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Effect of the native strain of the predator *Nesidiocoris tenuis* Reuter and the entomopathogenic fungi *Beauveria bassiana* and *Lecanicillium muscarium* against *Bemisia tabaci* (Genn.) under greenhouse conditions in Tunisia

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Abstract

Background: The misuse of chemical insecticides has developed the phenomenon of habituation in the whitefly *Bemisia tabaci* (Gennadius) causing enormous economic losses under geothermal greenhouses in southern Tunisia.

Results: In order to develop means of biological control appropriate to the conditions of southern Tunisia, the efficacy of the native strain of the predator *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) and two entomopathogenic fungi (EPF) *Beauveria bassiana* and *Lecanicillium muscarium* was tested against *Bemisia tabaci* (Gennadius). Indeed, the introduction of *N. tenuis* in doses of 1, 2, 3, or 4 nymphs per tobacco plant infested by the whitefly led to highly significant reduction in the population of *B. tabaci*, than the control devoid of predator. The efficacy of *N. tenuis* was very high against nymphs and adults of *B. tabaci* at all doses per plant with a rate of 98%. Likewise, *B. bassiana* and *L. muscarium*, compared to an untreated control, showed a very significant efficacy against larvae and adults of *B. tabaci*. In addition, the number of live nymphs of *N. tenuis* treated directly or introduced on nymphs of *B. tabaci* treated with the EPF remained relatively high, exceeding 24.8 nymphs per cage compared to the control (28.6).

Conclusions: It can be concluded that the native strain of *N. tenuis* and the EPF tested separately were effective against *B. tabaci*. Their combined use appears to be possible.

Keywords: *Bemisia tabaci*, Biological control, *Nesidiocoris tenuis*, Geothermal, *Beauveria bassiana*, *Lecanicillium muscarium*

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Background

Despite its importance, the geothermal greenhouse sector faces several phytosanitary constraints, including the whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) the devastator of several agricultural crops. This insect is one of the most destructive pests in the world (Oliveiraa et al., 2001). It transmits more than 200 plant viruses (Polston et al., 2014) and causes severe economic damage to more than 60 crop plants. In southern Tunisia, this whitefly causes harmful damage to crops heated by geothermal waters (Bel Kadhi, 2004).

Control of this pest has been based primarily on the use of conventional broad-spectrum chemical pesticides. As a result, *B. tabaci* developed resistance against different groups of insecticides following their intensive use (Sain et al., 2019). In addition to marketing problems, the use of chemicals in a heated greenhouse could pose poisoning problems for farmers (Bel Kadhi, 2004). The development of biological control methods is crucial, since the introduction of biological control agents in pest management programs provides a more stable results (Aggarwal et al., 2016). The use of as many natural enemies as possible in agricultural production systems can replace the use of pesticides (Trdan et al., 2020). Among other natural agent antagonists of the whitefly *B. tabaci*, *Nesidiocoris tenuis* Reuter (Hemiptera: Miridae) is a predatory zoophytophagous bug, naturally present in tomato crops in the Mediterranean basin (Perdikis and Arvaniti, 2016). It is used to control whiteflies (Hemiptera: Aleyrodidae), tomato leaf miner, and mites (Sanchez et al., 2014 and Yano et al., 2020). Similarly, the use of EPF is a relevant tool in biological control of whitefly (Faria and Wraightb, 2001). Some EPF, such as *Beauveria* spp., *Metarhizium* spp., *Lecanicillum* spp., and *Isaria* spp., were developed as mycoinsecticides (de Faria and Wraight, 2007). They are often used as an effective alternative for controlling insect pests throughout the world (de Faria and Wraight, 2007). In the quest for an integrated pest management program, based on the use of biological control agents, appropriate to the conditions of geothermal greenhouse, in southern Tunisia and meeting the "Global Gap" standards that govern the export niche, this study evaluated the efficacy of the two EPF *B. bassiana* and *L. muscarium* and the native predatory bug *N. tenuis*. In addition, the effect of these fungi on the survival of the predator was studied.

Methods

Insect

B. tabaci and the native predator *N. tenuis* were collected from oases in southern Tunisia. The predator and the pest were reared on a tomato crop in the same greenhouse of 9 × 30 m managed in organic mode, located at the Technical Center for Protected and Geothermal Crops in Chenchou Tunisia-Gabès (TCPG) (latitude, 33° 53′ 13.3″ North; longitude, 9° 53′ 37.1″ East; elevation 78 m). The insects were reared for 2 years from March 2016 until March 2018. The whiteflies collected from the biological greenhouse using a manual aspirator were introduced into previously prepared tobacco plants in pots in a glasshouse. Three weeks later, the mirid bug was collected from TCPG's greenhouse and subsequently released on plants infested with *B. tabaci*.

Preparation of entomopathogenic fungi

Two biological formulations based on EPF were tested on *B. tabaci* under controlled conditions. One was based on *Lecanicillium muscarium* strain Ve6 (19-79) with a water-soluble granular formulation of 10^{10} spores/g, and the other was based on *Beauveria bassiana* strain R 444 in wettable powder with a concentration of 2×10^9 conidia/g.

Experimental method

Effect of N. tenuis on B. tabaci

The study of the efficacy of 5 doses of treatment with *N. tenuis* nymphs was carried out on *Nicotiana tabacum* plants, previously prepared in pots and installed in cages in a glasshouse, under controlled conditions, at a temperature of 27 ± 1°C and RH of 75 ± 5%.

