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RESEARCH

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Biological control and field evaluation of the predatory mite, *Typhlodromus setubali* Dosse (Acari: Phytoseiidae) in apple orchards

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Abstract

Background: *Typhlodromus setubali* Dosse (Acari: Phytoseiidae) is an indigenous predatory mite in Morocco. This study aimed to evaluate its efficacy, through 3 release rates on 2 apple varieties, namely Skarlet and Golden delicious, with different initial densities of the European red spider mite, *Panonychus ulmi* Koch (Acari: Tetranychidae).

Results: The efficacy of the predator was significantly influenced by the initial *P. ulmi* density. Furthermore, the release rate of 50 individuals/tree did not provide effective control of *P. ulmi*. When it was released at 100 and 200 individuals per tree, *T. setubali* maintained the *P. ulmi* population below the economic threshold of 5 motile stages per leaf.

Conclusions: When the initial *P. ulmi* density is above the economic threshold, the lower release rate of *T. setubali* must be applied with high frequency or conciliated with a selective miticide treatment at a suitable dose for a successful biological control program. Further field-based studies are needed for perfect conclusions.

Keywords: Apple orchard, *Typhlodromus setubali*, *Panonychus ulmi*, Release rate, Biological control

Background

The European red spider mite, *Panonychus ulmi* Koch (Acari: Tetranychidae), is the most common phytophagous mite of various crops throughout the world (Kasap and Atlihan 2021). *Panonychus ulmi* attacks reduce photosynthesis and adversely affect vegetative growth, fruit yield, and fruit quality attributes such as size, firmness flavour, and storage life (Thistlewood et al. 2013). High *P. ulmi* density through its high reproductive rate, short generation time, multivoltinism, and the ability for rapid evolution of resistance to intensively used pesticides, deteriorates the production chain in commercial orchards, especially in apple and grapevine crops (Silva et al. 2021).

On various apple varieties, the development of *P. ulmi* from egg to adult stage has been clarified (Yin et al. 2013). Most tetranychid mites achieve 6–8 generations per season, inflicting considerable devastation at active developmental stages (Herbert 1970).

Despite phytosanitary advances and chemical control rationalization, *P. ulmi* can develop resistance to frequently used acaricides (Khajehali et al. 2021). The unintended effects of pesticides on natural enemy populations, including predatory mites, remain a major challenge for successful biological control programs, in strict accordance with the standard norms of integrated pest management (IPM) (Yang et al. 2014).

In Morocco, biological control against pests has recently emerged and natural enemies that reduce their abundance have been considered as a key component of IPM programs (Smaili et al. 2020). The native predatory mite, *Typhlodromus setubali* Dosse (Acari: Phytoseiidae), is one of the most adapted species to the Moroccan

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climate, being naturally abundant in its central plateau (Tixier et al. 2016).

In the frame of biological control experiments, this study aimed to evaluate the efficacy of *T. setubali* against *P. ulmi* on 2 apple varieties namely: Golden delicious and Skarlet, using different release rates.

Methods

Experimental site

Two separate release experiments were conducted in August 2019 in the Arbor orchard in the Oulmes region at an altitude of 1240 m (33° 26′ 19.6″ N, 5° 58′ 35.7″ W). The first experiment was carried out on the Skarlet apple plot, abbreviated 19B with approximately 4 m tree high, and the Jeromine rootstock. The second one was conducted in the Golden delicious plot, namely 6A, with 300 trees 4 m high. Both plots have histories of high *P. ulmi* infestations and were commonly managed by 3–5 applications of acaricides. All selected trees in both release experiments are of similar age (6 years) and were not treated with acaricides at least 40 days prior to the experiments.

The initial population of the predatory mite, *T. setubali*, was obtained from the Riyad fruit orchard in the Tiddas region at 500 m of altitude (33° 33′ 37.0″ N, 6° 15′ 40.8″ W), where a population had naturally maintained on the Anna apple cultivar a few years ago. Both plots are located on the northern talus of the Moroccan Middle Atlas within a range of 50 km (Fig. 1).

