



The potential of *Beauveria bassiana* to control *Raoiella indica* (Acari: Tenuipalpidae) and its compatibility with predatory mites

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ABSTRACT

The red palm mite, *Raoiella indica* (Acari: Tenuipalpidae), is a serious pest of coconut plantations in tropical countries such as Brazil. Ecologically-sound control strategies have been sought for this pest; however, the potential of entomopathogenic fungi such as *Beauveria bassiana* (Hypocreales: Cordycipitaceae) in controlling this pest, as well as its compatibility with co-occurring natural enemies, remains poorly investigated. Thus, the present study was conducted to investigate the potential of four *B. bassiana* strains in controlling *R. indica*, as well as the compatibility of the most pathogenic fungal strain with the predatory mites *Amblyseius largoensis* and *Typhlodromus ornatus* (Acari: Phytoseiidae). Briefly, the pathogenicity of four *B. bassiana* strains (Bb-032, Bb-053, Bb-057 and Bb-086), isolated from coconut pests, were tested against *R. indica*. The *B. bassiana* strain Bb-032 was selected for further bioassays for being the most pathogenic to *R. indica*. The red palm mite survival and mean survival time drastically decreased when exposed to *B. bassiana* strain Bb-032 at both LC₅₀ and LC₈₀. In contrast, neither survival nor mean survival time of *A. largoensis* and *T. ornatus* were affected by the exposure to LC₅₀ and LC₈₀ of *B. bassiana* strain Bb-032. The fungal strain showed considerable selectivity to the two predatory mites; however, at its estimated LC₈₀ for *R. indica*, reduced oviposition, egg viability and the instantaneous rate of increase (r_t) were generally observed for both natural enemies. Therefore, *B. bassiana* holds potential promise for controlling *R. indica* in addition to being generally selective to non-target predatory mites.

1. Introduction

The red palm mite, *Raoiella indica* Hirst (Acari: Tenuipalpidae), is a polyphagous species that can colonize plants of several families (Carrillo et al., 2012; Gómez-Moya et al., 2017). The ability to survive under adverse conditions, high reproductive capacity, and rapid spread and adaptation to new hosts favoured the invasion of *R. indica* onto the American continent, which culminated in a high economic impact in coconut-producing countries (Domingos et al., 2013; Melo et al., 2018). High infestations of *R. indica* in coconut palms may cause severe yellowing of the leaves followed by tissue necrosis (Flechtmann and Etienne, 2004). However, information on economic losses is scarce, and is based on estimates (Navia et al., 2013). For instance, Trinidad and Tobago have been heavily affected by *R. indica*, with some plantations

suffering a reduction in coconut production of about 70 % (Navia et al., 2013). In Brazil, the red palm mite was first reported in 2009 (Navia et al., 2011) and has since spread throughout the country causing significant losses to agriculture due to the potential damage to plants of economic importance, such as coconut, banana, several species of palm-producing oil or wax, and ornamentals (Melo et al., 2018).

Raoiella indica is usually controlled with acaricides, which potentially increase production costs, contaminate the environment, and negatively affect human health and non-target organisms (Geiger et al., 2010; Roubos et al., 2014). Entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin (Hypocreales: Cordycipitaceae) have been used effectively for the control of agricultural pests as they are broad-spectrum pathogens, capable of infecting insects (Dhawan and Joshi, 2017; Mascarin et al., 2019) and mites (Draganova and Simova,

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2010; van Lenteren et al., 2018; Chavan et al., 2019). In addition to fungi, predatory mites of the Phytoseiidae family, such as *Amblyseius largoensis* Muma (Acari: Phytoseiidae) and *Typhlodromus (Anthoseius) ornatus* Denmark and Muma (Acari: Phytoseiidae), naturally inhabit coconut plantations in Brazil (Morais et al., 2016; Navia et al., 2005; Reis et al., 2008). The predatory mite *A. largoensis* has also been emphasized as an important natural enemy of *R. indica* in many tropical countries (Carrillo and Pena, 2012; Domingos et al., 2013). Since the combined use of *B. bassiana* and predators can synergistically act on mite pests (Midthassel et al., 2016), this study aimed to investigate the potential of four *B. bassiana* strains in controlling *R. indica*, as well as the compatibility of the most pathogenic fungal strain with the predatory mites *A. largoensis* and *T. ornatus*.