Fifteen wooden cages measuring 1 × 1 × 1.2 m, covered on all three sides with an insect proof canvas (20/10 threads/cm), were prepared and installed in a glasshouse compartment. Each cage containing 5 tobacco plants was assigned to one of the 5 doses of 0, 1, 2, 3, 4, and 5 *N. tenuis* nymphs per plant. All 3 cages correspond to one treatment, and the whole was arranged in a randomized complete block design. At the 6–7 leaf stage, the plants in each cage were infested by adults of *B. tabaci*. A week after the infestation, control of the population level of *B. tabaci* began. When the pest population reached 9 individuals/leaf, inoculation by *N. tenuis* nymphs was carried out. A week later, until the 14th week, monitoring of the population level of nymphs and adults of *B. tabaci* was carried out (Calvo et al., 2009). Using a hand-held magnifying glass, an on-site analysis of a randomly selected leaf on each plant allowed the number of adults and larvae of *B. tabaci* and *N. tenuis* to be counted. Counting was done early in the morning and turning the leaves gently to avoid disturbance of mirid bug and whitefly adults.

The follow-up of the test was stopped at week 14, when it was noted that the vegetative state of the plants no longer allows the normal development of the whitefly due to lack of food, which can distort the efficacy of *N. tenuis*.

Efficacy of B. bassiana and L. muscarium on B. tabaci

The pathogenicity of *B. bassiana* and *L. muscarrium* was tested against the population of *B. tabaci* on *Cucumis sativus*, "Vigorex variety". To do this, cucumber plants were transplanted into 2-l plastic pots, filled with organic peat, and fertilized with a standard nutrient solution. Twelve wooden cages measuring 1 × 1 × 1.2 m, covered on all three sides with an insect proof canvas (20/10 threads/cm), were prepared and installed in a glasshouse compartment. In each cage, 5 cucumber plants were introduced. Four treatments were evaluated: 1 (1 g *B. bassiana* + 1 l sterile distilled water + 0.1% Tween 80); 2 (1 g *L. muscarium* + 1 l sterile distilled water + 0.1% Tween 80); 3 (0.5 g of *B. bassiana* + 0.5 g of *L. muscarium*+ 1 l sterile distilled water + 0.1% Tween 80); 4 (1 l sterile distilled water + 0.1% Tween 80). Therefore, all the 3 cages correspond to a treatment, and the whole was arranged in a randomized complete block design.

The infestation of cucumber plants with *B. tabaci* was previously accomplished. When plants reached stages 5 to 6 leaves, *B. tabaci* adults were introduced to tobacco plants at the rate of 50 adults per leaf. Three weeks later, when the different stages of *B. tabaci* were present, the plants were treated with a sprayer of 1 l volume until runoff. Each cage was treated with 0.4 l of the suitable preparation of the EPF. During the trial, these treatments were repeated 3 times with 10-day intervals.

To avoid any risk of contamination of the plants of a well-defined cage by the products intended for the plants of the neighboring cages, during the treatments, the cages of the plants not concerned were protected by plastic sheeting. Seven days later, the assessment began with a random weekly sampling by collecting 15 leaves per treatment at a rate of one leaf per plant.

Four sampling spaced 7 days intervals were conducted. Immediately after collection, the leaves were examined in the laboratory under binocular loupe to count the number of individuals of the different stages by identifying the dead individual. Nymph mortality was either a whitish or reddish color depending on the strain of the fungus in question, or a mycelial development on the cadavers (Quesada-Moraga et al., 2006).

Effects of EPF on the survival of N. tenuis nymphs

At this level, tomato plants (*Solanum lycopersicum*) were previously infested with adults of *B. tabaci*. Trials began when the *B. tabaci* population reached 30–40 nymphs per leaf. To study the effect of EPF on the survival of *N. tenuis* nymphs, the same treatments used in the fungus pathogenicity study were tested but in two different cases. In the first step, to test the effect of direct spraying of fungi on the survival of *N. tenuis*, the predator was introduced at the rate of 3 individuals per plant infested with *B. tabaci*. Twenty-four hours later, the plants were treated by the fungi. For each treatment, 10 plants were protected by a cage, replicated by 5. The whole was arranged in a randomized complete block design. Each cage was treated by 0.8 l using a 1-l sprayer. In the second step, to evaluate the effect of fungi already installed on a nymphal population of *B. tabaci* on the survival of *N. tenuis* nymphs, whitefly infested plants were treated by different EPF. Nymphs of *N. tenuis*, at a rate of 3 individuals per plant, were introduced at different times after treatment by fungi. Thus, the first lot was brought after 1 h, the second after 24 h, the third after 3 days, the fourth after 5 days, and the fifth after 7 days. This controlled test was repeated 5 times in succession. In all trials, counting of live *N. tenuis* nymphs was performed 3 days after their introduction. The experiments were conducted in separate compartments of the glasshouse.

Statistical analyses

For variance analysis (ANOVA), nymphal and adult populations of the whitefly and nymphs of *N. tenuis*, efficacy rates of *N. tenuis* against *B. tabaci*, and number of live predator nymphs were subjected to unidirectional analysis associated with a Tukey HSD test ($p < 0.05$) using the XLSTAT 2019 software, Microsoft Excel. Reduction of *B. tabaci* by the predator and treated pathogens verified according to the Henderson and Tilton formula was calculated (Henderson and Tilton, 1955).

