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The Survival and Parasitism Rate of *Tamarixia radiata* (Hymenoptera: Eulophidae) on Its Host Exposed to *Beauveria bassiana* (Ascomycota: Hypocreales)

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Abstract: The effect of the entomopathogenic fungus *Beauveria bassiana* (BB-12) on the biological characteristics of *Tamarixia radiata* parasitizing *Diaphorina citri* was studied under laboratory conditions. Twenty 3rd–5th instar nymphs were exposed to a single already-mated female parasitoid (1 day old) and removed after 24 h. Subsequently, the nymphs were sprayed at 1, 24 and 48 h post-exposure with 1×10^8 conidial mL^{-1} suspension. The percentage of parasitism recorded was 22%, 35% and 41% at 1, 24 and 48 h, respectively. The emergence rate varied between 28%, 51% and 49% at 1, 24 and 48 h, respectively. In a subsequent experiment, nymphs of *D. citri* were sprayed with 1×10^8 conidial mL^{-1} suspension and then exposed to *T. radiata* at 1, 24 and 48 h post-spraying to allow for parasitism to occur. The percentage of parasitism recorded was 18%, 27% and 28% at 1, 24 and 48 h, respectively, significantly lower than the parasitism rate recorded in the control (48%). The percentage of emergence varied between 24%, 44% and 45% at 1, 24 and 48 h, respectively. In both experiments, no significant difference was observed in the pre-adult duration (days) and the sex ratio of F1 generation. Meanwhile, significant differences were observed in the longevity of the F1 generation of females and males of *T. radiata* in a treatment consisting of spraying the fungal suspension and the control. Overall, the findings of the current study revealed a negative interaction between *T. radiata* and *B. bassiana* in controlling *D. citri* nymphs. This outcome is believed to be a result of the antagonistic effects of *B. bassiana* on the developmental process of the pre-adult stages of the parasitoid. However, our results also show that with a properly timed application (allowing parasitism to occur over an extended period of time before the application of the fungus), *T. radiata* could potentially be used in combination with *B. bassiana* for the successful biological control of *D. citri*. This should be carried out in order to minimize the potentially negative interactions between these two biological agents.

Keywords: entomopathogenic fungus; *Diaphorina citri*; biological control; parasitism; survival rate

1. Introduction

Diaphorina citri Kuwayama (Hemiptera: Liviidae) is the primary vector of the citrus greening disease, otherwise known as Huanglongbing (HLB) [1–3]. The disease of HLB has severely affected the citrus industry worldwide [4]. Chlorpyrifos, imidacloprid, and bifenthrin are the synthetic insecticides most frequently used to protect the citrus plantation

from *D. citri* [5–7]. Their overuse, however, has led to developed resurgence problems, pesticide residues and insecticide resistance in *D. citri* populations [8,9]. The current food market preferences increasingly require farmers growing horticulture to use pesticide-free products and to reduce the use of conventional broad spectrum chemical pesticides [10]. Consequently, a suitable alternative to chemical insecticides is the use of biological control agents, mainly entomopathogenic fungi [11–14] and the primary-associated ectoparasitoid *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) [15].

In the past 15 years, the development and use of entomopathogens as classical, conservational and augmentative biological control agents have included a number of successes and some setbacks [10]. Several studies suggested the use of entomopathogenic fungi against *D. citri*, including *Isaria fumosorosea* [14,16,17]; *Metarhizium anisopliae* and *Cordyceps bassiana* [18]; and *Hirsutella citriformis* [19,20]. The ectoparasitoid *T. radiata* is a well-known and principal natural enemy used for the biological control of the major citrus pest *D. citri* [21–23]. *T. radiata* has been extensively used in many countries as a classical biological control agent due to its high parasitism capacity and field adaptation [24–27]. Other control agents include predators such as lady beetles, lacewings and spiders [28].