Experimental design

A single release of *T. setubali* at 50, 100 and 200 individuals per tree was carried out on the 2 apple varieties with different initial *P. ulmi* densities. Before the beginning of experiments, each plot was sampled using a binomial procedure to determine initial prey densities. The first experiment carried out on the Skarlet cultivar has moderate initial *P. ulmi* density (4–6 *P. ulmi* motile stages/leaf), whilst the second was performed on a highly infested Golden delicious plot, comparatively to the economic threshold of 5 motiles/leaf, proposed by Nyrop et al. (1994) as a critical density related to *P. ulmi* in July. For each experiment, a control plot of 5 apple trees was selected for similarity in the varietal system, age of plantation, and field management.

A factorial design combining all modalities of release rate and sampling date within paired groups was adopted because it improves the experiment and reduces residual errors. Each plot is designed as a randomized complete block (RCBD), in which treatment levels are assigned to similar experimental units.

Release of *T. setubali*

The release timing was the same on both plots. One release point per tree test was selected in each of the two

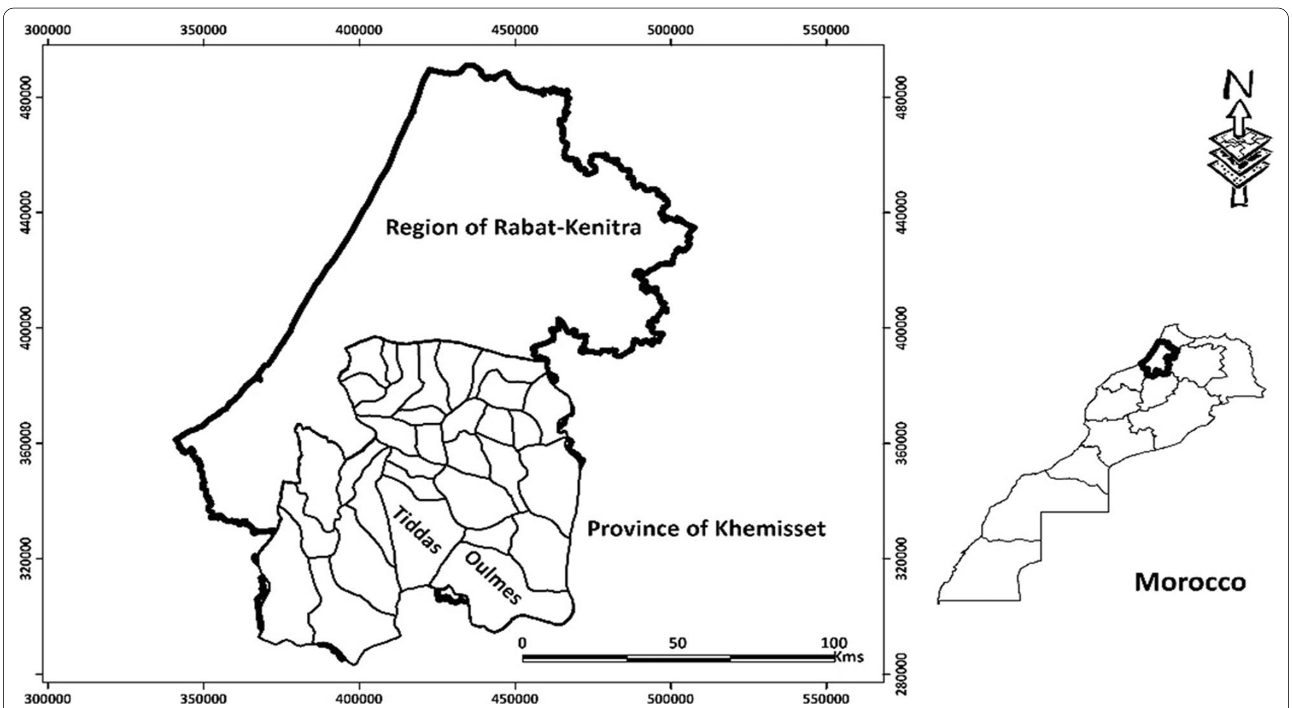

Fig. 1 Mapping of the experimental site

experiments. The release rate tested was 0 (no release control), 50, 100, and 200 predators per single test tree. The efficacy of *T. setubali* was evaluated based on the surviving number of *P. ulmi* eggs and motile stages every week after the initial release.