Reduction (%) = (1 − (No. in control before treatment × No. in treatment after treatment)/(No. in control after treatment × No. in treatment before treatment))*100.

Results

Effect of *N. tenuis* on *B. tabaci*

Evolution of B. tabaci according to treatments

In the absence of *N. tenuis*, the level of the larval population of *B. tabaci* progressed gradually until the end of the test, recording a maximum of 612.00 ± 68.48 nymphs per leaf at the 14th week (Fig. 1a). On the other hand, after treatment with nymphs of the *N. tenuis* bug, the numbers of nymphs of the whiteflies decreased significantly depending on the inoculation dose. Indeed, at the end of the test, there were numbers of nymphal populations of the whitefly of 360.40 ± 34.78, 299.06 ± 12.07, 93.13 ± 7.51, and 33.13 ± 7 individuals with an efficacy rate, respectively, of 98.60, 98.50, 98.44, and 98.80% for 1, 2, 3, and 4 *N. tenuis* nymphs per plant (Table 1). Thus, the analysis of the variance of the mean numbers of larvae of *B. tabaci* showed highly significant difference among the effect of the different doses of treatment by *N. tenuis* ($p < 0.0001$).

As for the adult population of *B. tabaci*, at the end of the test, the maximum number of individuals per leaf at the control level (no *N. tenuis*) is 97.73 ± 7.28 (Fig. 1b).

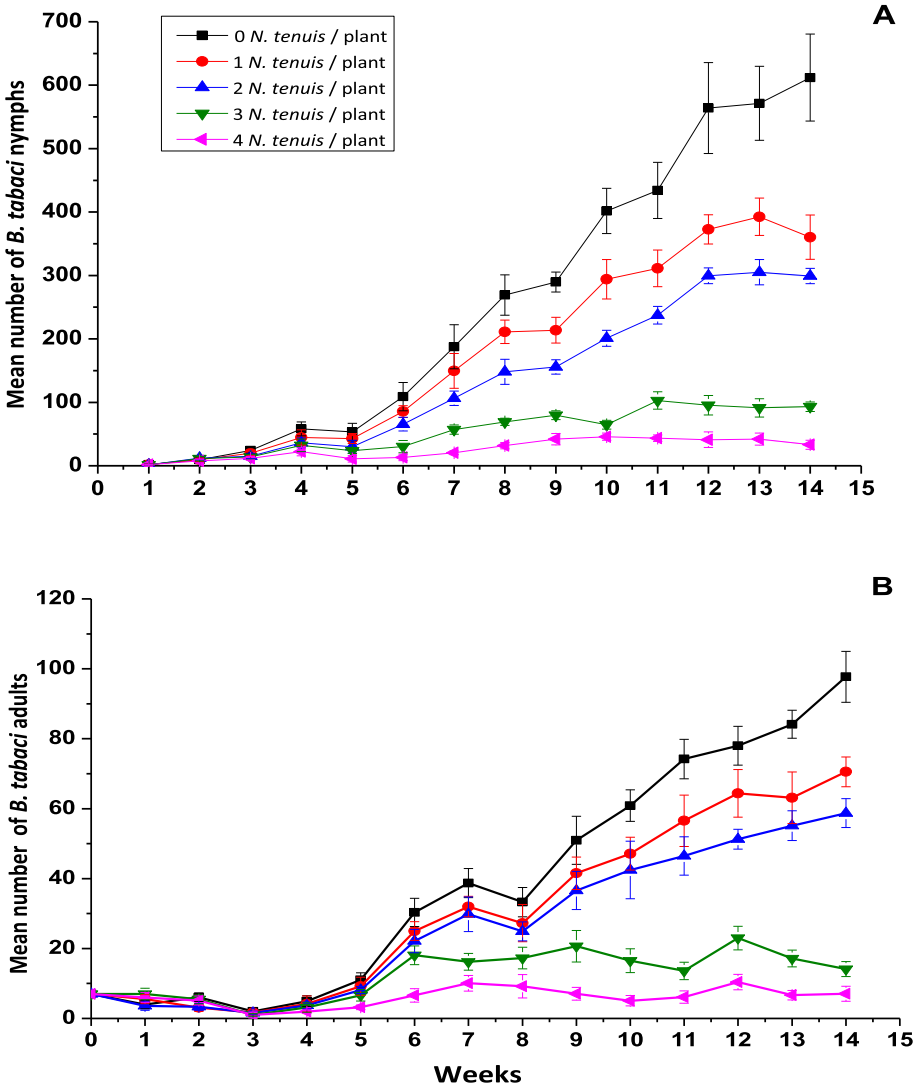

Fig. 1 Mean (±SD) number of *Bemisia tabaci* nymphs and adults as a function of treatment concentrations with (0, 1, 2, 3, and 4) *Nesidiocoris tenuis* nymphs per plant for 14 weeks (**a** nymph population, **b** adult population)

On the other hand, treatment of the *B. tabaci* population with different doses of *N. tenuis* showed a significant decrease in the adult population because of an increase in the number of introduced predator nymphs. The numbers of adults per leaf were 70.53 ± 4.27, 58.73 ± 4.11, 14.13 ± 2.16, and 7.06 ± 2.12 ($p < 0.0001$), respectively, for treatment doses per plant of 1, 2, 3, and 4 nymphs. The efficacy rates of *N. tenuis* against adults of *B. tabaci* are 98.25, 98.33, 98.71, and 98.34% for treatment doses per plant of 1, 2, 3, and 4 nymphs, respectively (Table 1).