2. Materials and methods

2.1. Rearing of *R. indica* and predators

Coconut leaflets containing teliochrysalids of *R. indica* were collected from coconut palms in Aracaju city (10° 57' 03" S; 37° 03' 07" W), Sergipe state, Brazil. The leaflets were kept in open transparent plastic tubes (52 cm long x 5.5 cm in diameter), placed in glass jars (10.1 × 10.1 × 13.7 cm, Invicta®, MG, Brazil) with water up to the base of the leaflets to maintain their turgidity until the emergence of the adult females (Lira et al., 2021). Mites fed on leaves and females less than 48 h old were used in all bioassays. The colony of *A. largoensis* and *T. ornatus* was started with adult females and nymphs collected from unsprayed coconut leaflets in the same plantation as described for *R. indica*. Individuals of *R. indica*, *A. largoensis* and *T. ornatus* were identified using taxonomic keys, and voucher specimens were deposited in the mite collection of Maranhao State University (UEMA), Sao Luıs, Maranhao, Brazil.

The colonies were maintained at 27 ± 3 °C and 70 ± 10 % R.H. with a 12 h scotophase. Arenas consisted of PVC pieces (24 cm long x 10 cm wide) on a polyurethane foam (26 cm long x 12 cm wide x 3.0 cm thick) with a barrier of cotton wool soaked in water around the edge to further prevent the predatory mites from escaping. Cotton threads underneath a glass slide (18 × 18 mm) were placed on the arenas to serve as shelter and oviposition sites (Teodoro et al., 2017). The predatory mites were fed with castor bean pollen, *Ricinus communis* L. (Malpighiales: Euphorbiaceae), eggs, protonymphs, deutonymphs (immature stages) and adults (males and females) of *R. indica* and diluted honey (10 %), which were replenished every two days.

2.2. Selection of *B. bassiana* strain

The pathogenicity of four *B. bassiana* strains, isolated from coconut pests and belonging to Embrapa Tabuleiros Costeiros, Aracaju - Brazil, were tested against adult stage of *R. indica*: [Bb 032- germination rate 95.8 %, isolated from *Rhynchophorus palmarum* Linnaeus (Coleoptera: Curculionidae) collected in Aracaju city, Sergipe state; Bb 053- germination rate 93.4 %, isolated from *Brassolis sophorae* Linnaeus (Lepidoptera: Nymphalidae) collected in Moju municipality, Para state; Bb 057- germination rate 93.5 %, isolated from *Homalinotus coriaceus* (Gyllenhal) (Coleoptera: Curculionidae) collected in Neopolis municipality, Sergipe state; and Bb 086- germination rate 98.6 %, isolated from *Rhinostomus barbirostris* Linnaeus (Coleoptera: Curculionidae) collected in Valencia municipality, Bahia state].

The arenas (experimental units) consisted of coconut leaflet sections placed upside down inside Petri dishes (15 cm diameter x 1.3 cm depth) in which a layer of solidified agar (5 %) was immersed, with 0.3 % methylparaben (Nipagim™) as fungicide and distilled water (Santos et al., 2019). Thirty adult females of *R. indica* were transferred to arenas (2.0 cm diameter), opened with the aid of a circular mold, thereby exposing the area of the coconut leaflet epidermis. Subsequently, the arenas were sprayed with the four *B. bassiana* strains at a standard