To determine *P. ulmi* eggs and motile stages densities, 16 randomly selected leaves (4 leaves per orientation) were taken from each test tree at 0, 7, 14, 21, 28, 35 and 42 days after release. The collected leaves were kept in coolers and transferred to the laboratory, where they were examined under a binocular stereomicroscope. The number of survived individuals after each treatment was minutely counted, and the results were recorded as prey density and the occupancy rate by one or more individuals per leaf.

Predatory mites were released between 8 and 10 am when the temperature and relative humidity were between 24 and 26 °C and 60 and 70%, respectively. Each release rate experiment was replicated on 5 test trees, separated by 2 buffer trees serving as windbreaks to reduce a possible wind-favoured mite dispersal. The average ambient temperature and relative humidity for the experiments were 30 °C and 55–65%.

Statistical analysis

Statistical analysis was performed in the R program (R Core Team 2019). Two-way multivariate analysis of variance (MANOVA) was used to test whether eggs and mobile stages of *P. ulmi* are influenced by the difference in release rate and sampling date. When the null hypothesis H_0 was rejected, the normality of residuals was checked and normal distribution was approximated through square root transformation. Wilk's lambda test was used to test whether there are differences between the mean densities of *P. ulmi* eggs and motiles. Furthermore, if the test revealed significant effects of treatment, sampling date, and treatment/sampling date interaction, a two-way analysis of variance (ANOVA), followed by a Tukey/Kramer HSD comparison test, was fitted to determine differences among release rates at each sampling date.

At each treatment, the Student *t*/test or Mann–Whitney nonparametric test was used, according to the Shapiro–Wilk test and Levene test for normality and homogeneity of variances to separate both groups.

Results

Release of *T. setubali* on Skarlet

Because the data were not normally distributed, a square root transformation was used to approximate the normal distribution. The MANOVA result revealed significant effects of the release rate (Wilk's $\lambda = 0.187$, $df = 2, 23$, $P < 0.0001$), and sampling date on the numbers of *P. ulmi* eggs and motiles (Wilk's $\lambda = 0.621$, $df = 2, 23$, $P = 0.0042$), likewise of the release rate-sampling date interaction (Wilk's $\lambda = 0.506$, $df = 2, 23$, $P = 0.0004$).

Two-way ANOVA results showed non-significant differences among release rates at 0 days in *P. ulmi* eggs ($F = 0.748$, $df = 3, 19$, $P = 0.539$) and motile stages ($F = 0.863$, $df = 3, 19$, $P = 0.480$, respectively). The mean numbers of *P. ulmi* eggs and motile stages were significantly influenced by the release rate change from 7 to 42 days after the release test ($P < 0.0001$) (Tables 1 and 2). The mean densities of *P. ulmi* eggs and motile stages, recorded in the Skarlet plot, are displayed in Fig. 2a and b.

Release of *T. setubali* on Golden delicious

The sampling date-release rate interaction was found to have a significant effect in multivariate analysis of variance MANOVA ($\lambda = 0.680$; $df = 2, 23$; $P = 0.0119$). The sampling date and release rate both had a significant effect on the mean number of *P. ulmi* eggs and motile stages ($\lambda = 0.603$; $df = 2, 23$; $P < 0.0030$ and $\lambda = 0.238$; $df = 2, 23$; $P < 0.0001$, respectively). Two-way analysis of variance ANOVA showed no significant differences among mean numbers of *P. ulmi* eggs and motile stages at the beginning of the release test on Golden Delicious ($F = 0.471$; $df = 3, 19$; $P = 0.707$ and $F = 0.465$; $df = 3, 19$; $P = 0.711$, respectively) (Tables 1 and 2). The mean densities of *P. ulmi* eggs and motile stages, recorded on the Golden Delicious variety, are illustrated in Fig. 2c, d.