In integrated pest management control, including the use of parasitoids in combination with entomopathogenic fungi may improve pest management efficacy. However, interspecific competition between two or more species of natural enemies, interacting in the same host, could result in complex multitrophic interactions and outcomes which can be synergistic, addictive or antagonistic with respect to host mortality [29–31]. A pathogen with a broader host range in favorable environmental conditions can easily infect the natural enemies [30] but, if the pathogen has a specific host range, it could interact with the parasitoid and enhance the efficacy of controlling the host pests [32,33]. Therefore, the strategies that incorporate fungi in combination with arthropod predators and parasitoids need to be defined/explored to ensure compatibility and maximize efficacy. The present study provides information to better understand the potential interaction of the entomopathogenic fungus *Beauveria bassiana* on the survival and parasitism rate of the ectoparasitoid *T. radiata*, by controlling *D. citri* under controlled conditions.

2. Materials and Methods

2.1. Insects Culture

Diaphorina citri and *Tamarixia radiata* were obtained from the laboratory of Insect Ecology and the Biological Control of Fujian Agricultural and Forestry University (FAFU), Fuzhou, China. Both populations were maintained for about ten generations, prior to the commencement of the experiments on orange jasmine (*Murraya paniculata*) (Sapindales: Rutaceae), in mesh cages (0.60 m wide, 0.50 m deep, 0.50 m high) grown in a climate room at 25 ± 2 °C, 75 ± 5% relative humidity and under 14 h of photoperiod (from 6:00–20:00).

2.2. Fungal Strain and Conidia Suspension Preparation

Beauveria bassiana strain 12 (BB-12) was obtained from the fungal culture bank of the Insect Ecology and Biological Control laboratory of Fujian Agriculture and Forestry University, Fuzhou, China, based on the source and the findings of preliminary studies conducted in the laboratory [34–36]. The strain was identified using morphological description through the keys of Humber [37,38]. To confirm the identity of the isolate, molecular identification was carried out in addition to the morphological structures using the sequence data from the ITS region of nuclear rRNA (GenBank accession number MG844429). Fungal strain BB-12 was sub-cultured on Potato Dextrose Agar (PDA) culture media in 90 mm × 15 mm Petri dishes at a constant temperature of 25 ± 2 °C, 70–75% relative humidity (RH) in complete darkness for 18–20 days to obtain conidia. Under sterile conditions, conidia were harvested by scraping the fungal mycelia using a sterile spatula and then suspending in sterile distilled water (SDW) containing the surfactant Tween 80 (0.01%). Later on, conidia were removed from the hyphal and homogenized by vortexing the mixture for 4 min, then filtered using a sterile syringe and cotton wool to remove

hyphal debris. Finally, conidial concentration was adjusted to 1×10^8 conidia mL^{-1} under the microscope using a Neubauer hemocytometer. Conidia viability was determined by plating 100 µL of the first serial dilution on 2.5% water agar and then incubated at 25 °C for 24 h. The viability was assessed by counting the number of germinated conidia. A conidium was considered to be viable when it developed and projected a germ tube that was longer than half of its diameter.

2.3. Diaphorina Citri First Parasitized by Tamarixia radiata and Afterward Treated with Beauveria bassiana Suspension

The study investigated the effects of the entomopathogenic fungus *B. bassiana* on the survival and developmental rate of *T. radiata* feed on *D. citri* nymphs. The parasitized nymphs were treated by spraying 1×10^8 conidial ml^{-1} suspension of *B. bassiana* at 1, 24, and 48 h after exposure to the parasitoids. For the control, sterile distilled water containing the surfactant Tween 80 (0.01%) was sprayed.

For each treatment, 10 rearing bottles made of plastic (20 cm in height and 8 cm in diameter) were used. To permit air circulation, perforated openings were made, and then covered with a fine mesh. For each bottle, *M. paniculata* shoots, containing 20 3rd–5th instar nymphs of *D. citri* each, were used. Shoots were 15 cm in height and placed in small 25 mL Polypropylene Deli Containers containing cotton and socked with water to keep the shoots fresh. Afterward, one female of *T. radiata* (1 day old), previously mated, was released in the individual container to allow parasitism for 24 h, after which the parasitoid was removed. Subsequently, potentially parasitized nymphs were sprayed with the suspension at 1, 24, and 48 h after parasitism. Thereafter, containers were kept in climate chambers at 25 ± 2 °C, 70 ± 5% RH, and 14 h:10 h dark photoperiod to enable an evaluation of the emergence of adult parasitoids.

