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Evaluation of the Effect of Different Concentrations of Spirotetramat on the Diaspine Scale *Parlatoria ziziphi* in Citrus Orchards

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Abstract: The control of *Parlatoria ziziphi* (Lucas, 1853) was studied in citrus orchards at Mechraa Belksiri in the Gharb area of Morocco. Three concentrations of spirotetramat T0 = 0 L/Ha as a control experiment, T1 = 0.625 L/Ha, T2 = 0.755 L/Ha, and T3 = 1 L/Ha, were applied on 4 ha of Valencia late orchard (each dose for 1 ha of Valencia late). The effect of spirotetramat was evaluated on the mortalities and survival rates of *P. ziziphin* during the stages of larvae (first instar and second instar) and females (F1, F2, and F3). Results showed that the spirotetramat was effective on larvae and females of *P. ziziphi*. Among the 11,229 females recorded, 93% were inhibited, while only 7% were intact after the treatment period. Finally, our study highlights that all concentrations tested were effective on the *P. ziziphi* population; besides, all three concentrations of this product tested were equally effective on larvae and females of *P. ziziphi*. Thus, during the spread period, spraying a low concentration of this product (0.625 L/Ha) will better control this pest and reduce the environmental impact.

Keywords: *Parlatoria ziziphi*; spirotetramat; pest control; Valencia late; crop protection; concentrations; management

1. Introduction

In Morocco, the citrus sector is of great importance to socio-economic development, with an estimated production of 2.2 million tons per year [1]. It is mainly produced in Souss, Berkane, Tadla, and Haouz regions, with a total surface area of 126.600 ha [2]. Despite the importance of the citrus sector in the national economy and its particular interest,

this sector has been seriously affected in recent years by biotic and abiotic factors [3]. In addition to the complications of production and marketing, numerous pest species and diseases reduce the quantity and quality of citrus fruits [4–6].

Among all, the black scale *Parlatoria ziziphi* (Lucas 1853) (Hemiptera: Diaspididae) is the most destructive pests to citrus. It is a cosmopolitan species and very abundant in citrus orchards in several countries. It originated from Europe and, later, from tropical and subtropical parts of the world on numerous hosts, especially on citrus [7–9].

The black parlatoria scale is one of the most significant pests attacking citrus trees in Morocco [10–12]. This pest causes considerable damage to citrus fruits in the Mediterranean basin and has a negative economic impact that translates into a loss of production in the Maghreb countries, including Morocco [13]. The damage caused by *P. ziziphi* consists of the weakening and drying of aerial parts of the tree, discoloration of the leaves and fruits, and, most importantly, a commercial loss in the fruits due to the scales fixed on it. Its specific resistance to fruit cleaning aggravates this cosmetic damage. Further, it is one of the most difficult armored scales to control with insecticides [14].

The life cycle of *P. ziziphi* including the duration of each larval (L1: Mobile larvae on the leaf; L2: Larvae fixed on the leaf), female (F1: Young female of small size with small black scale cover; F2: Female of medium size and medium scale cover; and F3: Adult female with a large scale cover, often carrying eggs), and male (pre-pupa, pupa, and adult) stage is influenced by the host plant, climatic conditions, and interaction with parasitoids [15,16]. *P. ziziphi* lays 10 to 20 eggs. There are two to five generations per year, sometimes six in countries where conditions are more favorable [17].

Spirotetramat (Movento 100 SC, BAYER SA) is a systemic insecticide that contains an active ingredient with a mode of action classified as Group 23 of the Insecticide Resistance Action Committee. It is a compound that belongs to the chemical class of ketoenols, and it is a lipid biosynthesis inhibitor (LBI) [18]. It is active against piercing–sucking insects by acting as an acetyl-CoA carboxylase (ACC) inhibitor, interrupting lipid biosynthesis in the insects [19,20].

In this study, we used field monitoring to investigate the appropriate dose of spirotetramat to control *P. ziziphi* without disturbing the natural functioning of the agroecosystem. More specifically, we investigated the low effective concentration of spirotetramat on *P. ziziphi* larvae and females inside citrus orchards.

2. Materials and Methods

2.1. Study Area

This work was carried in Bel Ksiri, Northeast of the Kenitra province, situated on the Gharb plain, North of Morocco (Figure 1). The study area was geographically located at a low altitude ranging from 300 to 500 m above sea level. The Gharb region is Morocco's largest agricultural area (600 Km^2) [21]. This region is well known for the production of citrus, cereals, and vegetable cultures due to the appropriate climate and soil properties [22]. The Gharb zone is characterized by the Mediterranean climate with annual precipitations ranging between 480 and 600 mm, and an average temperature of 27 °C during summer and 13 °C during winter.