Analysis of the whitefly population level at the end of the 1st week for the control showed a low number of first-instar nymph of about two nymphs per leaf. This period of about 7 days was the time required for the incubation of the eggs and the appearance of the first-stage nymphs. The nymphal population, all stages combined, began to increase from the 2nd week onwards in a remarkable way reaching 9 nymphs per leaf. Thus, at the end of the 2nd week, the starting point of the *N. tenuis* nymph treatment trial, all fertile eggs resulted in a large nymphal population, formed by the different nymphal instars. At the end of the 3rd week, the adult population became small and showed a marked change after this period. This coincided with the emergence of first-generation adults and the disappearance of adults introduced about 21 days ago. A further decline in adult numbers was recorded at week 8, followed by a gradual increase in nymphs and adults expressing the staggered development of new generations.

After establishment of the predator, the number of larvae and adults of *B. tabaci* decreased with the number

Table 1 Efficacy rate of *Nesidiocoris tenuis* against *Bemisia tabaci* nymphs and adults as a function of treatment and time

	Nymphs				Adult			
Weeks	**1 N/PL**	**2 N/PL**	**3 N/PL**	**4 N/PL**	**1N/PL**	**2 N/PL**	**3 N/PL**	**4 N/PL**
3	19.36	51.71	51.94	87.17	43.48	52.00	81.71	80.52
4	63.56	61.47	63.04	70.09	26.04	27.64	2.82	40.04
5	83.13	85.48	87.00	91.29	69.23	70.00	70.88	77.12
6	82.95	81.30	89.21	89.74	86.29	86.22	86.19	89.60
7	91.29	91.94	90.60	92.31	94.94	94.68	96.46	93.98
8	95.11	95.17	95.80	94.62	96.08	96.15	95.10	95.80
9	96.74	96.61	96.29	95.73	95.42	95.59	96.40	97.69
10	96.80	97.00	98.11	97.46	97.15	97.08	97.99	98.20
11	97.72	97.46	96.58	97.96	97.53	97.74	98.30	97.50
12	98.02	97.91	98.46	98.44	97.77	97.84	96.68	96.67
13	98.28	98.34	98.44	98.33	98.22	98.04	98.65	98.83
14	98.60	98.50	98.44	98.80	98.25	98.33	98.71	98.34

N/PL number of *Nesidiocoris tenuis* per plant

of introduced *N. tenuis* nymphs (Fig. 1). For treatment doses of one and two *N. tenuis* per plant, the whitefly population followed the same pattern as the control population up to the 12th week. Thereafter, the number of nymphs decreased from the 12th week for the dose of 2 nymphs and from the 13th for the dose of one nymph of *N. tenuis* per plant. While for 3 and 4 *N. tenuis* nymphs per plant, changes in larval and adult populations remained relatively low compared to the control.

Evolution of N. tenuis population according to treatments

Once settled on the whitefly larval population, the level of the *N. tenuis* population changed according to their number of leaves starting at the level of each leaf following an exponential function (Fig. 2). The analysis of the variance of the means of the populations of the predator *N. tenuis* associated with the population of *B. tabaci* showed that there was a highly significant difference among the populations of the different doses of treatments ($p < 0.0001$). For high doses of 3 and 4 nymphs per plant, at week 14, *N. tenuis* exceeded 2 individuals per leaf: 2.06 and 2.4 individuals, respectively. While for the doses of 1 and 2 nymphs, the number remained relatively low, not exceeding 0.6 individuals per leaf were respectively 0.53 and 0.66 individuals per leaf. The whitefly nymphal population at doses 1 and 2 did not decline until the 13th week in which *N. tenuis* began to control the pest population (Fig. 2). However, with the same treatment doses, the number of adults of *B. tabaci* remained in increasing evolution. The exponential

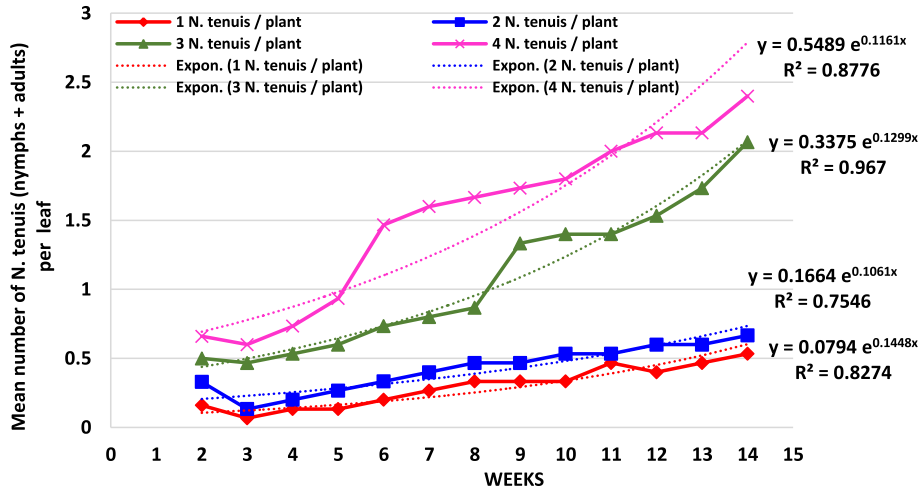

Fig. 2 Mean number of *Nesidiocoris tenuis* (nymphs + adults) per leaf and exponential analysis of the mean as a function of treatment concentrations with (1, 2, 3, and 4) predator nymphs per plant for 14 weeks

