Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2016; 4(6): 692-695

Received: 01-09-2016
Accepted: 02-10-2016

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Study of the biotic potential of indigenous predator *Nesidiocoris tenuis* on *Tuta absoluta* pest of geothermal culture in the south of Tunisia

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Abstract
Nesidiocoris tenuis is a predator bug spontaneously present in horticultural crops. Recently, it is used for several pests' biological control. In this work we evaluated its predator effect to control *Tuta absoluta* in geothermal waters heated tomato greenhouses, at various stages of their life cycles. Our results showed that *N. tenuis* preferentially feeds on *T. absoluta* eggs, and the predation effect significantly diminished within advanced developmental stages of the pest. In addition, L1 and L2 larvae of *T. absoluta* were the most attacked by the third nympha of *N. tenuis* (1.5 ± 0.57 and 1.25 ± 0.50. respectively) in comparison to other stages e.g. N1 0.50 ± 057 and 0.25 ± 0.50, respectively at $p \leq 0.05$). These findings spotted the importance of indigenous N. tenuis as biological agent to manage the ravaging insect *T. absoluta* in geothermal-greenhouses. The predator should be introduced, in a manner where N. tenuis third nymphs meet the L1 and L2 developmental stages of *T. absoluta*, in order to maximize the effect.

Keywords: Green houses, *Nesidiocoris tenuis*, biotic potential, *Tuta absoluta*, predation

1. Introduction

Greenhouse crops are vulnerable to fungal and viral diseases and pest attacks, due to humidity and ambient temperature. The main pests that develop on tomato are nematodes, insects and many other arthropods [1]. In Tunisia, tomato producers are facing a new serious pest, known as *Tuta absoluta* due to the extensive damage it induces [2], as well as the low efficiency of chemical pesticides [3, 4]. *T. absoluta* life cycle, is fascinatingly synchronous to which of tomato, fact that potentiated its specific induced damages coming to 100% [3].

T. absoluta is a new colonizer of Mediterranean coasts where it finds favorable conditions to proliferate [5]. It was discovered for the first time in the province of Castellon, in Spain, in 2006. Soon, this pest settled in all Mediterranean regions [6, 7]. In 2008, it was noticed in Morocco, Algeria and France (Corsica, Var and Bouches-du-Rhône) [1].

In Tunisia, it was firstly observed in the Sahel region and specifically in the towns of Akouda and Chatt-Mariem, in October 2008 [8]. The appearance of *Tuta absoluta* has motivated scientific research in order to control this pest; and various protection ways were envisaged [5]. Chemical control is often doomed to failure because of the resistance of *Tuta absoluta* to many pesticides. Hence, several auxiliaries of biological management are reported. The life cycle of this *T. absoluta* varies from 67 days at 15 °C to 21 days at 27 °C [9]. This pest is a polyvoltine specie; and gives 10 to 12 generations per year. Each female can deposit from 40 to more than 200 eggs, preferentially, on the shadowed side of leaves or tender young stems and sepals of immature fruits. After hatching, the young larvae penetrate the leaves, stems or fruits. The larvae of *T. absoluta* transform to pupa, inside galleries into the surfaces of host plants or in soil. The insect is resistant to the cold season as an egg, pupa or adult [9].

Lycopersicum esculentum is the major host of *T. absoluta,* but it also attacks potato (*Solanum tuberosum*), eggplant (*Solanum melongena*) and several other species of the same family as black nightshade (*Solanum nigrum*) and Jimson weed (*Datura stramonium*) [10]. Tomato plants can be attacked at any stage of development, from seedlings to maturity, and one larva can cause damages to many fruit in the same package [11]. To manage *T. absoluta* sex pheromone traps, with glue or water and light traps have been used; but their usefulness was limited by their difficult maintenance and expensive costs [12].

Given the risks of large-scale use of some specific chemicals on the environment and the auxiliary fauna, the biological control is sought as the best alternate processes to manage Pests [13].

N. tenuis is a predatory mirid bug that appears spontaneously in cultures. In recent years, it is sought as important biological agent of controlling pests in vegetable crops. It is a very active polyphagous predator. So, this work aims to outline the predatory effect of the indigenous N. tenuis on T. absoluta, at various developmental stages, as a way of controlling this pest.