Significant differences were observed in *P. ulmi* eggs among treatments beyond the 7th day of experiments (Table 1). In the same way, the mean density of *P. ulmi* motile stages differed among release rates from 7 to 42 days after release (Table 2). In the experiment of T/200, the predatory mite was more efficient to high initial *P. ulmi* density, holding the *P. ulmi* population below 5 motile forms per leaf (Nyrop et al. 1994), since the first week of the test.

Regardless of the apple cultivars tested in this study, the *P. ulmi* population showed a fluctuation during the study period. The number of *P. ulmi* eggs was significantly higher in the control than in the treatments over time, suggesting an accurate control by *T. setubali* against the *P. ulmi* population. On the other hand, the maximum number of *P. ulmi* eggs produced was observed on the Golden Delicious variety, due to the fast development of individuals with optimal female fecundities.

Discussion

The present study revealed that the predatory mite, *T. setubali*, showed an effective response to *P. ulmi* when it was released at a sufficient rate on apple trees. The effectiveness of *T. setubali* was significantly influenced by

Table 1 Mean numbers per leaf (± SE) of *P. ulmi* eggs recorded every week at different release rates of *T. setubali* on two apple varieties

DAR	Variety	T/0	T/50	T/100	T/200	*F*	*df*	*P*
0	Skarlet	24 ± 1.87 Aa	21.46 ± 6.87 Aa	20.37 ± 2.84 Aa	22.38 ± 2.08 Aa	0.748	3, 19	0.539
	Golden delicious	26 ± 3.16 Aa	24.80 ± 4.38 Aa	23.83 ± 3.80 Aa	25.20 ± 4.96 Aa	0.471	3, 19	0.707
	t/z/U	2.306	2.306	2.306	1.960			
	P	0.258	0.387	0.251	0.242			
7	Skarlet	30 ± 2.54 Aa	16.28 ± 0.85 Ba	13.28 ± 1.50 Bab	8.82 ± 3.04 Cb	87.820	3, 19	< 0.0001***
	Golden delicious	32 ± 2.82 Aa	17.40 ± 1.81 Ba	14.60 ± 1.51 Bab	10.00 ± 1.22 Cb	119.611	3, 19	< 0.0001***
	t/z/U	2.306	2.306	2.306	2.306			
	P	0.274	0.260	0.205	0.444			
14	Skarlet	42 ± 3.16 Aa	12.32 ± 2.25 Ba	8.98 ± 1.98 BCa	4.92 ± 1.20 Cb	279.139	3, 19	< 0.0001***
	Golden delicious	30 ± 2.54 Ab	11.60 ± 1.51 Ba	10.80 ± 1.30 BCa	7.20 ± 1.64 Ca	159.091	3, 19	< 0.0001***
	t/z/U	25.000	0.593	1.960	1.960			
	P	0.014*	0.570	0.087	0.012*			
21	Skarlet	39 ± 3.39 Ab	15.98 ± 1.08 Ba	8.82 ± 4.19 Ca	4.26 ± 1.29 Ca	148.958	3, 19	< 0.0001***
	Golden delicious	48 ± 6.12 Aa	8.60 ± 1.34 Bb	5.20 ± 0.83 Ca	3.60 ± 0.89 Ca	220.371	3, 19	< 0.0001***
	t/z/U	1.960	25.000	1.892	0.936			
	P	0.004**	0.012*	0.095	0.349			
28	Skarlet	53 ± 7.51 Aa	9.78 ± 2.63 Ba	2.10 ± 0.49 BCb	4.86 ± 1.49 Ca	173.581	3, 19	< 0.0001***
	Golden delicious	47 ± 6.89 Aa	10.40 ± 2.19 Ba	5.00 ± 1.22 BCa	1.60 ± 0.89 Cb	138.440	3, 19	< 0.0001***
	t/z/U	1.960	1.960	0.0001	25.000			
	P	0.207	0.686	0.011*	0.012*			
35	Skarlet	34 ± 4.58 Ab	10.60 ± 2.68 Ba	3.14 ± 0.57 Ca	3.78 ± 1.04 Ca	141.531	3, 19	< 0.0001***
	Golden delicious	55 ± 4.30 Aa	6.40 ± 0.54 Bb	2.00 ± 0.70 BCa	3.40 ± 0.89 Ca	652.060	3, 19	< 0.0001***
	t/z/U	1.960	3.429	2.208	0.617			
	P	< 0.0001***	0.001**	0.123	0.537			
42	Skarlet	32 ± 4.52 Aa	7.40 ± 1.50 Ba	3.34 ± 1.18 Ba	4.38 ± 1.01 Ba	146.713	3, 19	< 0.0001***
	Golden delicious	37 ± 5.61 Aa	4.60 ± 0.54 Bb	1.60 ± 0.89 Bb	2.20 ± 0.44 Bb	179.323	3, 19	< 0.0001***
	t/z/U	1.960	25.000	2.621	25.000			
	P	0.121	0.011*	0.009**	0.010*			