concentration of 3.3 × 10⁸ conidia/mL through a Potter tower using a 1.7 mL aliquot at 34 kPa (0.34 bar) pressure, rendering a 1.8 ± 0.1 mg/cm² residue, which is in line with the recommendation of IOBC/WPRS (International Organization for Biological Control of Noxious Animals and Plants/West Palearctic Regional Section) (Hassan et al., 1994). The solution was previously prepared in an aseptic chamber by adding 150 mL of distilled and sterile water, and 0.05 % Tween® 80 (Sigma Aldrich) to a vial with the matrix culture and stirring it well with the aid of a sterile glass rod to release the conidia from the respective medium (Ferreira 2004). Ten replicates were performed for each fungal strains (Bb-032, Bb-053, Bb-057 and Bb-086), and control arenas were sprayed with Tween® 80 (0.05 %) solution.

Thirty minutes after spraying, the arenas were covered with perforated PVC film and maintained under controlled conditions (27 ± 3 °C, 70 ± 10 % relative humidity, 12 h scotophase). Mortality was recorded daily for a period of eight days, and dead mites were individually transferred to Petri dishes (6 cm diameter x 1 cm depth) containing water-soaked cotton and daily examined under optical microscope. Individual mites that developed fungal mycelia were considered to have been killed as result of fungal infection (Wu et al., 2020).

2.3. Acute toxicity bioassays

Further bioassays were carried out with the *B. bassiana* Bb-032 strain since it was shown to be the most pathogenic to adult stage of *R. indica*. Acute toxicity bioassay followed the methodology described above; however, fifteen adult females of *R. indica* were used in the bioassay. Adult females of *R. indica* were sprayed with increasing concentrations of *B. bassiana* (10¹, 10², 10³, 10⁴, 10⁵, 10⁶, 10⁷, 10⁸, 10⁹ and 10¹⁰ conidia/mL), which were selected in a previous bioassay that had a range of 1.1 × 10⁻³ to 7.6 × 10¹⁵ conidia/mL of solution.

The toxicity of *B. bassiana* to the predatory mites *A. largoensis* and *T. ornatus* was assessed by transferring ten adult females of each predator to PVC discs (7 cm in diameter) placed on a polyurethane foam (8 cm diameter x 4.5 cm thick) saturated with distilled water in a plastic container (9 cm diameter x 7 cm depth). Predatory mites were sprayed with the LC₅₀ (9.15 × 10⁵ conidia/mL) and LC₈₀ (1.55 × 10⁹ conidia/mL) of the fungal solution, *B. bassiana* Bb-032 strain. Cotton threads underneath a glass slide (18 × 18 mm) were transferred to arenas to serve as shelter and oviposition sites. Ten replicates (arenas) were used for each concentration tested. The evaluations were performed every 24 h for a period of eight days, and dead mites were placed in a humid chamber until fungal emergence. The predatory mites were fed with castor bean pollen *R. communis* L. and 10 % honey placed on unsprayed 0.5 cm² PVC pieces, and these sources were replenished every two days.

2.4. Sublethal effects of *B. bassiana* strain Bb-032 on life history parameters of *R. indica* and predatory mites

The bioassays were performed with the LC₅₀ and LC₈₀ of *B. bassiana* strain Bb-032, as estimated for *R. indica*. The following parameters were observed: a) survival of *R. indica*; b) survival, reproduction and egg viability of *A. largoensis* and *T. ornatus*. The arenas were prepared as described previously for both *R. indica* and predatory mites, and control arenas were sprayed with Tween® 80 (0.05 %). Fifteen adult females of *R. indica* and ten adult females and five males of *A. largoensis* and *T. ornatus* were used in the bioassays. Ten replicates (arenas) were used for each LC (LC₅₀; LC₈₀ and control) tested. The mites were evaluated every 24 h for a period of eight days. Dead individuals were placed in a humid chamber until fungal emergence and the eggs laid by either *A. largoensis* or *T. ornatus* females were separated to assess egg viability. Every 24 h, the numbers of eggs laid by *A. largoensis* or *T. ornatus* females from each replicate per treatment were separated and placed in new arenas to be evaluated until the larvae hatched.