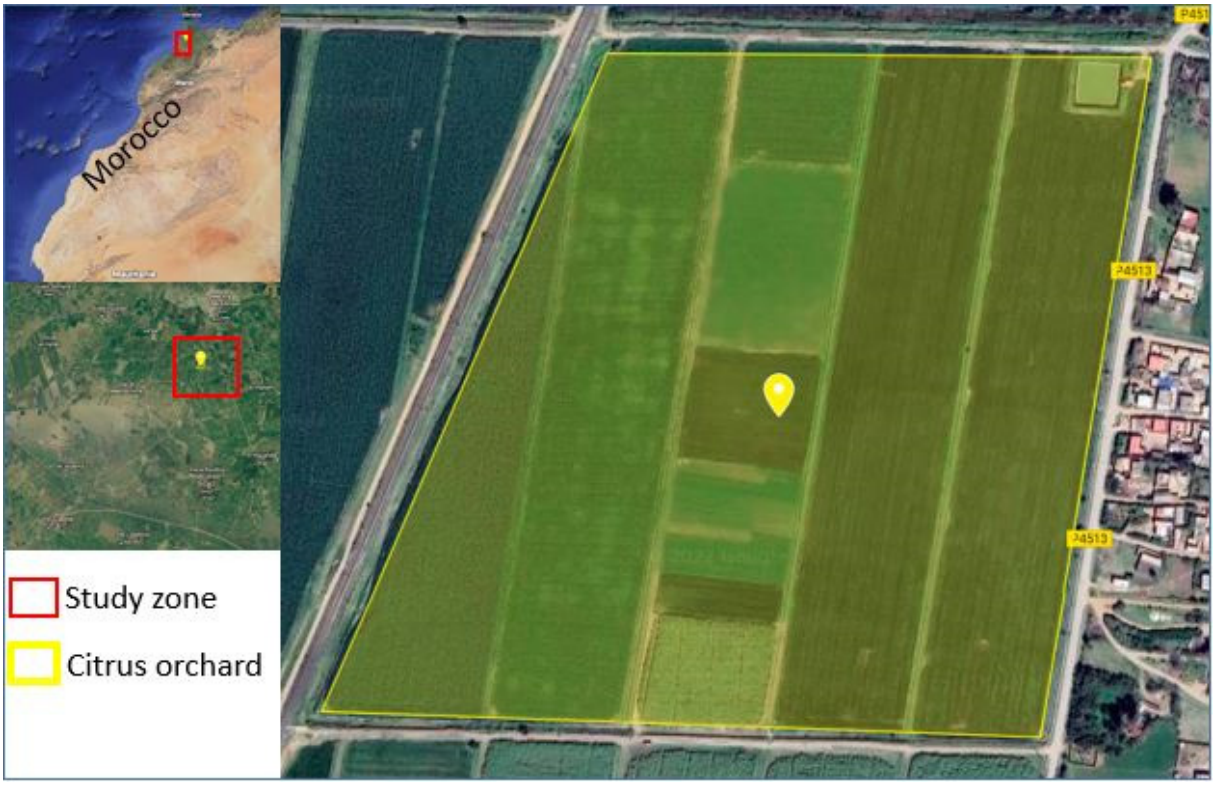

Figure 1. Location of studied orchards [23].

2.2. Sampling Design

To investigate the impact of spirotetramat on *P. ziziphi*, an orchard of 'Valencia Late' (*Citrus sinensis*) was selected and followed [22]. This product systemic (spirotetramat) has been used because of its effectiveness against a wide range of pests, including the diaspine scale insects, and its reduced damage to the environment and natural enemies. The orchard covers 4 ha on Valencia Late trees. The orchard was divided into 4 plots of 1 ha (Figure 2), and each plot was treated by a specific dose of the pesticide: (i) T0 treated only by water (as a control experiment), (ii) T1 = 0.625 L/ha, (iii) T2 = 0.755 L/ha, and (iv) T3 = 1 L/ha. In addition, the Teyme Eolo sprayer (Teyme Tecnologia Agricola, Girona, Spain), with its turbulent nozzle and exit diameter of 12 mm, delivered 1.55 L/min at a pressure of 20 bars. In addition, 2500 L of spray liquid were sprayed by a towed atomizer on each plot (1 ha), at a rate of 6 L of spray for each tree (Figure 2). These 3 concentrations were used based mainly on the quantities of pesticides used by local farmers.

