 0.33-0.34: 0.39-0.40: 0.24-0.25; hindleg (femur: tibia: tarsus): 0.44-0.45: 0.50-0.52: 0.32 - 0.34.

## Second instar (Fig. 1B)

Body generally oval, length 1.74–1.76. COLORATION: generally greenish brown. **Head**: yellowish green; compound eyes dark brown; tylus slightly brown; antennae generally pale brown, middle part of first antennal segment dark brown, base and apex of second and third antennal segment dark brown; rostrum generally pale brown, 1/3 apical part of fourth rostral segment dark brown. **Thorax**: almost yellowish green; legs almost pale brown, third tarsal segment dark brown. **Abdomen**: almost yellowish green. SURFACE AND VESTITURE: glossy, head and pronotum covered with sparse pubescences; tibiae with two rows of erect setae, abdomen covered with somewhat dense pubescences. STRUCTURE: **Head**: relatively as big as pronotum; ocelli absent; vertex width longer than first antennal segment; antennae

somewhat long and slender, proportion of first to fourth antennal segments 0.1:0.3:0.3:0.3; rostrum reaching to hindcoxae, proportion of first to fourth rostral segments 0.1:0.1:0.1:0.2. **Thorax**: pronotum almost trapezoid; scutellum absent; forewing pad slightly developed; legs generally slender. **Abdomen**: rounded, apical segment of abdomen slightly protuberant.

**MEASUREMENTS**: (n=3): Body length, tylus–apex of abdomen: 1.74-1.76; head length, excluding collar: 0.34-0.35; head width, including compound eyes: 0.35-0.37; vertex width: 0.32-0.34; 1st antennal segment length: 0.15-0.16;  $2^{nd}$  antennal segment length: 0.30-0.32;  $3^{rd}$ antennal segment length: 0.37-0.38; 4th antennal segment length: 0.33-0.34; total antennal length: 1.15-1.20; 1<sup>st</sup> rostral segment length: 0.15–0.16; 2<sup>nd</sup> rostral segment length: 0.14–0.16; 3<sup>rd</sup> rostral segment length: 0.12–0.13; 4<sup>th</sup> rostral segment length: 0.18–0.19; total rostral segment length: 0.59-0.64; anterior pronotal margin width (straight): 0.27-0.28; mesal pronotal length: 0.27-0.29; basal pronotal margin width (straight): 0.39-0.40; foreleg (femur: tibia: tarsus): 0.38-0.39: 0.40-0.41: 0.32-0.33; midleg (femur: tibia: tarsus): 0.38-0.39: 0.49-0.51: 0.31-0.32; hindleg (femur: tibia: tarsus): 0.51-0.53: 0.56-0.58: 0.38 - 0.39.

## Third instar (Fig. 1C)

Body suboval, length 2.02–2.05. COLORATION: generally greenish brown. Head: yellowish green; compound eyes dark brown; tylus slightly brown; antennae generally pale brown, middle part of first antennal segment dark brown, base and apex of second and third antennal segment dark brown; rostrum generally pale brown, 1/3 apical part of fourth rostral segment dark brown. **Thorax**: almost yellowish green, both sides slightly yellowish green; legs almost pale brown, third tarsal segment dark brown. Abdomen: almost yellowish green. SURFACE AND VESTITURE: glossy, head and pronotum covered with sparse pubescences; tibiae with two rows of erect setae, abdomen covered with somewhat sparse pubescences. STRUCTURE: Head: relatively as big as pronotum; ocelli absent; vertex width longer than first antennal segment; antennae somewhat long and slender, proportion of first to fourth antennal segments 0.1:0.3:0.3:0.3; rostrum reaching to hindcoxae, proportion of first to fourth rostral segments 0.2:0.2:0.2:0.2. Thorax: pronotum almost trapezoid; scutellum absent; hindwing pad slightly developed; legs generally slender. Abdomen: rounded, apical segment of abdomen slightly protuberant.