Means within rows followed by the same capital letter are not significantly different, according to Tukey's test at $\alpha = 0.05$, whereas the means within columns followed by the same small letter are not significantly different ($P > 0.05$) according to Student t/test (Values in bold font), or Mann–Whitney test (Values in normal font)

T/0: Control; T/50: one release of *T. setubali* at the rate of 50/tree; T/100: one release of *T. setubali* at the rate of 100/tree; T/200: one release of *T. setubali* at the rate of 200/tree

* $P < 0.05$, **$P < 0.01$ and ***$P < 0.001$

initial *P. ulmi* densities and release rate. The release rate of 50 individuals conducted on high initial *P. ulmi* density maintained the population below the economic threshold for a few days after release, because injurious *P. ulmi* densities can be fastly reached. The release rate of 50 predators/ tree was not sufficient on Golden delicious rather than on Skarlet. Therefore, the biological control using *T. setubali* cannot be achieved with such a release rate, which can be recommended at low levels of the *P. ulmi* population.

The release rate of 100 individuals/tree prevented *P. ulmi* from exceeding the economic threshold after 14 days of experiments in both plots, suggesting a poor response of predators during the 2 weeks following release. For both varieties, similar mean numbers of *P. ulmi* eggs and motile stages from 7 to 42 days. On the other hand, the release of 200 predators/tree was effective for controlling high initial *P. ulmi* densities, without the need for any supplementary treatment over the study period. Moreover, lower mean numbers of *P. ulmi* eggs and motile stages were recorded soon after release, suggesting a successful control of the *P. ulmi* population.

The use of *T. setubali* at a low rate can be helpful for sustainable control when it is safely combined with scheduled acaricide treatments (Kang et al. 2018), or released with a high frequency. However, making a reasonable release rate depends on whether the biological control is performed with augmentative, inundative, or

Table 2 Mean numbers per leaf (± SE) of *P. ulmi* motile stages recorded every week at different release rates of T. setubali on two apple varieties