2. Materials and Methods

2.1 *N. tenuis* breeding

N. tenuis (Hemiptera Miridae) males and females were reared in a greenhouse of tobacco plants infested by *Trialeurodes vaporariorum*, *Bemisia tabaci* and *Myzus persicae* [14. 15].

N. tenuis was firstly released in specific areas of the greenhouse where pries were largely distributed,

2.2 Protocol

2.3.1 Infested leaves' collection

Infested leaves with *T. absoluta* were distinguished by the shipshape of their galleries (leaf mines). The presence of the developmental stages of the ravaging insect has been confirmed using magnifying- glass, to not confuse *T. absoluta* with other tomato pests as the fly miner *Liriomyza spp*.

2.3.2. Leaf analysis and identification of various stages

Observation of samples was done using a binocular microscope. The transparency of mines in the leaves requires a superior and another inferior lighting.

The identification of larval stages rested on color and size. The larvae are cream color then greenish and light pink. Once the larvae is demonstrated, using a scalpel, cut a circle of 4 cm diameter surrounding the mine, have retaining ribs.

For pupae, they have cylindrical shape of 4.3 mm wide and 1.1 mm in diameter and brown in color. Pupation may take place on the ground, on the leaves or inside mines. They were usually covered by a white silky cocoon. Indeed, the pupa stage was a form of *T. absoluta* resistance at low temperatures (21 days at 15 °C). However, the weather conditions at the geothermal greenhouse (± 23 °C) shorten the length of the stadium, as the multiple positions pupae; these two factors minimize the number of pupae found in the leaves. To this end it has retained larvae stage 4 to complete the pupa stage.

For the egg stage, the identification is based on their cylindrical shape and brownish-yellow color with a soft sheen. The underside of young leaf is carefully deposited on the plate slide. pieces of infested leaves with *T. absoluta* eggs were cut using scalpel.

2.3.3 Collection of the auxiliary

To capture *N. tenuis* in the green house tobacco, the following process were followed:

The first step was to gently tap at the floral bouquet and tobacco plant leaves with a white sheet below for clear observation of the insect. During installation of the insect on the white paper, verification of eye location based on size, color, and on the presence or absence of the wings. During the first two nymphal stages nymphs were sized from 1 to 2 mm and in greenish-yellow color and with the total absence of wings. For the third stage, the nymph was in size of 3 mm and Veronese green. In the last two nymphal stages the beginning of the development of the wings were seen.

The adult was 3 to 4 mm long, green in color with black spots on the wings and legs. It has a characteristic black ring around his head. After verification, the designated stage was aspired very carefully, using a mouth aspirator. Then it was ejected in a box designed for *N. Tenuis* and brought to the laboratory.

The adopted devices were plastic containers, 14 cm wide, 25 cm long and 12 cm height. Outside of each vessel were pasted stickers, which contain the following indicators: The stages of *Tuta absoluta* (bottom), stage of *Nesidiocoris tenuis* and date (top). Inside the container was placed a filter paper soaked to support the tomato leaves humidity.

In each vessel was put 5 individuals of each stage of *Tuta* [5 eggs + 5 L1 + 5 L2+ 5L3+ 5L4 + 5 pupae]. Then, using the vacuum one of the stages of the predator was introduced. This process is repeated 4 times for each stage of *N. tenuis* [4 × 6 replicates stages]. The estimated time for the study of the performance of the predator is 48 hours. It refers to the longevity of each stage *N. tenuis* (± 2.6 days for nymphal stages and adults) and greenhouse conditions (25 °C).

2.4 Statistical Analysis

Non parametric Mann-Whitney test was used to compare the obtained results. The significance was considered at $p \leq 5\%$. In addition, spearman simple correlation was cheked to evaluate the larval predation preferences.

3. Results and discussion

Ravaging insects are responsible for important reduction of crops' production, especially under greenhouses. Because of the augmented air humidity and temperature in geothermal water- heated greenhouses these pests' development and proliferation are enhanced. As alternate to chemical pesticides, the use of natural enemies of pests is envisaged to protect crops and vegetables productivity in such conditions. Among pests, *T. absoluta* causes important damages in tomato production in Mediterranean regions. In the context of its biological management, the effectiveness of using the endogenous *N. tenuis* at various developmental stages, to predate this whitefly was evaluated. Our results showed that both adult and nymphs of *N. tenuis* feed on all developmental stages of *T. absoluta*, with great preference to eggs (total predated eggs and L1 were of 17.75 ± 0.95 and 3.25 ± 0.50, respectively with p = 0.019 (Table 1)) ; as shown by the drastic decrease of predation within advancement of the pest development (eg, spearman simple correlation showed significant reduction of the predated individual respectively by adult and the second nymph of *N. tenuis*; Rh-2 of 0.49 and 0.51, respectively (Fig. 1)). The third nymph of the predator presented the greatest effect on both L1 and L2 larvae of *T. absoluta*, (1.5 ± 0.57 and 1.25 ± 0.50 predated individuals, respectively) when compared to other nymphs and adults of *N. tenuis* (Fig. 2).