**MEASUREMENTS**: (n=3): Body length, tylus–apex of abdomen: 2.02–2.05; head length, excluding collar: 0.37–0.39; head width, including compound eyes: 0.35–0.37; vertex width: 0.22–0.24; 1<sup>st</sup> antennal segment length: 0.15–0.16; 2<sup>nd</sup> antennal segment length: 0.30–0.33; 3<sup>nd</sup> antennal segment length: 0.37–0.39; 4<sup>th</sup> antennal segment length: 1.15–1.22; 1<sup>st</sup> rostral segment length: 0.20–0.21; 2<sup>nd</sup> rostral segment length: 0.21–0.22; 3<sup>nd</sup> rostral segment length: 0.18–0.20; 4<sup>th</sup> rostral segment length: 0.24–0.25; total rostral segment length: 0.83–0.88; anterior pronotal margin width (straight): 0.27–0.29; mesal pronotal length: 0.27–0.29;

basal pronotal margin width (straight): 0.40–0.42; foreleg (femur: tibia: tarsus): 0.39–0.40: 0.51–0.52: 0.33–0.35; midleg (femur: tibia: tarsus): 0.42–0.43: 0.55–0.57: 0.33–0.35; hindleg (femur: tibia: tarsus): 0.52–0.54: 0.79–0.82: 0.40–0.42.

#### Fourth instar (Fig. 1D)

Body elongated oval, length 2.71-2.75. COLORATION: generally greenish brown. **Head**: yellowish green; compound eyes dark brown; sometimes base of tylus slightly brown; antennae generally pale brown, middle part of first antennal segment dark brown, base and apex of second and third antennal segment dark brown; rostrum generally pale brown, 1/3 apical part of fourth rostral segment dark brown. Thorax: almost yellowish green, both sides slightly yellowish green; legs almost pale brown, third tarsal segment dark brown. Abdomen: almost yellowish green. SURFACE AND VESTITURE: glossy, head and pronotum covered with sparse pubescences; tibiae with two rows of erect setae, abdomen covered with sparse short pubescences. STRUCTURE: Head: relatively as big as pronotum; ocelli absent; vertex width longer than first antennal segment; antennae long and slender, proportion of first to fourth antennal segments 0.2:0.4: 0.5:0.4; rostrum reaching to hindcoxae, proportion of first to fourth rostral segments 0.3:0.3:0.2:0.2. **Thorax**: pronotum almost trapezoid; scutellum absent; hindwing pad well developed; legs generally slender. Abdomen: rounded, apical segment of abdomen slightly protuberant.

**MEASUREMENTS**: (n=3): Body length, tylus–apex of abdomen: 2.71-2.75; head length, excluding collar: 0.40-0.42; head width, including compound eyes: 0.45-0.47; vertex width: 0.26–0.28; 1st antennal segment length: 0.20-0.21;  $2^{nd}$  antennal segment length: 0.46-0.49;  $3^{rd}$ antennal segment length: 0.53–0.55; 4<sup>th</sup> antennal segment length: 0.41-0.43; total antennal length: 1.60-0.68; 1st rostral segment length: 0.30-0.32; 2<sup>nd</sup> rostral segment length: 0.24–0.25; 3<sup>rd</sup> rostral segment length: 0.22–0.23; 4th rostral segment length: 0.27-0.28; total rostral segment length: 1.03-1.08; anterior pronotal margin width (straight): 0.35-0.37; mesal pronotal length: 0.36-0.38; basal pronotal margin width (straight): 0.57–0.59; foreleg (femur: tibia: tarsus): 0.54-0.56: 0.55-0.58: 0.34-0.35; midleg (femur: tibia: tarsus): 0.54-0.56: 0.62-0.64: 0.38-0.40; hindleg (femur: tibia: tarsus): 0.77-0.79: 1.18-1.24: 0.49 - 0.53.