DAR	Variety	T/0	T/50	T/100	T/200	*F*	*df*	*P*
0	Skarlet	6 ± 1.58 Ab	7.17 ± 1.77 Ab	6.66 ± 0.95 Ab	5.98 ± 0.84 Ab	0.863	3, 19	0.480
	Golden delicious	11 ± 1.36 Aa	11.40 ± 2.07 Aa	12.20 ± 2.28 Aa	12.40 ± 2.60 Aa	0.465	3, 19	0.711
	t/z/U	1.960	2.306	0.00001	0.0001			
	P	< 0.0001***	0.010*	0.011**	0.012*			
7	Skarlet	8 ± 1.87 Ab	5.92 ± 1.01 ABb	5.38 ± 0.90 Ba	4.04 ± 0.88 Ba	8.825	3, 19	0.0011**
	Golden delicious	15 ± 1.67 Aa	7.40 ± 0.89 Ba	6.40 ± 0.54 Ba	4.20 ± 0.44 Ca	91.819	3, 19	< 0.0001***
	t/z/U	1.960	1.960	2.306	2.306			
	P	< 0.0001***	0.014*	0.064	0.728			
14	Skarlet	14 ± 1.58 Aa	4.92 ± 0.52 Ba	3.74 ± 0.61 BCa	2.34 ± 0.73 Ca	151.205	3, 19	< 0.0001***
	Golden delicious	15 ± 1.29 Aa	5.80 ± 0.83 Ba	3.00 ± 1.41 Ca	1.40 ± 0.54 Ca	134.352	3, 19	< 0.0001***
	t/z/U	1.960	2.306	1.960	2.306			
	P	0.317	0.082	0.282	0.050			
21	Skarlet	17 ± 2.12 Aa	6.28 ± 1.80 Ba	3.06 ± 1.37 Ca	1.29 ± 0.63 Ca	98.598	3, 19	< 0.0001***
	Golden delicious	18 ± 1.55 Aa	4.80 ± 0.83 Ba	2.20 ± 1.64 Ca	1.18 ± 0.46 Ca	198.434	3, 19	< 0.0001***
	t/z/U	1.990	1.960	0.897	0.332			
	P	0.398	0.091	0.396	0.749			
28	Skarlet	15 ± 1.81 Aa	5.64 ± 1.07 Bb	2.06 ± 0.61 Ca	1.21 ± 0.29 Ca	155.690	3, 19	< 0.0001***
	Golden delicious	14 ± 2.34 Aa	7.40 ± 0.89 Ba	3.20 ± 1.09 Ca	1.04 ± 0.08 Ca	86.778	3, 19	< 0.0001***
	t/z/U	1.960	1.960	2.306	1.251			
	P	0.456	0.005**	0.079	0.246			
35	Skarlet	15 ± 2.91 Aa	5.26 ± 2.23 Ba	3.78 ± 1.71 Ba	1.61 ± 0.46 Ba	42.082	3, 19	< 0.0001***
	Golden delicious	15 ± 3.16 Aa	4.74 ± 0.91 Ba	2.66 ± 1.01 Ba	1.80 ± 0.83 Ba	59.087	3, 19	< 0.0001***
	t/z/U	1.930	0.481	1.258	1.960			
	P	0.996	0.643	0.244	0.641			
42	Skarlet	15 ± 1.89 Aa	4.92 ± 2.15 Ba	2.38 ± 1.53 BCa	1.74 ± 0.53 Ca	70.389	3, 19	< 0.0001***
	Golden delicious	16 ± 1.58 Aa	4.20 ± 1.09 Ba	1.60 ± 0.54 BCa	0.86 ± 0.57 Ca	202.220	3, 19	< 0.0001***
	t/z/U	1.850	0.667	1.071	1.826			
	P	0.361	0.524	0.284	0.105			

Means within rows followed by the same capital letter are not significantly different, according to Tukey's test at α = 0.05, whereas the means within columns followed by the same small letter are not significantly different ($P > 0.05$) according to Student t/test (Values in bold font), or Mann–Whitney test (Values in normal font)

T/0: Control; T/50: one release of *T. setubali* at the rate of 50/tree; T/100: one release of *T. setubali* at the rate of 100/tree; T/200: one release of *T. setubali* at the rate of 200/tree

* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$

inoculative release (Crowder 2007). In such a biological treatment, some requirements must be taken into consideration, such as an appropriate economic threshold and a perfect knowledge of predator–prey interactions.

Several phytoseiid mites have been successfully tested on different crops. However, the successful separate or combined releases of predatory mites depend on multivarious factors that can impact their efficacy (Maoz et al. 2014). In this study, the obtained results are consistent with those obtained for *Galendromus occidentalis* Nesbitt and *Phytoseiulus persimilis* Athias-Henriot at 100 and 200 individuals/tree on satsuma trees, with moderate initial densities of *Panonychus citri* McGregor (Acari: Tetranychidae) (Fadamiro et al. 2013).

Conclusions

The release rate of 50 T. *setubali* per tree did not achieve perfect protection against the *P. ulmi* population on both apple varieties, but it seems to be an adequate biological treatment at the beginning of the season when *P. ulmi* wintering eggs hatch and diapausing adult females migrate toward the canopy.