To evaluate the effect of each concentration after treatment (1 to 8 weeks), 200 leaves of Valencia late trees in each plot were collected weekly and randomly from the different directions of the tree (North, East, South, and West), for a total of 20 trees by the plot that belonged to a square block (3 repetitions were performed independently). We left 2 lines between the different plots treated with spirotetramat. Then, we count the mortalities (M) and survival rates (V) of *P. ziziphi* at two stages: Larvae (L1 and L2) and females (F1, F2, and F3) on each leaf surface.

In the laboratory, the different stages of *P. ziziphi* on each leaf were determined and counted on both leaf surfaces; we counted the survival rates (V) and mortalities (M) of *P. ziziphi* at two larval stages (L1 and L2) and three generations of female (F1, F2, and F3) using a binocular microscope.

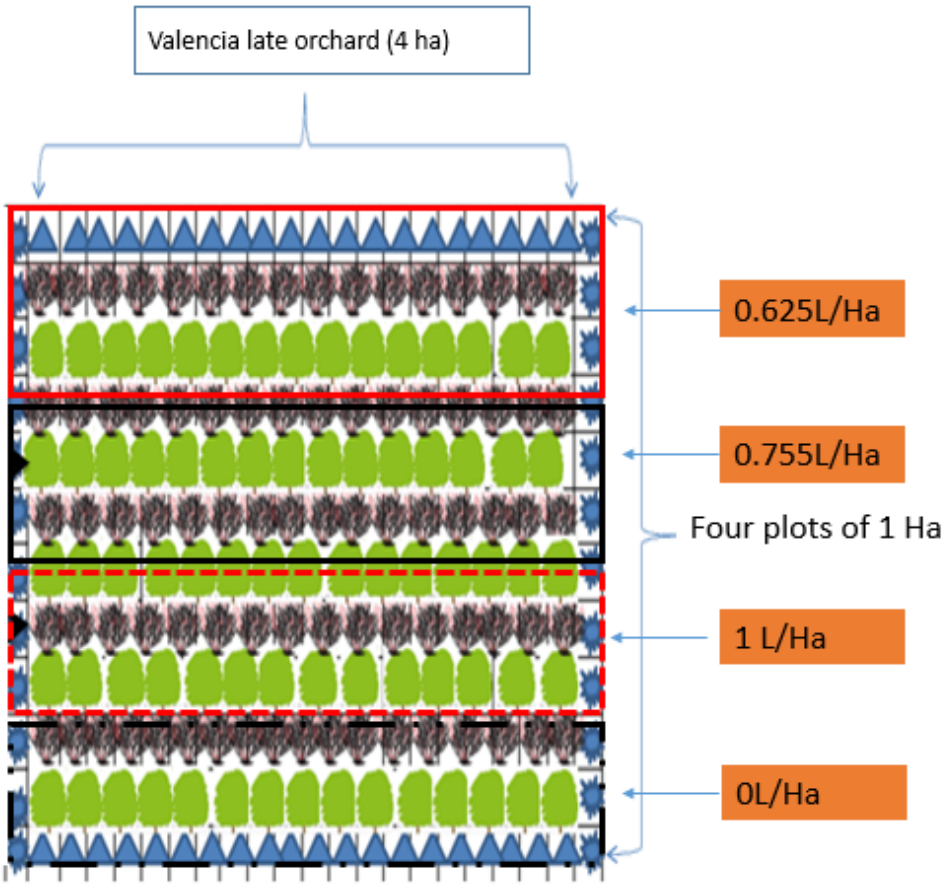

Figure 2. Model of the monitored orchard (divided into four small plots of 1 ha) and used doses of spirotetramat (T0 = 0 L/ha, T1 = 0.625 L/ha, T2 = 0.755 L/ha, and T3 = 1 L/ha).

2.3. Statistics

Statistics of data were done in Minitab software, version 1.1.19, LLC, USA. The results were given as mean ± SD. Moreover, to evaluate the effectiveness of spirotetramat, we calculated the survival (survived specimens/total sampled specimens) and mortality (inhibited specimens/total sampled specimens) of *P. ziziphi* for both larvae and females [23]. We checked for normality and homogeneity of variance for all variables with the Kolmogorov–Smirnov test. To assess the difference between the survival rates and mortalities between different stages of *P. ziziphi*, we used the independent t-test, considering the two stages as unrelated variables, while the effect of different doses of the systemic product was tested by the ANOVA One-way test followed by a post hoc Tukey test at $p < 0.05$. The linear correlation between different larvae and female stages was obtained by the Pearson correlation coefficient at $p < 0.05$. Principal component analyses were accomplished to elucidate the relationship between the treatment and different stages of *P. ziziphi*.