Each treatment was replicated 10 times, with each replicate consisting of 20 *D. citri* nymphs per each *M. paniculata* shoot. In total, 200 nymphs were examined per treatment. The developmental parameters estimated were: percent of parasitism, preadult duration, percent of emergence, sex ratio, survival and longevity. The percentage of parasitism was evaluated on the 6th day based on the mummies formation. The sex ratio was determined by counting the total female individuals and dividing this by the total offspring for each replicate. For the longevity of *T. radiata*, emerged females and males per treatment were each individually kept in 50 mL Polypropylene Deli Containers with the openings covered with a fine mesh for air circulation. Honey was provided as food and placed in a climate chamber at 25 ± 2 °C, 70 ± 5% RH, for 14 h of photoperiod, and the longevity was monitored until the death of all individuals.

2.4. Diaphorina Citri First Sprayed with Beauveria bassiana and Afterward Exposed to Tamarixia radiata

For this experiment, *D. citri* nymphs were treated first with the suspension of *B. bassiana* by spraying 1×10^8 conidial mL^{-1} and at 1, 24, and 48 h before spraying, *T. radiata* was released to allow parasitism for 24 h. For this study, the methodology previously described above was used, where 20 *D. citri* nymphs were used per one female of *T. radiata*, with 10 replicates per treatment and sterile distilled water was used as the control. Similar developmental parameters, as described above, were evaluated. The study was set up using a complete randomized design (CRD); consisting of three treatment groups (1, 24 and 48 h) and 10 replicates per treatment.

2.5. Statistical Analysis

Raw data obtained were subjected to a test of the normality and homoscedasticity of the variance before the statistical analysis. To test normality, the Anderson Darling test was used ($p > 0.05$); where raw data was abnormal ($p < 0.05$), the Bliss transformation was used. To test homoscedasticity, the Bartlet test was used when the data were normal, while the Levene test was used when the data were not normal. For all the analyses, the statistical software, Minitab 16, was used. Survival statistics were performed using Kaplan-Meier-

analysis and subsequent Log-Rank-test for the F1-progeny. Data were analyzed using analysis of variances (ANOVA, $\alpha = 0.05$), followed with pairwise comparison of means post hoc with Tukey ($p < 0.05$). These statistical analyses were performed using SPSS 19.0 (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Diaphorina Citri Parasitized by Tamarixia radiata and Subsequently Treated with Beauveria bassiana (BB-12)

The parasitism rate showed significant differences between the control and the treatment consisting of spraying the fungal suspension 1 h after parasitism ($F_{3.36} = 38.92$; $p < 0.0001$), Figure 1A. The parasitism rates at 24 and 48 h were 39% and 42%, respectively. The fungal suspension application after parasitism had no influence on the emergence rate of *T. radiata* F1 generation. The emergence rates at 1, 24, and 48 h were 29%, 60%, and 55%, respectively. However, significant differences were observed between the control and the treatment consisting of spraying the fungal suspension ($F_{3.36} = 63.18$; $p < 0.0001$). In addition, no significant differences ($F_{3.36} = 63.18$; $p = 0.6722$) were observed in the emergence rate in the treatment consisting of spraying the fungal suspension 24 and 48 h after parasitism (Figure 1B). The nymphs that were not parasitized during the 24 h that the parasitization was allowed, were infected by *B. bassiana*; some of them died because of fungal infection while others emerged as adults and died a few days later.