## Fifth instar (Fig. 1E)

Body elongated oval, length approximately 2.99–3.02. COLORATION: generally greenish brown. **Head**: yellowish green; compound eyes dark brown; sometimes base of tylus slightly brown; antennae generally pale brown, middle part of first antennal segment dark brown, base and apex of second and third antennal segment dark brown; rostrum generally pale brown, 1/3 apical part of fourth rostral segment dark brown. **Thorax**: almost yellowish green; legs almost pale brown, third tarsal segment dark brown. **Abdomen**: almost yellowish green. SURFACE AND VESTITURE: glossy, head and pronotum covered with sparse pubescences; tibiae with two rows

of erect setae, posterior part of abdomen covered with sparse short pubescences. STRUCTURE: **Head**: relatively as big as pronotum; ocelli absent; vertex width longer than first antennal segment; antennae long and slender, proportion of first to fourth antennal segments 0.2:0.4: 0.5:0.4; rostrum reaching to hindcoxae, proportion of first to fourth rostral segments 0.3:0.3:0.2:0.2. **Thorax**: pronotum almost trapezoid; scutellum absent; wing pads elongated and thick; legs generally slender. **Abdomen**: rounded, apical segment of abdomen slightly protuberant.

**MEASUREMENTS**: (n=3): Body length, tylus-apex of abdomen: 2.99-3.02; head length, excluding collar: 0.44–0.46; head width, including compound eyes: 0.44– 0.46; vertex width: 0.21–0.23; 1st antennal segment length:  $0.20-0.21; 2^{nd}$  antennal segment length:  $0.46-0.48; 3^{rd}$ antennal segment length: 0.55-0.57; 4th antennal segment length: 0.41-0.42; total antennal length: 1.62-1.68; 1<sup>st</sup> rostral segment length: 0.30–0.31; 2<sup>nd</sup> rostral segment length: 0.29-0.30; 3<sup>rd</sup> rostral segment length: 0.20-0.21; 4<sup>th</sup> rostral segment length: 0.26–0.27; total rostral segment length: 1.05-1.09; anterior pronotal margin width (straight): 0.34–0.36; mesal pronotal length: 0.38–0.41; basal pronotal margin width (straight): 0.56-0.57; foreleg (femur: tibia: tarsus): 0.68-0.70: 0.76-0.78: 0.41-0.43; midleg (femur: tibia: tarsus): 0.72-0.74: 0.78-0.81: 0.39-0.41; hindleg (femur: tibia: tarsus): 0.97-1.03: 1.24-1.31: 0.49 - 0.53.

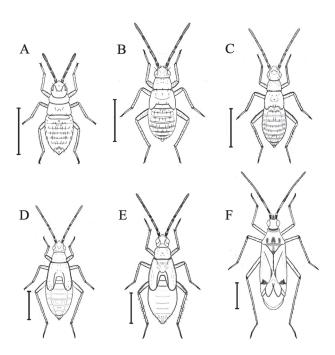
#### Adult (Fig. 1F)

Male: Body elongated oval, length 3.01-3.08. COLORATION: generally greenish brown with dark spots. **Head**: greenish brown; compound eyes dark brown; tylus entirely dark brown; antennae generally pale brown, middle part of first antennal segment dark brown, base and apex of second antennal segment dark brown, base of third antennal segment dark brown; rostrum generally pale brown, 1/2 apical part of fourth rostral segment dark brown. Thorax: almost yellowish green; carina dark brown; collar greenish pale brown; posterior part of pronotum generally brown with dark spots; mesoscutum greenish brown, scutellum pale brown, apex of scutellum dark brown; hemelytra generally greenish brown; commissure dark brown; corium tinged with dark brown, posterior part of corium with dark brown spots; membrane grayish brown with dark brown spots, vein dark brown; legs almost pale brown, base of tibiae dark brown; third tarsal segment dark brown. Abdomen: almost yellowish green. SURFACE AND VESTITURE: glossy, hemelytra covered with sparse pubescences; tibiae with two rows of erect setae. STRUCTURE: Head: relatively smaller than pronotum; ocelli absent; vertex width longer than first antennal segment; antennae somewhat long and slender, proportion of first to fourth antennal segments 0.2:0.6: 0.7:0.4; rostrum reaching to hindcoxae, proportion of first to fourth rostral segments 0.3:0.3:0.2:0.3. Thorax: pronotum almost trapezoid; scutellum equilateral; lateral margin of hemelytra straight; cuneal fracture developed; legs generally slender. Abdomen: rounded, almost reaching to base of membrane. GENITALIA: gonopore relatively short and rounded, with curved par316 J. KIM et al.