3. Results

3.1. Impact on Larvae

Out of 3965 larvae, 83% were inhibited, and only 17% survived after the treatment period. Specifically, during the first instar larval (L1) stage, 620 survived (16%), while 2234 were suppressed (56%). Moreover, in the second instar larval (L2) stage, 53 larvae survived (1%) and 1058 larvae were inhibited (27%). On the other hand, survival and mortality rates were very high during larval stage 1 (Figure 3), while these two parameters were low during larval stage 2. Mortalities and survival rates were uncorrelated during both stages (Table 1).

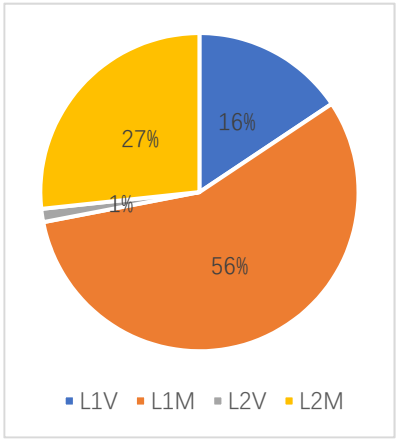

Figure 3. Comparison of mortalities (M) and survival rates (V) during two larvae stages of *P. ziziphi* (L1V: Surviving larvae of stage 1; L1M: Dead larvae of stage 1; L2V: Surviving larvae of stage 2; L2M: Dead larvae of stage 2).

Table 1. Relationship between the different stages of *P. ziziphi*.

	L1V	L1M	L2V	L2M	F1V	F1M	F2V	F2M	F3V
L1M	−0.197								
L2V	0.344	−0.390							
L2M	−0.396	0.036	−0.178						
F1V	0.445	−0.406	0.779	−0.352					
F1M	−0.244	−0.122	−0.323	0.256	−0.268				
F2V	0.269	−0.462	0.550	−0.204	0.580	−0.170			
F2M	−0.389	0.117	−0.451	0.440	−0.452	0.599	−0.269		
F3V	0.348	−0.296	0.542	−0.275	0.589	−0.283	0.860	−0.429	
F3M	−0.336	0.126	−0.390	0.296	−0.374	0.312	−0.099	0.617	−0.103

Regarding the efficacy of the doses on the different larval stages, all concentrations of spirotetramat tested were effective on stage 1 larvae survivors (L1V) with the means of T1 = 10.00 ^{B} ± 3.30, T2 = 7.75 ^{B} ± 3.17, and 8.08 ^{B} ± 2.49, while the T3 dose was very effective on stage 1 dead larvae (L1M) with an average of 41.13 ^{B} ± 10.82. Further, all the tested doses (T1, T2, and T3) were effective against stage 2 larvae (Table 2). Furthermore, all doses of this systemic product were positively correlated with second instar larval Inhibited (L2M) specimens. The T3 concentration was positively correlated with first instar larval Inhibited (L1M) specimens. In contrast, all doses tested were negatively correlated with surviving larvae of the first instar and second instar (L1V and L2V) (Figure 4).

Table 2. Survival (V) and mortality (M) of *Parlatoria ziziphi* larvae (L1,L2) treated with different doses of spirotetramat T0 = 0 L/ha, T1 = 0.625 L/ha, T2 = 0.755 L/ha, and T3 = 1 L/ha.

	L1V	L1M	L2V	L2M
T0	14.13 ^{A} ± 4.79	21.13 ^{A} ± 7.96	3.42 ^{A} ± 1.67	9.13 ^{A} ± 5.90
T1	10.00 ^{B} ± 3.30	27.92 ^{A} ± 10.60	1.00 ^{B} ± 1.38	14.42 ^{B} ± 5.24
T2	7.75 ^{B} ± 3.17	24.04 ^{A} ± 7.32	0.67 ^{B} ± 0.87	13.71 ^{B} ± 4.51
T3	8.08 ^{B} ± 2.49	41.13 ^{B} ± 10.82	0.54 ^{B} ± 0.59	15.96 ^{B} ± 4.43

Means in the same column with different superscripts are significantly different ($p < 0.05$).