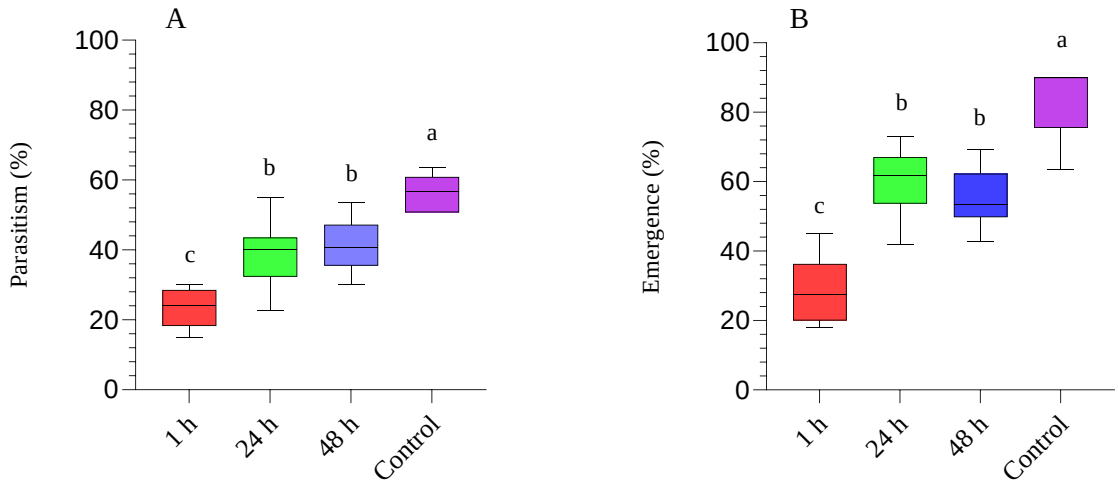

Figure 1. Percentage of parasitism (**A**) and emergence (**B**) of *Tamarixia radiata* from nymphs where Diaphorina citri nymphs were first parasitized by *Tamarixia radiata* and subsequently treated with Beauveria bassiana (BB-12) at 1, 24, and 48 h after parasitism. Means followed by the same letter are not significantly different (Tukey, $p < 0.05$).

There was no statistical difference in the pre-adult duration of the parasitoid (days) among the treatments and the control ($p > 0.05$). The sex ratio of the F1 generation of *T. radiata* was statistically similar between treatment and control ($p > 0.05$) (Table 1).

Table 1. Means and SE of the pre-adult duration, sex ratio and longevity of *Tamarixia radiata* when *Diaphoria citri* nymphs were first parasitized by *Tamarixia radiata* and subsequently treated with *Beauveria bassiana* (BB-12).

Treatment	n° Nymphs Exposed	Pre-Adult Duration		Sex Ratio
		Female	Male	
1 h	20	10.35 ± 0.11a	9.15 ± 0.10a	0.48 ± 0.06a
24 h	20	10.85 ± 0.25a	9.45 ± 0.15a	0.56 ± 0.04a
48 h	20	10.90 ± 0.10a	9.60 ± 0.16a	0.53 ± 0.04a
Control	20	10.20 ± 0.16a	9.40 ± 0.12a	0.58 ± 0.01a

Means in the same column with the same letter are not significantly different (Tukey, $p < 0.05$).

The values of the survival rate of *T. radiata* females and males are plotted in Figure 2. Results showed that when applying *B. bassiana* after parasitism, the median survival time of *T. radiata* females was significantly reduced ($p < 0.0001$), from 31 days in the control to 14, 20 and 27 days at 1, 24 and 48 h, respectively (Figure 2A). The median survival time of *T. radiata* males was also significantly reduced ($p < 0.0001$), from 19 days in the control to 11 and 14 days at 1 and 24 h, respectively. Meanwhile there were no significant differences ($p = 0.3208$) between the male median survival in the control and at 48 h of parasitism, with the median survival time of 19 days seen in both treatments (Figure 2B).