N. tenuis is a piercing- sucking insect fact that explains its preference to *T. absoluta* eggs (Fig. 3) which are immobile and have a distinguishable color. The tender cuticle of the second and third larval stages of the pest is an also a prominent factor facilitating the feeding process of the predator [13]. These findings outline the potential use of the endogenous *N. tenuis* to manage *T. absoluta* pest. It is suggested to release the predator in a manner that its third nymph coincides with the ravaging insect eggs and first and second larval stages. To confirm this hypothesis more deeper studies are envisaged.

Table 1: Total predation variation along *N. tenuis* and *T. absoluta* developmental stages.

N. tenuis	**Adult**	**N1**	**N2**	**N3**	**N4**	**N5**
Means± SD	3.75±0.95	3.5±1.29	5±1.15	6±1.15	3.75±0.95 (*)	3±0.81 (*)
T. absoluta	Eggs	L1	L2	L3	L4	pupae
Means± SD	17.75±0.95 (c,d,e,f)	3.25±0.5 (a,e,f)	2.5±1.73 (a,e,f)	1.5±1.29 (a,e,f)	00 ±00 (a,b,c,d)	00 ±00 (a,b,c,d)

a, b, c, d, e and f represent significant differences at p = 0.05 from *T. absoluta* eggs, L1, L2, L3, L4 and pupae. (*) represents significant difference in comparison to the third nymph of *N. tenuis* (L3).

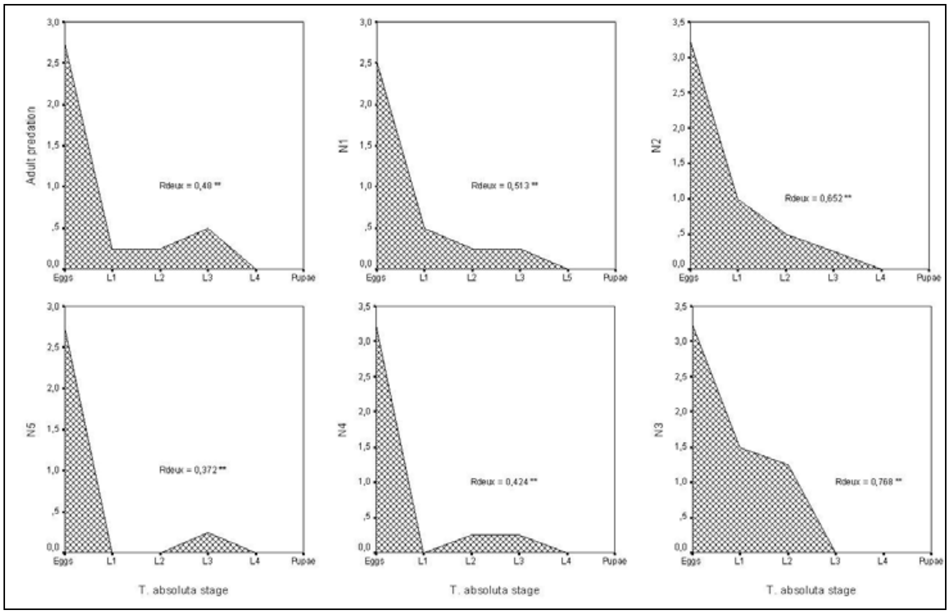

Fig 1: Mortality average of each stage *T. absoluta* by each stage of *N. tenuis*