Figure 5 shows that the survival means of the larval stage in the untreated plot increased significantly from week 1 (15.67 ^{AB} ± 2.06) to week 8 (22.67 ^{A} ± 1.15). On the other hand, the average for surviving larvae remained low for all weeks after treatment with all concentrations of spirotetramat (Table 3).

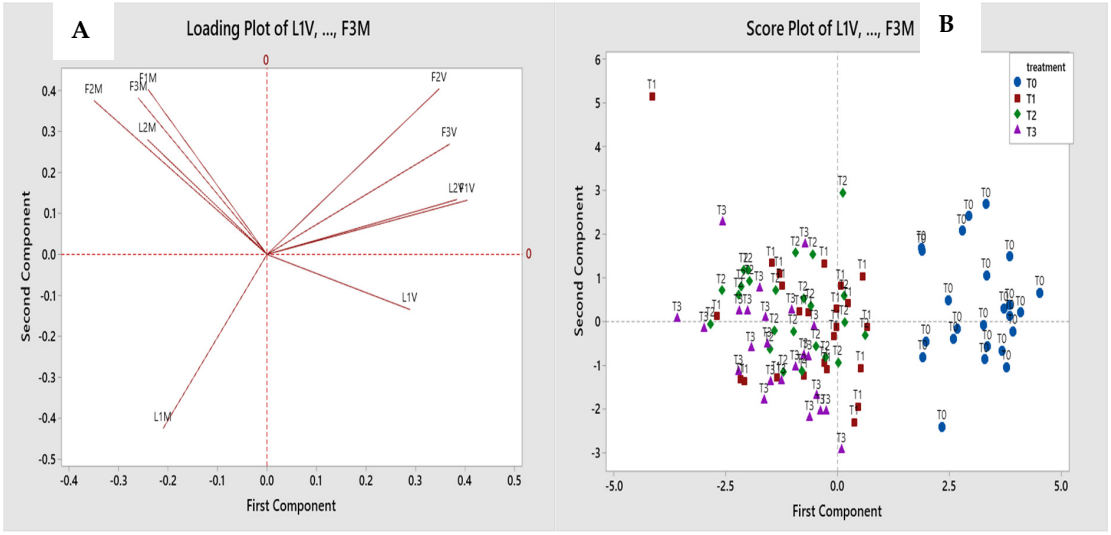

Figure 4. Principal component analysis (PCA) of the different stages of *P. ziziphi* according to the different treatment rates (**A**); double projection diagram for the two components (**B**).

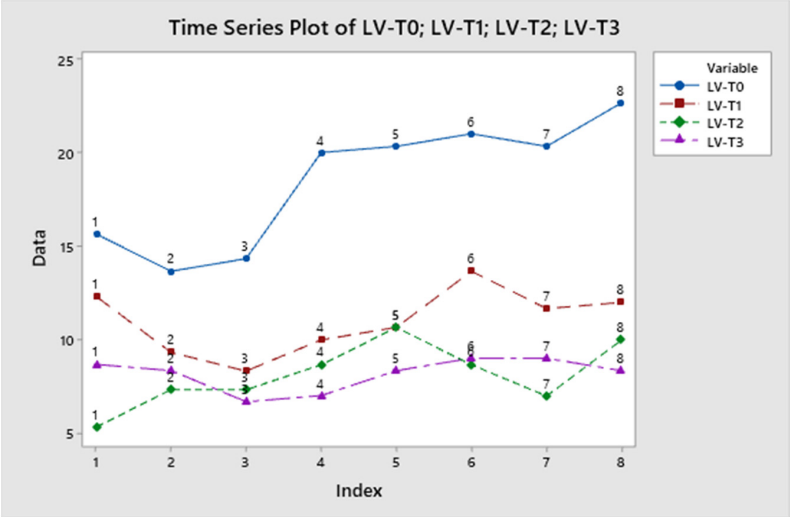

Figure 5. Evolution of the larvae survival of *Parlatoria ziziphi* according to different follow-up dates after treatment.

Table 3. Survival (V) of *Parlatoria ziziphi* larvae treated with different doses of spirotetramat (T0 = 0 L/ha, T1 = 0.625 L/ha, T2 = 0.755 L/ha, and T3 = 1 L/ha) according to different follow-up dates after treatment.