ameres (Fig. 2A–B); left paramere with slender hypophysis with setae and sensory lobe (Fig. 2A); right paramere with slender hypophysis and sensory lobe (Fig. 2B); endosoma membranous, densely with small protuberances (Fig. 2C).

Female: Body elongated oval, length 3.22–3.26. COLORATION: as in male. SURFACE AND VESTITURE: as in male. STRUCTURE: somewhat more rounded oval and larger size in overall shape than male. GENITALIA: Ovipositor with small toothes (Figs. 2D, E); posterior wall with lined membrane (Fig. 2F (arrow)); genital chamber with sclerotized rings (Fig. 2G) and median process projecting into genital chamber (Fig. 2H (arrow)) with membrane connecting to median process.

**MEASUREMENTS**: Male (n=3)/female (n=3): Body length, tylus-apex of membrane: 3.01-3.08/3.22-3.26; head length, excluding collar: 0.39-0.45/0.40-0.41; head width, including compound eyes: 0.46-0.47/0.46-0.49; vertex width: 0.16-0.19/0.19-0.20; 1st antennal segment length: 0.27-0.29/0.24-0.25; 2<sup>nd</sup> antennal segment length: 0.56–0.57/0.50–0.54; 3<sup>rd</sup> antennal segment length: 0.61-0.70/0.56-0.61; 4th antennal segment length: 0.34-0.35/0.32-0.40; total antennal length: 1.55-1.91/1.62-1.80; 1st rostral segment length: 0.25-0.34/0.30-0.33; 2nd rostral segment length: 0.31-0.34/0.30-0.32; 3<sup>rd</sup> rostral segment length: 0.23-0.25/0.24-0.26; 4th rostral segment length: 0.30-0.31/0.29-0.30; total rostral length: 1.09-1.24/1.13-1.21; anterior pronotal margin width: 0.33-0.38/0.32-0.33; mesal pronotal length: 0.41-0.43/0.37-0.39; basal pronotal width: 0.73-0.74/0.72-0.73; outer embolial margin length: 1.45-1.55/1.51-1.54; outer cuneal margin length: 0.43-0.52/0.46-0.49; maximum width across hemelytron: 0.42-0.44/0.42-0.45; foreleg (femur:

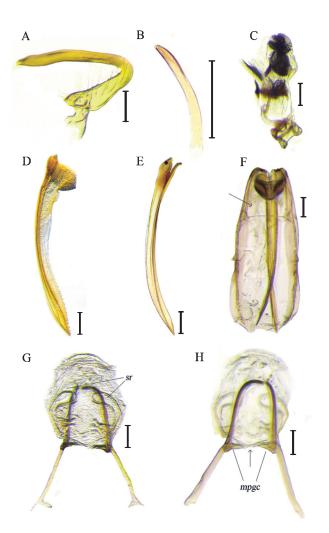


**Fig. 1.** Descriptions of nymphal stages and adult of *N. tenuis*. A: first instar; B: second instar; C: third instar; D: fourth instar; E: fifth instar; F: adult. Scale bar: 1 mm.

tibia: tarsus): 0.85-0.91: 0.82-0.90: 0.39-0.40/0.78-0.80: 0.82-0.87: 0.37-0.41; midleg (femur: tibia: tarsus): 0.88-0.89: 0.96-1.03: 0.37-0.38/0.78-0.83: 0.91-0.97: 0.39-0.40; hindleg (femur: tibia: tarsus): 1.12-1.23: 1.44-1.61: 0.44-0.50/1.09-1.10: 1.52-1.55: 0.45-0.48.