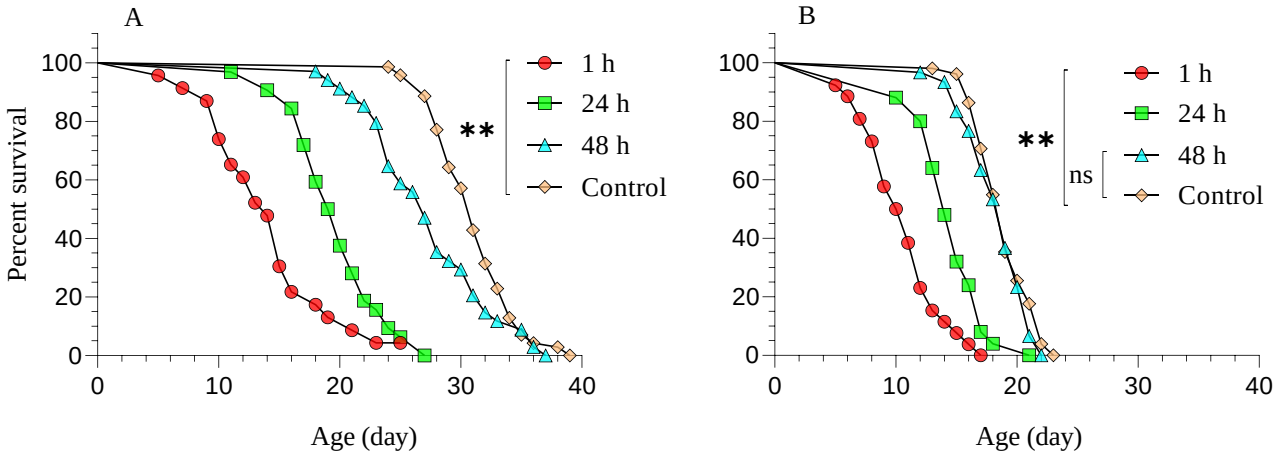

Figure 2. Survival rate for females (**A**) and males (**B**). Survival curves of *Tamarixia radiata* by log-rank analysis were generated where *Diaphorina citri* nymphs were first parasitized by *Tamarixia radiata* and subsequently treated with *Beauveria bassiana* (BB-12) (Females $n = 23$, 32, 34 and 70 at 1 h, 24 h, 48 h and control, respectively) (Males $n = 26$, 25, 30 and 51 at 1, 24, 48 h and control, respectively). (** $p < 0.001$).

There were statistically significant differences between group means per sample in the longevity of F1 generation of females and males of *T. radiata*, as determined by one-way ANOVA ($F_{3.13} = 57.59$; $p < 0.0001$) and ($F_{3,11} = 55.45$; $p < 0.0001$), respectively. However, no statistically significant differences were observed in the longevity of females ($p = 0.3502$) and males ($p = 0.9190$) in the treatment group consisting of spraying *B. bassiana* suspension 48 h after parasitism and the control group (Figure 2A,B).

3.2. Diaphorina Citri Treated with Beauveria bassiana (BB-12) and Parasitized by Tamarixia radiata

The parasitism rate by *T. radiata* showed a significant difference between the control and the treatment consisting of spraying the fungal suspension at 1, 24, and 48 h before parasitism ($F_{3.36} = 62.71$; $p < 0.0001$) (Figure 3A). However, no significant differences were observed when *T. radiata* was parasitized at 1, 24, and 48 h after the fungal suspension application ($p = 0.9526$). The parasitism rates at 1, 24 and 48 h were 16%, 20% and 22%, respectively.

The parasitoid releasing time after fungal suspension application had no influence on the emergence rate of the *T. radiata* F1 generation. The emergence rates at 1, 24, and 48 h were 13%, 40.18%, and 45.43%, respectively. However, significant differences ($F_{3.36} = 70.60$; $p < 0.0001$) were observed between the control and the treatment where *D. citri* was first treated with *B. bassiana* and subsequently parasitized by *T radiata*, while no significant differences ($p = 0.7096$) were observed at 24 and 48 h (Figure 3B).

Pre-adult duration of the parasitoid (days) among the treatment groups and the control were statistically similar. There was no statistical difference between treatment and control concerning the sex ratio of the F1 generation of *T. radiata*. ($p < 0.005$) (Table 2).