	LV-TO	LV-T1	LV-T2	LV-T3
Week 1 BT	15.67 ^{AB} ± 2.06	12.33 ^{A} ± 2.31	5.33 ^{A} ± 1.31	8.66 ^{A} ± 0.57
Week 2 BT	13.67 ^{B} ± 0.57	9.33 ^{A} ± 1.53	7.33 ^{A} ± 1.52	8.33 ^{A} ± 0.57
Week 3 BT	14.33 ^{B} ± 2.62	8.33 ^{A} ± 2.51	7.33 ^{A} ± 1.15	6.67 ^{A} ± 4.51
Week 4 BT	20.00 ^{AB} ± 2.65	10.00 ^{A} ± 2.57	8.67 ^{A} ± 2.52	7.00 ^{A} ± 1.00
Week 5 BT	20.33 ^{AB} ± 2.31	10.68 ^{A} ± 1.52	10.67 ^{A} ± 2.50	12.00 ^{A} ± 3.00
Week 6 BT	21.00 ^{AB} ± 2.65	13.67 ^{A} ± 2.21	8.67 ^{A} ± 1.16	9.00 ^{A} ± 1.73
Week 7 BT	20.33 ^{AB} ± 2.08	11.67 ^{A} ± 1.04	7.00 ^{A} ± 1.73	9.00 ^{A} ± 2.00
Week 8BT	22.67 ^{A} ± 1.15	12.00 ^{A} ± 2.45	8.33 ^{A} ± 1.15	8.33 ^{A} ± 1.55

Means in the same column with different superscripts are significantly different ($p < 0.05$).

3.2. Impact on Females

Among the 11,229 females recorded (Figure 6), 93% (n = 10,473) were inhibited, while only 7% (n = 756) were intact after the treatment period. Specifically, during the F1 generation, only 1% (n = 76) survived, while 14% (n = 1574) were inhibited. Furthermore, during the F2 generation, 2% (n = 225) survived and 30% (n = 3377) were eliminated. Similarly, 4% (n = 455) survived and 49% (n = 5522) were inhibited during the F3 generation.

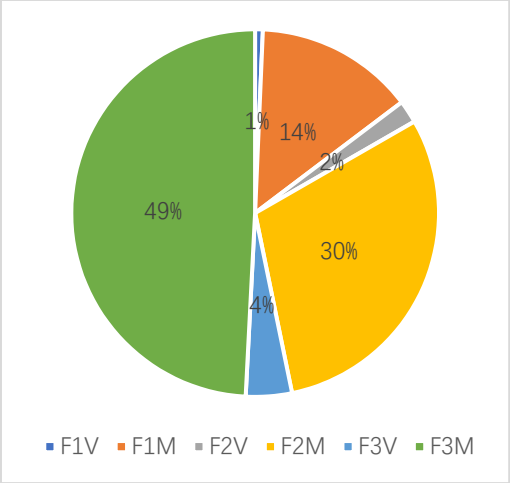

Figure 6. Comparison of mortalities (M) and survival rates (V) during three female stages of *P. ziziphi*. (F1V: Survived females of stage 1; F1M: Dead females of stage 1; F2V: Surviving females of stage 2; F2M: Dead females of stage 2; F3V: Surviving females of stage 3; F3M: Dead females of stage 3).

In terms of comparisons between stages, the means of surviving females were higher in F3, followed by F2 and then F1. Similarly, inhibited females were more numerous in F3, followed by F2 and F1. On the other hand, survival averages were variable for all periods. Similarly, the means of females eliminated from the three stages were significantly different during the entire follow-up period (Table 4).

Table 4. Survival (V) and mortality (M) of *Parlatoria ziziphi* females (F1, F2, and F3) treated with different doses of spirotetramat (T0 = 0 L/ha, T1 = 0.625 L/ha, T2 = 0.755 L/ha, and T3 = 1 L/ha).

	F1V	F1M	F2V	F2M	F3V	F3M
T0	6.08 ^{A} ± 2.59	13.38 ^{B} ± 4.86	9.43 ^{A} ± 3.51	27.88 ^{A} ± 6.40	16.88 ^{A} ± 5.71	64.50 ^{A} ± 15.71
T1	1.38 ^{B} ± 1.17	23.21 ^{A} ± 11.33	3.29 ^{B} ± 2.45	43.42 ^{B} ± 14.27	6.67 ^{B} ± 3.10	76.54 ^{A} ± 22.41
T2	1.13 ^{B} ± 0.99	24.00 ^{A} ± 6.08	3.50 ^{B} ± 2.41	49.92 ^{B} ± 11.55	5.92 ^{B} ± 3.41	77.04 ^{A} ± 22.01
T3	0.67 ^{B} ± 0.90	18.38 $^{A\,B}$ ± 7.57	2.58 ^{B} ± 2.55	47.38 ^{B} ± 16.05	6.83 ^{B} ± 2.20	76.50 ^{A} ± 23.29

Means that do not share a letter are significantly different.