**DISTRIBUTIONS**: Asia, Australia, Europe, North Africa, North America, Pacific Islands, tropical Africa, West Indies (Kerzhner and Josifov, 1999).

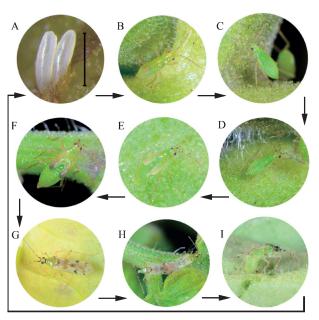
**HOSTS**: Capparidaceae (Lindberg, 1958), Euphorbiaceae (Lindberg, 1958), Solanaceae (Carvalho, 1956; Alayo, 1974), Asteraceae (Linnavuori, 1986), Pedeliaceae (Alayo, 1974).



**Fig. 2.** Parameres and genitalia of male and female of *N. tenuis*. A: left paramere; B: right paramere; C: endosoma; D–E: ovipositor in lateral view; F: posterior wall; G–H; genital chamber; *sr*: sclerotized rings; *mpgc*: median process projecting into genital chamber; arrow: membrane. Scale bar: 0.1 mm.

36°22'06"N; 127°21'10"W, 11.viii.2014, on *Humulus japonicus* Siebold & Zucc. (Cannabaceae), Junggon Kim; 1\$\mathcal{J}\$, Gyejok—san (Mt), Jang—dong, Daeduk—gu, Daejeon—si, Korea, 36°23'07"N; 127°26'22"W, 22.viii.2014, Light trap, Junggon Kim; 1\$\mathcal{J}\$, Gung—dong, Yuseong—gu, Daejeon—si, Korea, 36°22'06"N; 127°21'10"W, 12.viiii. 2014, Light trap, Junggon Kim; Compared with Japanese specimens: 1\$\mathcal{J}\$, Awa, Tokushima, Japan, 34°06'10"N; 134°17'49"W, 29.vii.2014, on *Sesamum indicum* (Pedaliaceae), K. Matsuo.

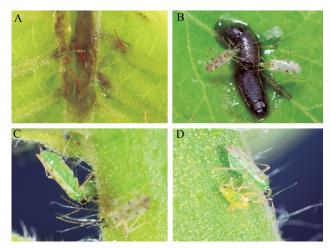
**REMARKS**: Length of each antennal segment and each rostral segment of fourth instar are approximately equal to those of fifth instar. It is thought that length of body and legs increases mainly after fourth instar.



**Fig. 3.** Life cycle of *N. tenuis*. A: eggs (Scale bar: 0.5 mm); B: first instar; C: second instar; D: third instar; E: fourth instar; F: fifth instar; G: adult; H: mating of adult; I: oviposition of female.

#### DISCUSSION

In the two host plants we used in this study, immature stages of *N. tenuis* developed into adult successfully, which was subsequently confirmed to mate and oviposit (Fig. 3). Traces of oviposition were found on the underside of young leaves (Fig. 4A). Nymphs and adult fed on their prey wandering host plants from the stem to flowers. N. tenuis fed on not only tiny prey such as aphids and whitefly but also somewhat large prey such as moth larvae (Fig. 4B). In the case of lack of prey, N. tenuis gained nutrients from host plants for survival, which indicates that they are zoophytophagous as (Fig. 4C). In addition, cannibalism was also observed in adults that had fed on fourth instar immature (Fig. 4D) as an opportunist (Wheeler, 2001). Therefore, this mirid bug is assumed to be predominantly predaceous, but it becomes injurious to plants if its prey population density is low.



**Fig. 4.** Egg and traces of oviposition and prey types of *N. tenuis*. A: egg and traces of oviposition on underside of tomato leaf (red arrows); B: *N. tenuis* feeding on moth larva; C: adult feeding on its host plant (tomato); D: adult feeding on its nymph (cannibalism).

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