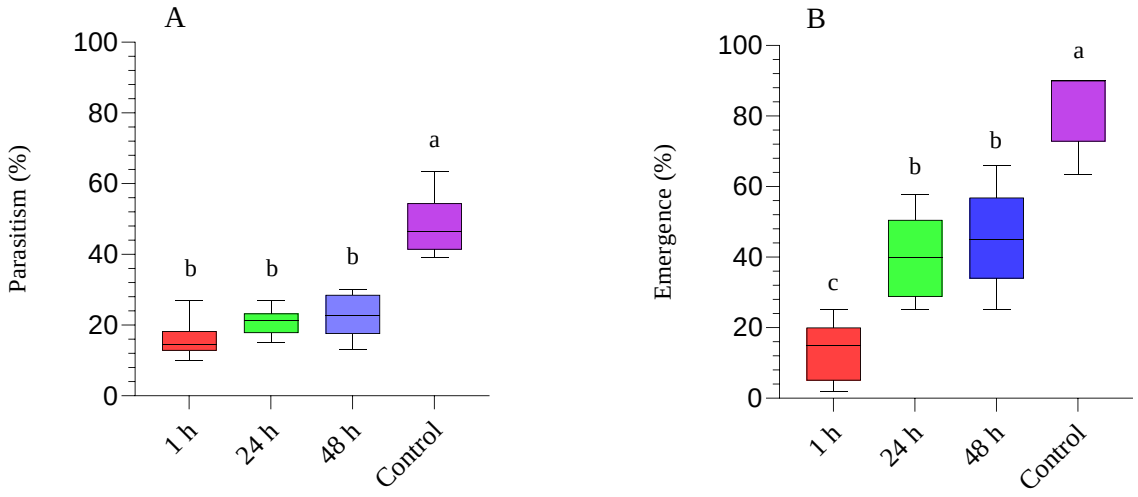

Figure 3. Percentage of parasitism (**A**) and emergence (**B**) of *Tamarixia radiata* from nymphs where *Diaphorina citri* first were treated with *Beauveia bassiana* (BB-12) and subsequently exposed to *Tamarixia radiata* (parasitoids were released at 1, 24, and 48 h after *Beauberia bassiana* application). Means followed by the same letter are not significantly different (Tukey, $p < 0.05$).

Table 2. Means and SE of the pre-adult duration, sex ratio and longevity of *Tamarixia radiata* when *Diaphorina citri* nymphs were first treated with *Beauveria bassiana* (BB-12) and subsequently parasitized by *Tamarixia radiata*.

Treatment	n° Nymphs Exposed	Pre-Adult Duration		Sex Ratio
		Female	Male	
1 h	20	10.35 ± 0.16a	9.25 ± 0.13a	0.28 ± 0.13a
24 h	20	10.80 ± 0.20a	9.30 ± 0.13a	0.31 ± 0.10a
48 h	20	10.50 ± 0.19a	9.40 ± 0.12a	0.41 ± 0.11a
Control	20	10.40 ± 0.16a	9.45 ± 0.11a	0.51 ± 0.02a

Means in the same column with the same letter are not significantly different (Tukey, $p < 0.05$).

In the experiment where *T. radiata* were released after *B. bassiana* spraying, the median survival rate of *T. radiata* females was significantly reduced ($p < 0.0001$) to 9, 12, and 11 days at 1, 24, and 48 h, respectively, in comparison to 29 days in the control; however, there were no significant differences ($p = 0.1984$) between the different parasitoid's realizing time (Figure 4A). The median survival rate of *T. radiata* males was significantly reduced ($p < 0.0001$) to 6, 10, and 10 days at 1, 24, and 48 h, respectively, from 16 days in the control; however, there were no differences ($p = 0.5817$) between the median survival at 24 and 48 h (Figure 4B).

There were statistically significant differences between group means per sample in the longevity of the F1 generation of females and males of *T. radiata*, as determined by one-way ANOVA ($F_{3.80} = 122.9$; $p < 0.0001$) and ($F_{3.69} = 35.66$; $p < 0.0001$), respectively. Meanwhile, no significant differences ($p = 0.6389$), ($p = 0.7708$) and ($p = 0.9890$) were observed in the longevity of females between the treatment groups at 1, 24 and 48 h, respectively. Additionally, the male population showed no significant differences ($p = 0.9794$) between 24 and 48 h.