The three doses were all effective on the survival rates of females in all three stages (F1V, F2V, and F3V) with averages of T1 = 1.38 ^{B} ± 1.17, T2 = 1.13 ^{B} ± 0.99, and T3 = 0.67 ^{B} ± 0.90. The concentrations T1 = 23.21 ^{A} ± 11.33 and T2 = 24.00 ^{A} ± 6.08 were effective on stage 1 dead females (F1M). What is more, all doses of this systemic product tested were effective on dead females of stage 2 (F2M) with the averages T1 = 43.42 ^{B} ± 14.27, T2 = 49.92 ^{B} ± 11.55, and T3 = 47.38 ^{B} ± 16.05, while spirotetramat showed no effect on stage 3 females (F3M) (Table 4). In addition, the doses of this systemic product were positively correlated with inhibited females of all stages (F1M, F2M, and F3M), while all doses tested were negatively correlated with surviving females (F1V, F2V, and F3V) (Figure 4).

Figure 7 shows that female survival in the untreated plot decreased significantly from week 1 (46.00 ^{A} ± 5.56) to week 8 (28.00 ^{B} ± 2.00 14.33 ^{AB} ± 2.45). While the mean survival

of females in the 0.625 L/ha treated plot was very high in the first week after treatment (21.00 $^{A} \pm 2.31$), the means then decreased significantly over the last 7 weeks after treatment to (14.33 $^{AB} \pm 2.45$) in week 8 (Table 5). The survival averages of the females remained low for all 8 weeks after treatment with the concentrations 0.755 L/he and 1 L/he (Table 5).

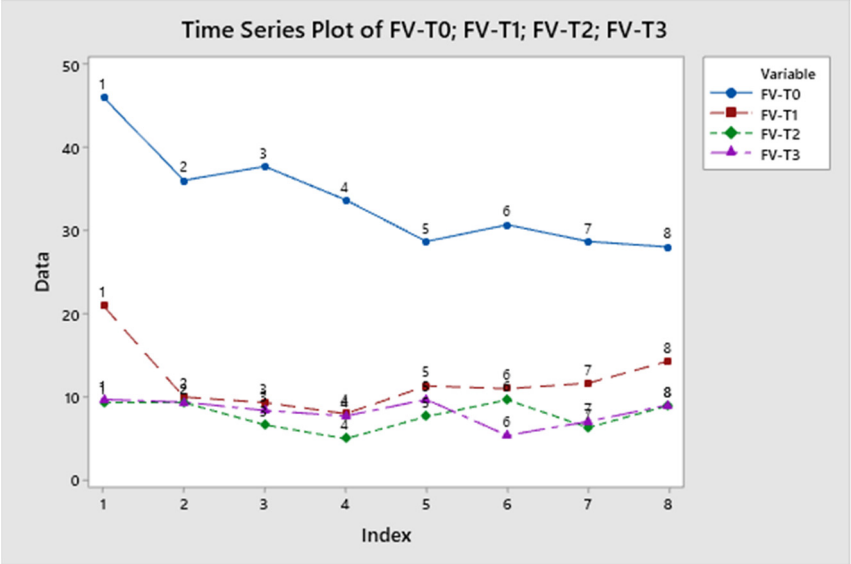

Figure 7. Evolution of females' survival of *Parlatoria ziziphi* according to the different follow-up dates after treatment.

Table 5. Survival (V) of *Parlatoria ziziphi* females treated with different doses of spirotetramat (T0 = 0 L/ha, T1 = 0.625 L/ha, T2 = 0.755 L/ha, and T3 = 1 L/ha) according to different follow-up dates after treatment.