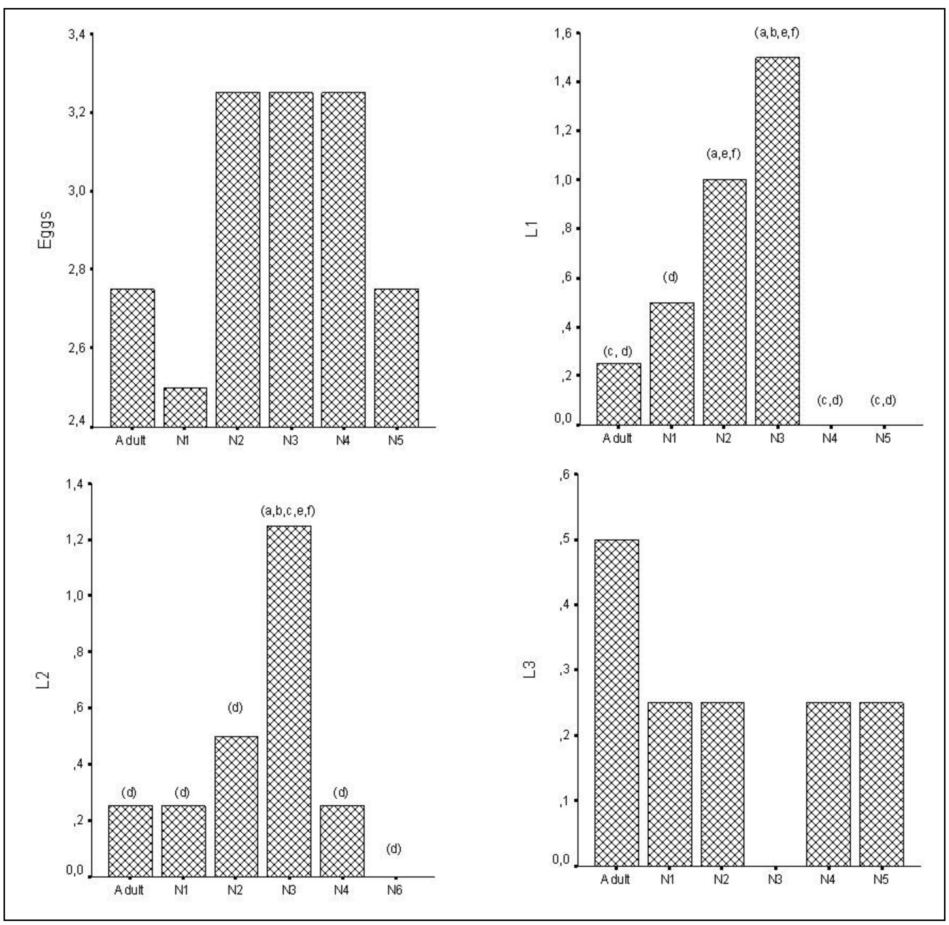

Fig 2: Predation average of each stage of *N. tenuis* by attacked stages of *T. absoluta*

4. Discussions

The predator *Nesidiocoris tenuis* is a Hemiptera with a piercing-sucking mouthpiece. It stings and sucks their prey with a rostrum. Consequently, the mortality of the host is affected by two factors which are mobility and color of prey.
Indeed it is known for its greed for eggs and larvae of *Tuta absoluta* whiteflies [13]. The female deposits her eggs *Tuta absoluta* with micropyles vertically upwards. The surface of eggs has very fine sculptures. Thus the large numbers of eggs has nutritional values and represents a free and attractive food source for predators. In the beginning of the attack the egg appears transparent (Fig.5.) and then it becomes blackish.

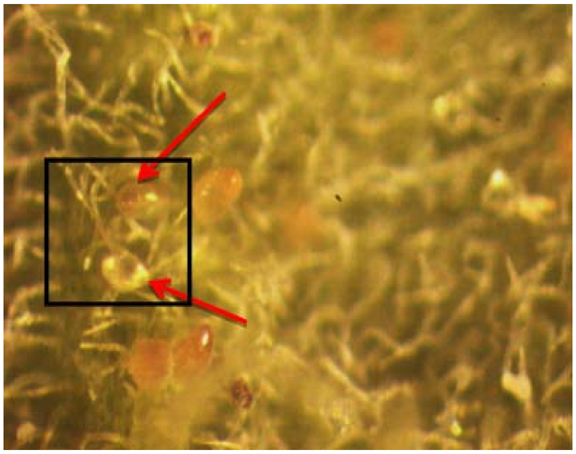

Fig 3: Two attacked eggs of *Tuta absoluta* by *Nesidiocoris Tenuis* (photo taken in DC Laboratory (IRA Kebili) 2014).

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