functions for doses 1, 2, 3, and 4 nymphs per leaf were respectively $y = 0.0794\ e^{0.1448x}$, $y = 0.1664\ e^{0.1061x}$, $y = 0.3375\ e^{0.1299x}$, and $y = 0.5489\ e^{0.1161x}$.

Analysis of correlations between the various factors

Positive linear relationships were established between the efficacy rates of *N. tenuis* against whitefly larval population and the population density of the mirid bug at each treatment dose (Table 2). To estimate the density needed to control the entire whitefly population, equations corresponding to the different doses used were developed. These equations for doses 1, 2, 3, and 4 nymphs per leaf are Y1 = 43.43 X1 + 87.80, Y1 = 83.13 X1 + 51.71, Y1 = 7.78 X1 + 85.18, and Y1 = 6.92 X1 + 83.09, respectively. The calculation of the density required to eradicate the entire whitefly population was respectively 0.28, 0.58, 1.9, and 2.44 individuals per leaf for 1, 2, 3, and 4 nymphs of *N. tenuis* per plant. To estimate the time required to reach the density of *N. tenuis* that allowed control of the entire whitefly population, positive linear relationships between the efficacy rates of *N. tenuis* against whitefly larval population and the time were developed. Equations corresponding to the different doses used were established (Table 2).

These equations for doses 1, 2, 3, and 4 nymphs per leaf were Y2= 0.8543 X2 + 87.6, Y2 = 0.7875 X2 + 88.348, Y2 = 0.8702 X2 + 87.454, and Y2 = 0.8638 X2 + 87.636, respectively. Of course, the time required to eliminate the entire population was closely related to the dose used at the start of treatment.

Efficacy of *B. bassiana* and *L. muscarrium* on *B. tabaci*

The successive treatments against *B. tabaci* by the EPF, *B. bassiana* and *L. muscarium*, demonstrated the efficacy of the different treatments against the developmental stages of *B. tabaci*. Indeed, the variance analysis of efficacy rates showed a significant difference between treatments (Fig. 3).

In the case of the 1st instar (L1) nymphs, the analysis of the variance of efficacy rates revealed that the effect of association of the 2 fungi was similar to that caused by *B. bassiana* alone over time, except for the 1st of follow-up, where the former was the most effective. In fact, efficacy rates from both fungi ranged from 64.97 to 77.05%, followed by efficacy rates from *B. bassiana* ranging from 44.22 to 72.86%, followed by efficacy rates from *L. muscarium* ranging from 38.58 to 61.04% ($p < 0.0001$, Fig. 3a). For the 2nd instar L2, variance analysis showed that mixing of the 2 fungi was most effective over time with efficacy rates ranging from 77.65 to 95.02%. *B. bassiana* and *L. muscarium* had a comparable effect ($p < 0.0001$, Fig. 3b).

For the 3rd nymphal instar (L3), variance analysis showed that the 2 associated fungi were most effective over time, except that in the 4th week of treatment, both fungi and *B. bassiana* had a similar effect. The efficacy rates ranged from 73.15 to 84.97% ($p < 0.0001$, Fig. 3c). As for the 4th instar L4, the analysis of the variance showed that the 2 associated fungi were the most effective over time, except that in the 3rd week of treatment, both fungi and *B. bassiana* had a similar effect ($p < 0.0001$, Fig. 3d). In the case of pupae, the analysis of variance showed that treatment by fungi had the same effect as that on 4th instar L4 ($p < 0.0001$, Fig. 3e). For the adult stage, variance analysis of efficacy rates showed that the mixture of the 2 fungi was the most effective with the rates varying between 49.63 and 61.05%. *B. bassiana* and *L. muscarium* had a comparable effect over time, except in the 4th week of treatment ($p < 0.0001$, Fig. 3f). It can be concluded that the 2nd nymphal instar of development revealed the highest sensitivity to different treatments, with efficacy rates reaching 95.02% at the 4th week of treatment. However, the pupal stage was the most resistant to different treatments resulting in low efficacy rates not exceeding 61.05%.