	FV-TO	FV-T1	FV-T2	FV-T3
Week 1 BT	46.00 $^{A} \pm 5.56$	21.00 $^{A} \pm 2.31$	9.33 $^{A} \pm 0.58$	9.68 $^{A} \pm 0.57$
Week 2 BT	36.00 $^{AB} \pm 5.33$	10.00 $^{B} \pm 1.53$	9.33 $^{A} \pm 1.53$	9.33 $^{A} \pm 0.57$
Week 3 BT	37.67 $^{AB} \pm 3.21$	9.33 $^{B} \pm 2.51$	6.67 $^{A} \pm 1.53$	8.33 $^{A} \pm 4.51$
Week 4 BT	33.67 $^{AB} \pm 2.52$	8.00 $^{B} \pm 0.57$	5.00 $^{A} \pm 1.73$	7.67 $^{A} \pm 1.00$
Week 5 BT	28.67 $^{B} \pm 2.08$	11.33 $^{B} \pm 1.52$	7.67 $^{A} \pm 1.52$	9.66 $^{A} \pm 3.00$
Week 6 BT	30.67 $^{B} \pm 2.89$	11.00 $^{B} \pm 2.21$	9.67 $^{A} \pm 2.31$	5.33 $^{A} \pm 1.73$
Week 7 BT	28.67 $^{B} \pm 4.04$	11.67 $^{B} \pm 1.04$	6.33 $^{A} \pm 1.15$	7.00 $^{A} \pm 2.00$
Week 8 BT	28.00 $^{B} \pm 2.00$	14.33 $^{AB} \pm 2.45$	9.00 $^{A} \pm 2.65$	9.00 $^{A} \pm 1.55$

Means that do not share a letter are significantly different.

4. Discussion

Based on our nationwide survey, this is the first research on the control of *P. ziziphi* using a systemic product, spirotetramat. Our main objective was to provide detailed and extensive data on the efficacy of this systemic product in controlling this pest. These results are the first and only data supplied in the control of *P. ziziphi* in citrus in North Africa, which is of great importance for further comparative research and the adoption of less environmentally damaging and more effective doses to control the pest.

Our results showed that in the larval stage, inhibition was nearly 83%, while in females, the inhibition rate was more than 92%. All three doses tested were effective on the survival rates of females in all three stages (F1V, F2V, and F3V) during the whole follow-up period, and the same results were reported by [24], where spraying spirotetramate on citrus fruit reduced the survival rate and fecundity of the mealybug (*Lepidosaphes beckii*) to 100%

172 days after treatment. In addition, spirotetramat showed no effect on the mortality rates of stage 3 females (F3M). This may be due to other unknown factors that contributed to the increased mortality in the untreated plot. In Egypt, the same results were reported on navel orange trees, *Citrus sinensis*, which reported high mortality due to unknown factors [7]. All doses of spirotetramat tested were effective on stage 1 larvae, while the T3 concentration was very effective on stage 2 larvae. According to the findings of Nauen [25], spirotetramat causes death in larvae deposited by adult female aphids after 24 h of its application. At the larval stages, the efficacy of the doses used was variable. Regarding the mortality rate, the T3 concentration (1 L/he) was effective against larva 1, while all three doses tested were effective on the survival rate of larva 1, while on larva stage 2, all concentrations showed the same efficacy. Our objective was to search for lower concentrations of pesticides that have effective control against *P. ziziphi*. This will reduce pesticide use and avoid pesticide resistance [26–28]. Therefore, for easy marketing, the remaining residues are suggested to be less on treated fruits [29,30].

Among the total population of 15,194 treated with this systemic product, 90.59% were inhibited, and only 9.41% were intact after treatment. Similar results were reported in Morocco and Algeria where spirotetramat controlled *Parlatoria pergandii* with encouraging results in the citrus orchard [23,31]. All doses of spirotetramat used showed a significant effect on larvae and females of *P. Ziziphi*. All the concentrations of spirotetramat used were effective on the population of *Parlatoria blanchardi* of date palm. Among all concentrations tested, we noticed that the survival rate remained low for 8 weeks after the application of the treatment. According to [24], spirotetramat spraying on citrus fruit resulted in 100% lower survival and fecundity of Lepidosaphes beckii for 172 days after treatment. Therefore, the choice of the right concentration remains a necessity in order to preserve the agroecosystem. In general, large amounts of pesticides control pests but negatively influence natural enemies and the environment [32]. In contrast, the combination of the effect of parasitism (natural enemies) and low doses of pesticides would provide effective and environmentally friendly pest control [23].

5. Conclusions

Our findings highlight two key messages: (1) The data indicated that spirotetramat was effective on larvae and females of *P. ziziphi* and (2) all doses tested were effective on the *P. ziziphi* population. Since spirotetramat is an effective and widely used pesticide, further studies on its effect on other natural enemy populations and the residues remaining on fruits are needed to establish appropriate management strategies.

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