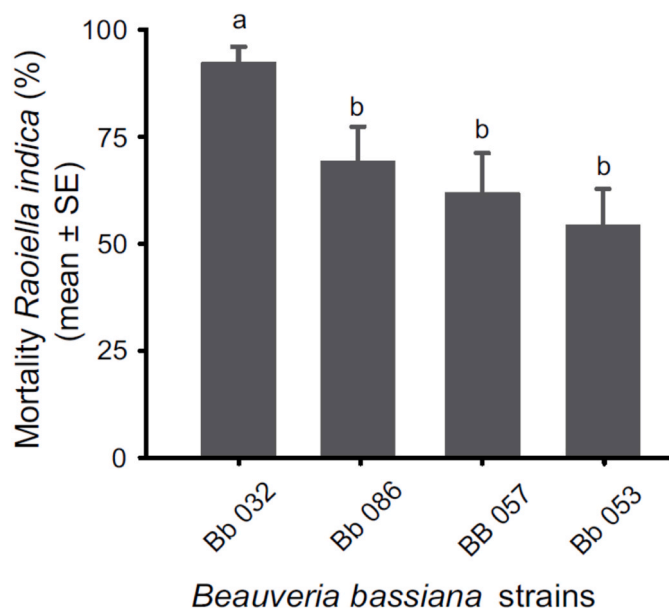


Fig. 1. Mortality (%) of *Raiiella indica* exposed to four strains of *Beauveria bassiana*. Different letters denote significant differences by the Fisher's LSD test ($P < 0.05$). Means \pm SE are given. Mortality was recorded daily for a period of eight days.

2.5. Sublethal effects of *B. bassiana* on population growth of *R. indica* and predatory mites

Procedures and spraying were performed as described in the pathogenicity bioassays, except that *R. indica* individuals were transferred to coconut leaflets (4 cm wide x 40 cm long) standing upright in distilled water-saturated polyurethane foam sitting inside plastic tubes (52 cm long x 5.5 cm in diameter). Twenty females and ten males of *R. indica* (adult stages) or ten females and five males of either *A. largoensis* or *T. ornatus* (adult stages) were sprayed with the LC_{50} and LC_{80} of *B. bassiana*, as estimated for *R. indica*. Adult females at the onset of their reproductive period were used in this bioassay. We carried out ten replicates per treatment; the control was sprayed with Tween® 80 (0.05 %). The numbers of eggs, immatures and adults of *R. indica* were counted after ten days of exposure. As for predatory mites, counting only occurred on the eighth day, which corresponds to peak oviposition (G.S Freitas et al., unpublished data). Predatory mites were fed as previously described. Data were used to calculate the instantaneous rate of increase (r_t) using the equation: $r_t = [\ln(N_f/N_i)]/t$, where N_f is the final number of live mites (including eggs and immatures), N_i is the initial number of live mites and t is the length of the experiment (Walthall and Stark, 1997).

2.6. Statistical analyses

The pathogenicity of the four *B. bassiana* strains were compared with one-way ANOVA followed by Fisher's LSD test. Beforehand, the normality of the residues was tested using the Kolmogorov-Smirnov test and the homogeneity of variances was checked using the Hurttley test. Median lethal concentrations of the most pathogenic strain of *B. bassiana* to *R. indica* (Bb-032) were estimated by Probit analysis using the PROC PROBIT (SAS Institute, 2013). Survival analyses were estimated with the Kaplan-Meier method (log-rank test, $P < 0.05$) using SigmaPlot 12.5 (Systat Software, San Jose, CA, USA). Cumulative oviposition data were subjected to non-linear regression analyses, whereas egg viability and differences in population growth were submitted to one-way ANOVAs followed by the Tukey test using SigmaPlot 12.5. Whenever data did not adjust to normality, the ANOVA ON RANK test was performed.