Table 2 Densities of *Nesidiocoris tenuis* nymphs and periods needed to control the entire population of *Bemisia tabaci* according to doses

		1 *N. tenuis* per plant	2 *N. tenuis* per plant	3 *N. tenuis* per plant	4 *N. tenuis* per plant
Efficacy of *N. tenuis* against *B. tabaci* nymphs	**Linear equation**	Y1= 43.43 X1 + 87.80	Y1 = 83.13 X1 + 51.71	Y1 = 7.78 X1+ 85.18	Y1 = 6.92 X1 + 83.09
	Coefficient of determination	$R^2 = 0.7947$	$R^2 = 0.8083$	$R^2 = 0.7524$	$R^2 = 0.7459$
	Density of *N. tenuis* reduces 100% of nymphs	0.28	0.58	1.9	2.44
Efficacy of *N. tenuis* against *B. tabaci* nymphs as a function of time	**Linear equation**	Y2 = 0.8543 X2+ 87.6	Y2 = 0.7875 X2 + 88.348	Y2 = 0.8702 X2 + 87.454	Y2 = 0.8638 X2 + 87.636
	Coefficient of determination	$R^2 = 0.756$	$R^2 = 0.7885$	$R^2 = 0.6455$	$R^2 = 0.853$
	Time required to reduce the *B. tabaci* population by 100% (Weeks)	14.51	14.79	14.41	14.31

X1, X2, reduction of *Bemisia tabaci*; *Y1*, the population density of *Nesidiocoris tenuis* per leaf; *Y2*, time (week)

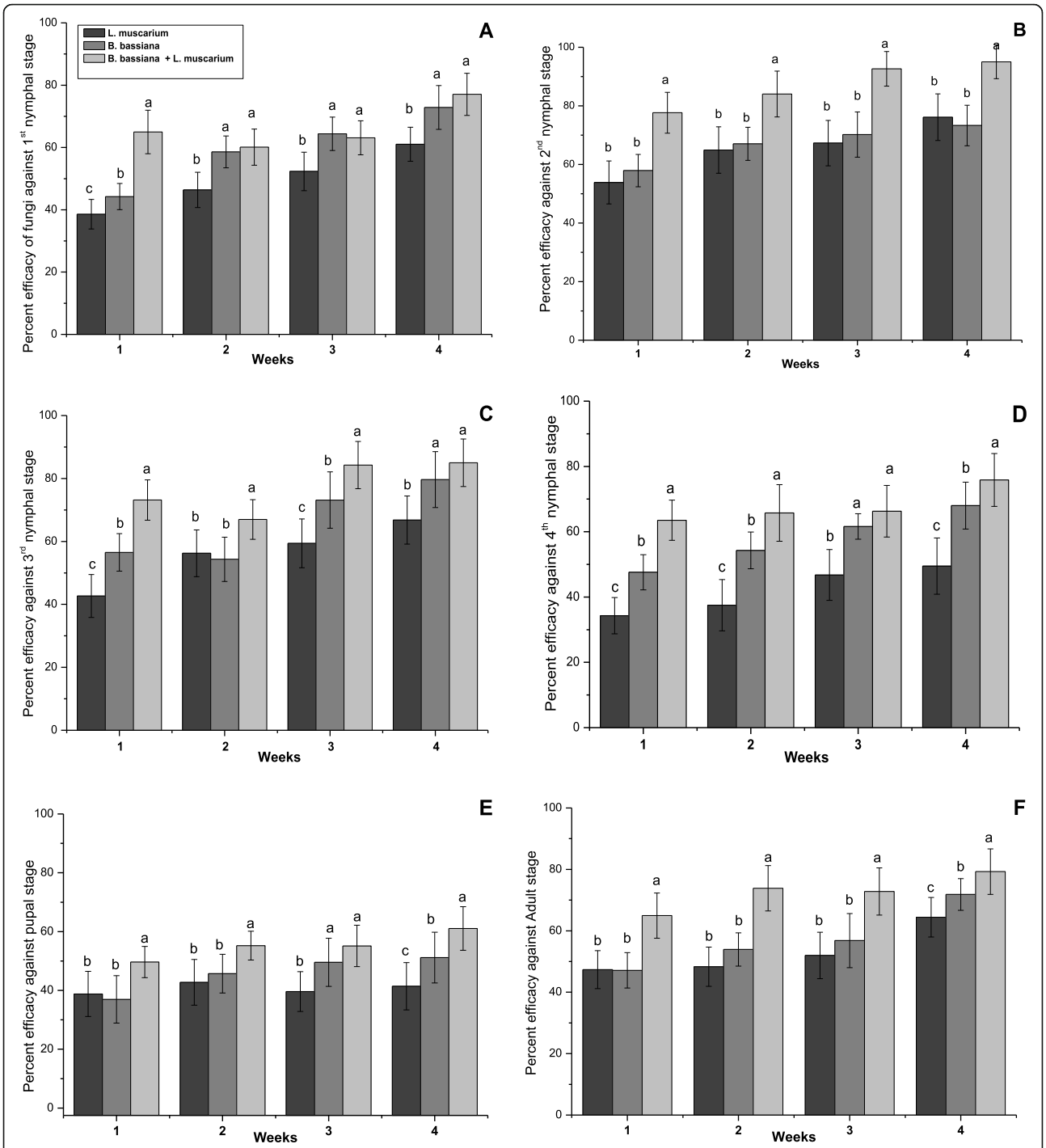

Fig. 3 Mean (±SD) efficacy of entomopathogenic fungi against different stages of development of *Bemisia tabaci* for 4 weeks. Different letters denote means are significantly different from one another, as determined by Tukey's HSD. **a** 1st nymphal instar L1; **b** 2nd instar L2; **c** 3rd instar L3; **d** 4th instar L4; **e** pupa, and **f** adult