The release rates of 100–200 predatory mites per tree are practically recommended. IPM programs including *T. setubali* as a biological control agent must take into consideration the initial *P. ulmi* population and require selective adjunctive treatments when it is released at low rates.

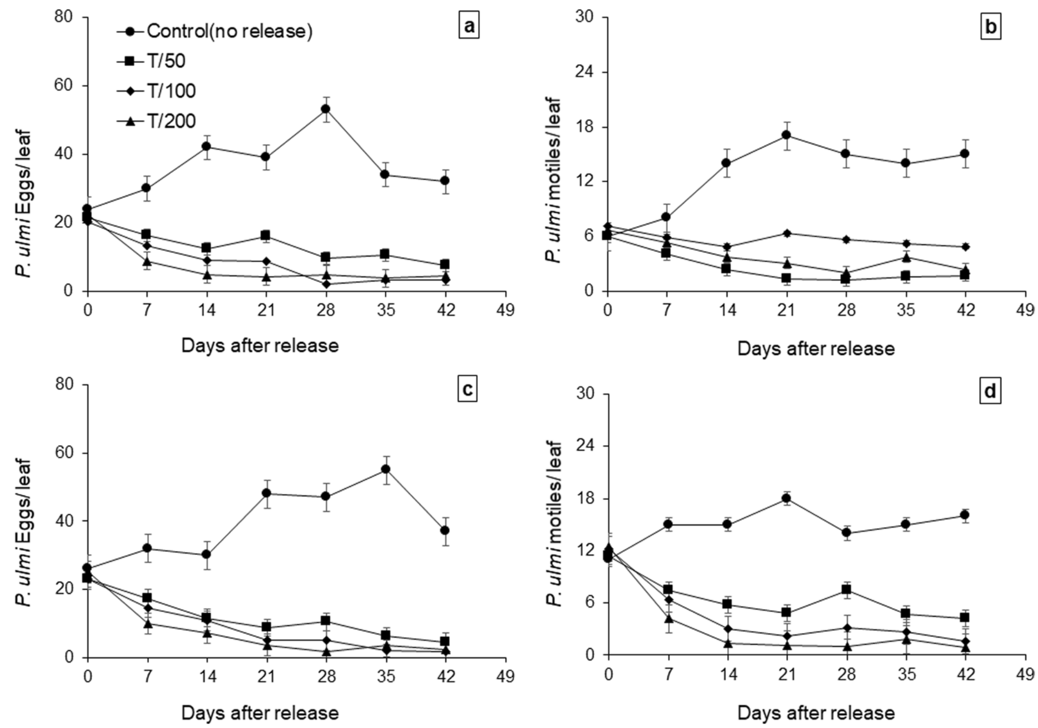

Fig. 2 Mean numbers of *P. ulmi* eggs and motile stages surviving after the single release of *T. setubali* treatment at different release rates. **a** and **b** illustrates the mean number of eggs and motile stages recorded on the Skarlet cultivar every week after predatory mite release (0 days), with moderate initial density. **c** and **d** illustrates the mean number of eggs and motile stages recorded on Golden delicious, with high initial prey density. T/50: one release of *T. setubali* at the rate of 50/tree; T/100: one release of *T. setubali* at the rate of 100/tree; T/200: one release of *T. setubali* at the rate of 200/tree; Control: no release. The predatory mite releases were carried out on the 7th day of study

The economic importance of *T. setubali* was proven when it was released at 200 predators per tree because it reduced costs related to acaricides by 50–60%.

Abbreviations

ANOVA: Analysis of variance; DAR: Days after release; HSD: Honestly significant difference; MANOVA: Multivariate analysis of variance; RCBD: Randomized complete block design; T/0: Control (no release of *T. setubali*).; T/50: Release rate of *T. setubali* with 50 individuals per tree; T/100: Release rate of *T. setubali* with 100 individuals per tree; T/200: Release rate of *T. setubali* with 200 individuals per tree.

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Authors' contributions

LA performed the experiment design and was a major contributor in writing the manuscript. SO analysed and interpreted data. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analysed during this study are included in this published article.

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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