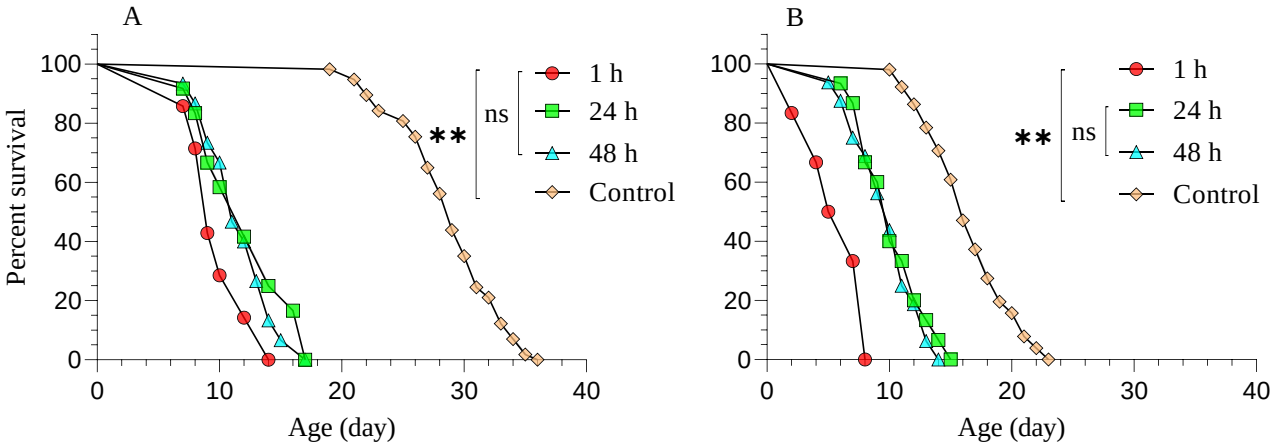

Figure 4. Survival rate for females (**A**) and males (**B**). Survival curves of *Tamarixia radiata* by log-rank analysis were generated where *Diaphorina citri* were first treated with *Beauveria bassiana* (BB-12) and subsequently exposed to *Tamarixia radiata* (Females n = 7, 12, 15 and 57 at 1 h, 24 h, 48 h and control, respectively) (Males n = 6, 15, 16 and 50 at 1, 24, 48 h and control, respectively). (** $p < 0.001$).

4. Discussion

The results indicated a lower percentage of parasitism and emergence in the treatments where the fungal suspension was sprayed immediately after parasitism (1 h post treatment). In concordance with our results, Martins et al. [39] reported that the parasitism and emergence of the aphid parasitoid *Diaeretiella rapae* McIntoch (Hymenoptera: Braconidae) was significantly lower in the treatments where *D. rapae* and *B. bassiana* were exposed within 0 and 24 h. Rashki et al. [40] also reported a reduction in the number of mummies and the percentage emergence of the F1 generation of *Aphidius matricariae* (Hymenoptera: Braconidae) when the time interval was short between application of the fungus and exposure to the parasitoid; likewise for the exposure to the parasitoid and subsequent fungal application. However, according to Lord [41] parasitoids evade direct competition with entomopathogens by detecting infected hosts using their antennae. According to Mann et al [42] *T. radiata* perceives the odor of *D. citri* nymphs before deciding to either feed or oviposit. In the same way, Chien et al [43] reported that females of *T. radiata* used the ovipositor to search for suitable hosts for oviposition.

Our results showed a negative effect of *B. bassiana* (BB-12) on the development and survival of the parasitoid larva when *D. citri* nymphs were first exposed to *T. radiata* and then treated immediately with the fungal suspension. This may suggest that the eggs or premature larval instars of *T. radiata* have vulnerability to *B. bassiana*. Additionally, our results showed that by extending the time interval between parasitoid parasitism and fungal infection, the survival of the parasitoid larva is enhanced. Similar trends were reported by Rashki et al [40] and Martins et al [39], where they found that the number of mummies formation, emergence rate, survival and parasitoid behavior are dependent on the time interval between parasitism and fungal spraying. This indicates that the number of host mummies obtained is directly related to the time interval between parasitioid release and fungal application. Additionally, Chow et al [44] reported that when a parasitoid and an entomopathogen were attacking the same host, the time and order among parasitism and infection commanded the interaction and consequence.