Effects of EPF on the survival of *N. tenuis* nymphs

In the first step, a trial to elucidate the possible relationship between the EPF and the predatory insect, the most frequently used, has been performed. Direct treatment of *N. tenuis* nymphs, already associated with *B. tabaci* nymphs, by EPF, affected their survival. There was a statistically significant difference ($p < 0.0001$) (Table 3) between the number of living predator nymphs per cage

Table 3 Mean number of live nymphs of *Nesidiocoris tenuis* (±SD) treated with entomopathogenic fungi

Treatments	Number of live nymphs of *N. tenuis* treated directly with fungi	Number of live nymphs of *N. tenuis* introduced on *B. tabaci* contaminated with fungi				
		1 HAT	1 DAT	3 DAT	5 DAT	7 DAT
Control	28.6 ± 0.28 a	29 ± 0.7 a	29.2 ± 0.83 a	29 ± 0 a	29.2 ± 0.44 a	29.2 ± 0.83 a
Lecanicillium muscarium	25.2 ± 1.27 b	27.6 ± 1.14 ab	28.6 ± 0.54 a	28.8 ± 0.83 a	26.4 ± 1.14 b	26 ± 1.87 b
Beauveria bassiana	25.2 ± 0.56 b	26.8 ± 0.83 b	28.6 ± 0.54 a	28.6 ± 0.89 a	26 ± 0.7 b	26.2 ± 1.48 b
B. bassiana + *L. muscarium*	24.8 ± 0.14 b	26.6 ± 0.89 b	28 ± 1.22 a	28.2 ± 0.83 a	25.6 ± 0.54 b	25.8 ± 1.92 b
F	16.188	7.192	1.714	1.061	23.188	5.122
P value	< 0.0001	0.003	0.204	0.393	< 0.0001	0.011

Different letters denote means are significantly different from one another, as determined by Tukey's HSD. *HAT* hour after treatment, *DAT* day after treatment

treated and untreated. However, the number of live nymphs remained high, over 24.8 nymphs per cage compared to the control, which was 28.6.

On the other hand, the introduction of *N. tenuis* nymphs into a population of *B. tabaci* previously contaminated by the strains of the fungi *B. bassiana* and *L. muscarium* led to a variation in the number of living nymphs depending on the duration of exposure after treatment. Indeed, the analysis of the variance of the means of the numbers of live nymphs of *N. tenuis* showed a significant difference only between the numbers of live nymphs of the mirid bug after 1 h of exposure ($p = 0.003$), 5 days ($p < 0.0001$), 7 days of exposure ($p < 0.0001$), and the untreated control. As for the numbers of live nymphs corresponding to 1 day and 3 days after contamination of the prey, the difference was not significant compared to the control.

Discussion

The efficacy study of the predator *N. tenuis* showed that the numbers of whitefly nymphs decreased remarkably depending on the inoculation dose. Concerning the monitoring of the evolution of nymphal and adult populations of the whitefly on tobacco in the absence of *N. tenuis* showed that the appearance of the 1st nymphal instar L1 requires about 7 days, and the lifespan of newly emerged introduced adults was almost equal to 21 days. These results were consistent with studies (Bel Kadhi, 2004), which showed that the development time of *B. tabaci*'s eggs varies between 4 and 6 days, and that of 1st nymphal instar was an average of 2 days and a preoviposition near zero under controlled conditions similar to that of this test.

As for the evolution of the population of *B. tabaci*, after the introduction of *N. tenuis*, it showed a significant decrease in nymphs and adults for the different doses of treatment by 1, 2, 3, or 4 mirid bug per plant compared to an untreated control under controlled conditions. These results are consistent with studies showing that *N. tenuis* was capable of controlling tobacco whitefly (Calvo et al., 2009). Similarly, the introduction of *N. tenuis* into the tomato greenhouse, from planting, could control populations of tobacco whitefly or *Tuta absoluta* (Calvo et al., 2012).

The efficacy rates of *N. tenuis* against nymphs and adults of whitefly were very high and reached 98% for all treatment doses at the end of the test. Likewise, Calvo et al. (2009) showed that *N. tenuis* treatment doses of 1 and 4 individuals per tomato plant had the same effect on the reduction of the population of *B. tabaci* with reduction of around 80%. Also, the specificity of the host plant and the diet could influence the survival time of *N. tenuis* (Urbaneja et al., 2005). A study carried out in a geothermal greenhouse showed that *N. tenuis* had a preference for tobacco over tomatoes and melons (Ben Belgacem et al., 2016).

However, in this trial, all doses could not control the whitefly population, which remained large at the end of the trial. This may be due to the evolution of the predator, which was slow for different doses over time and reached only 2.4 individuals per leaf for the best dose of treatment. In fact, its low fertility of 60 nymphs per female was very low compared to that of *B. tabaci,* which was 130 (Sanchez et al., 2009). In addition, the developmental time of the predator's larvae was 21.8 days (Sanchez et al., 2009) longer than that of the 17-day pest (Bel Kadhi, 2004). These differences in the biological parameters of the 2 insects certainly influenced the control power of the *B. tabaci* population in a very limited period. In addition, treatment initiation with *N. tenuis* nymphs coincided with a high level of the whitefly larval population assessed at 9 larvae per leaf.

In this trial, at best, the whitefly population was completely controlled after 14.31 weeks, with the highest dose used being 4 nymphs per plant. Based on the pre-established equations, the periods necessary for the total

elimination of whitefly populations were 14.51, 14.79, and 14.41 weeks for doses 1, 2, and 3, respectively. Thus, in the second step, it was able to determine the intervention period and the dose necessary to eliminate or even avoid the installation of a population of whiteflies.

In addition, the results of the trials undertaken to determine the level of pathogenicity of EPF *B. bassiana* and *L. muscarium* against *B. tabaci* associated with heated greenhouse crops in southern Tunisia were very interesting. In fact, the strains of *B. bassiana* and *L. muscarium*, used either separately or in a mixture, allowed to reduce the levels of the populations of the different development stages of *B. tabaci*. These results were in perfect agreement with that of several research studies that showed that EPF were effective at different stages of development of the whitefly (Quesada-Moraga et al., 2006; Park and Kim, 2010 and Polanczyk et al., 2019). The JAB07 isolates of *B. bassiana* and LCMAP3790 of *L. muscarium* resulted in more than 80% mortality of eggs and the 3rd instar nymphs of *B. tabaci* biotype B (Polanczyk et al., 2019). Similarly, *B. bassiana* strain Bb-202 caused a good pathogenicity for *B. tabaci* above 77% mortality (Ghulam et al., 2018).

In this study, *B. bassiana* was more effective than *L. muscarium* against all developmental stages of *B. tabaci*. This is consistent with the study of Mascarin et al. (2013), which showed that *B. bassiana* strain CG1229 was significantly more virulent to nymphs than all *L. muscarium* isolates, resulting in larval mortality rates greater than 71%. Similarly, the in vitro study of Keerio et al. (2020) showed the efficacy of 2 strains of *B. bassiana* and one strain of *L. lecanii* against *B. tabaci. L. lecanii* resulted in lower mortality rate than the two strains of *B. bassiana*. In contrast, the commercial isolate of *L. muscarium* tested against the different stages of development of *B. tabaci* on poinsettia showed low efficacy (20–40% mortality) (Cuthbertson et al., 2008). This can be explained by the influence of factors, other than isolate, on the efficacy of fungi, such as application method, environmental conditions, or morphology.

The results showed a high sensitivity of the 2nd and 3rd nymphal *B. tabaci* instars to the fungi tested. Similarly, the mortality of the 2nd instar *B. tabaci* nymphs was the highest following application of *L. muscarium* under controlled laboratory and greenhouse conditions (Cuthbertson and Walters, 2005).

In addition, a study (Poprawski et al., 2000) found that the 3rd nymphal instar of *Trialeurodes vaporariorum* was the most sensitive to *B. bassiana*. According to Malekan et al. (2015) the 3rd and 4th nymphal instars of *B. tabaci* were more sensitive to *B. bassiana* and *L. muscarium* than the 1st and 2nd nymphal instars with mortality percentages of 63.74 and 62.49% on young nymphs and 71.68 and 87.13% on old nymphs, respectively.

The study of the effect of the 2 fungi *L. muscarium* and *B. bassiana* on the survival of *N. tenuis* showed that the number of nymphs significantly reduced than the control, when they were sprayed directly by the fungi or introduced on *B. tabaci* nymphs treated for 1 h or 5 or 7 days.

The number of live nymphs of *N. tenuis* introduced after 1 h of treatment of the nymphs of *B. tabaci* by *B. bassiana* and *L. muscarium* was lower than that of the control. This may be due to the direct contact of *N. tenuis* nymphs with the fungal preparation. Likewise, the survival of *N. tenuis* was affected after 5 and 7 days of treatment when the fungi developed in the nymphs of *B. tabaci* and the mortality of the latter increased. However, the number of live nymphs remained relatively high, over 24.8 nymphs per cage than the control, which was 28.6 nymphs.

Conclusion

It can be inferred from the results that the native predator *N. tenuis* alone reduced the population of *B. tabaci*, and its introduction well before the appearance of the pest increased its effectiveness. Similarly, microbiological control of the same pest with *B. bassiana* and *L. muscurium* significantly reduced the populations of *B. tabaci*. The effect of EPF used in association with a nymphal population of *N. tenuis* on nymphs' survival was low. Thus, the use of these biological means in combination, as part of an integrated pest control program, is recommended taking into account the time interval necessary between the introduction of *N. tenuis* and the treatment with EPF.

Abbreviations

TCPG: The Technical Center for Protected and Geothermal Crops in Chenchou; N/PL: Number of *Nesidiocoris tenuis* per plant; HAT: Hour after treatment; DAT: Day after treatment; R^2: Coefficient of determination; *P*: *P*-value

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The plants used in this study were prepared at the technical center for protected and geothermal crops by sowing seeds of crops listed in the official catalog in the order of the Minister of Agriculture, Water Resources and Fisheries in Tunisia.

Authors' contributions

Conceptualization: BHA and MSB. Methodology: BHA and MSB. Formal analysis: BHA and SC. Investigation: BHA, SC, and RE. Resources: RE, NMB, and MSB. Data curation: BHA, SC, and RE. Writing—original draft preparation: BHA, SC, and RE. Writing—review and editing: BHA, NMB, and MSB. Visualization: BHA, SC, and MSB. Supervision: BHA, NMB, and MSB. Funding acquisition: BHA and MSB. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
All data of the study have been presented in the manuscript, and high quality and grade materials were used in this study.

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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