In the current study, where the fungal suspension was sprayed at 24 and 48 h after potential parasitism, the parasitoid emergence rate was higher, but when the fungal suspension was sprayed immediately after potential parasitism, the emergence rate was at the minimum level. Similarly, van Lenteren and Fransen [45] reported that the fungal infection level decreased as the time interval between parasitization and treatment with fungi increased. Additionally, Mesquita and Lacey [46] reported that if the parasitism had 48 h before exposure to the fungus, the parasitoid could escape the lethal action of the

entomopathogen and complete its development to the adult stage. According to Ibarra-Cortés et al [47], to reduce the negative interaction between these two biocontrol agents, it would be advisable to first release the parasitoids to allow parasitism and host feeding. Then, when a significant number of *D. citri* nymphs were carrying pupae, the fungi could be applied to infect remaining nymphs that were not parasitized. Moreover, according to Rashki et al [40] the percentage of parasitoid emergence was also influenced by competition for food between parasitoid's larvae and entomopathogenic fungi, especially when the time among oviposition and fungal spraying was less than 96 h. To cope with this competition, the parasitoid emitted fungicidally active substances within the host, thus preventing the development of mycosis via pathogens, allowing the normal development and emergence of their offspring [45,48].

In the experiment where *D. citri* nymphs were first treated with *B. bassiana* and afterward exposed to *T. radiata*, the parasitism rate, at the three times it was interval tested (1, 24, and 48 h), was at its minimum level, which was significantly lower than the parasitism rate recorded in the control. In concordance with our results, Mesquita and Lacey [46] reported the reduced parasitism of *Aphelinus asychis* (Hymenoptera: Aphelinidae) as a function of time between treatment with the entomopathogenic fungus *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes). This was because *A. asychis* could easily detect the presence of the hyphal from the fungi on the surface or the fungal metabolites into the hemolymph of the host and thus avoid the infected insect for oviposition. Brobyn et al [32] proved that parasitoids avoid ovipositing in aphids sprayed with fungi even after 72 h of being treated, while in fungi-free aphids the oviposition rate was high because the percentage of parasitism was lower compared with healthy aphids. According to Lord [41], this was because parasitoids could detect infected hosts by entomopathogens and avoid them for oviposition. This was probably the case for *T. radiata*, although parasitoid behavior toward treated hosts was not monitored in this experiment.

Our results have shown significant differences in both the longevity and reproductive potential of *T. radiata* among the treatment groups and the control in the experiment where *D. citri* nymphs were first exposed to *T. radiata* and afterward treated with *B. bassiana* suspensions. Similar results were reported by Rashki et al [40] and Martins et al [39]. These findings may suggest that, due to the exposure of the natural enemies of the pests to the entomopathogenic fungi, the emerged adult could become infected by *B. bassina* resulting in its early death. Similar trends were observed in the longevity of females where *D. citri* nymphs were first treated with *B. bassiana* and then exposed to *T. radiata*.

5. Conclusions

Our results showed a negative interaction between *T. radiata* and *B. bassiana* in controlling *D. citri* nymphs due to the antagonistic effects generated by *B. bassiana* on the parasitism percentage and normal development of parasitoid larvae. However, according to our findings, the application/interaction between *T. radiata* and *B. bassiana* could potentially be combined and applied for the management of *D. citri*, wherein effective time management would be required in order to minimize the potentially negative interactions between these two biological control agents; the most suitable strategy would be to allow parasitism to occur over an extended period of time (>48 h) before the application of *B. bassiana*. This was evident in the significant positive correlations between the time interval, the parasitism percentage and adults' emergence obtained in this study. The results from this study show the potential to design sustainable biological control programs to control Asian citrus psyllid and other economic pests, when combining the entomopathogenic fungi *B. bassiana* and parasitoids. The findings also demonstrate the importance of providing accurate information to farmers and stakeholders for the optimal release and application times for both biocontrol agents. Although the present study was carried out in controlled conditions, it provides significant information that can be fundamental for future field trial assessment and validation. Further studies are therefore warranted to verify conclusions made from the controlled environment where these experiments were conducted and to

evaluate the effectiveness of the combined use of these two biocontrol agents under field or greenhouses conditions. Large-scale field trials in citrus are needed to demonstrate the effective release/application rates and timing for effective integration into current *D. citri* management programs.

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