Table 1

Acute toxicity (conidia/mL) of *Beauveria bassiana* strain (Bb-032) to adult females of *Raiiella indica*. Mortality was recorded daily for a period of eight days.

Strain	^a LC_{50} (95 % CI) ^b	^a LC_{80} (95 % CI) ^b	Slope (\pm SE)	χ^2	P^c	df^d
Bb-032	9.15×10^5 ($3.52 \times 10^5 - 2.24 \times 10^6$)	1.55×10^9 ($4.32 \times 10^8 - 8.30 \times 10^9$)	0.026 ± 0.0023	5.37	0.25	5

^a Lethal concentrations.

^b Confidence intervals.

^c Probability value.

^d Degrees of freedom.

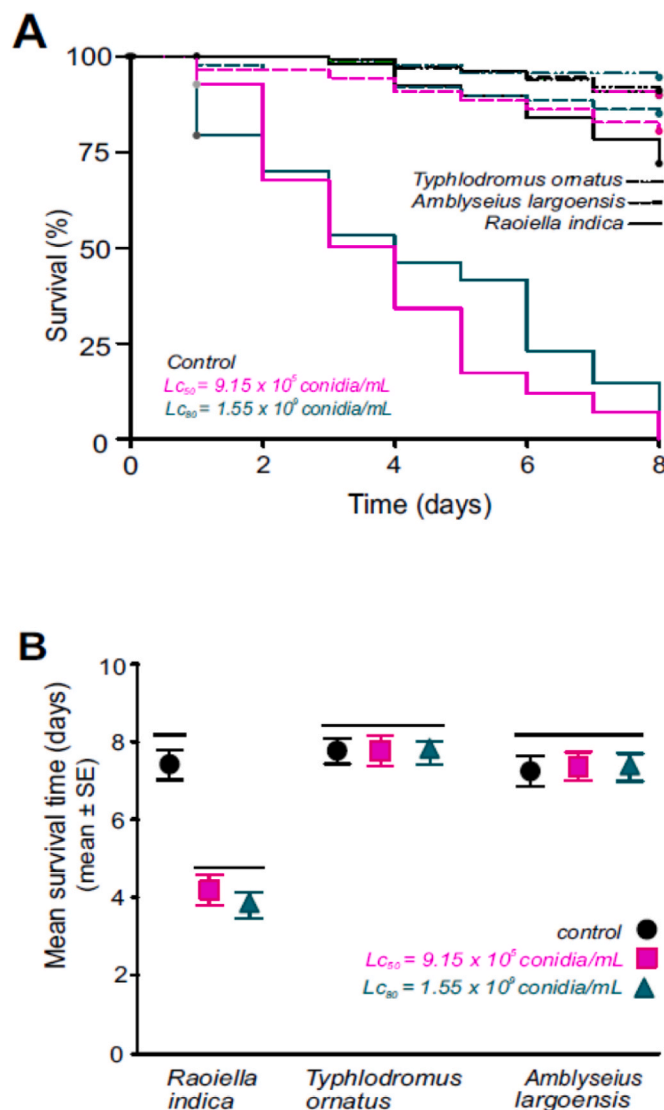


Fig. 2. Survival of *Raiiella indica* and the predatory mites *Typhlodromus ornatus* and *Amblyseius largoensis* exposed to the LC_{50} (9.15×10^5 conidia/mL) and LC_{80} (1.55×10^9 conidia/mL) of *Beauveria bassiana*, as estimated for *Raiiella indica* (A); and mean survival time (B). Treatments for each species under the same horizontal line do not differ statistically ($P < 0.05$).

3. Results

Three days after spraying the *B. bassiana* strains (Bb-032, Bb-053, Bb-057 and Bb-086), fungal mycelia were observed in some adult females of *R. indica* in all treatments. However, at the end of the bioassay (eight days), the *B. bassiana* strain Bb-032 was the most pathogenic to *R. indica*

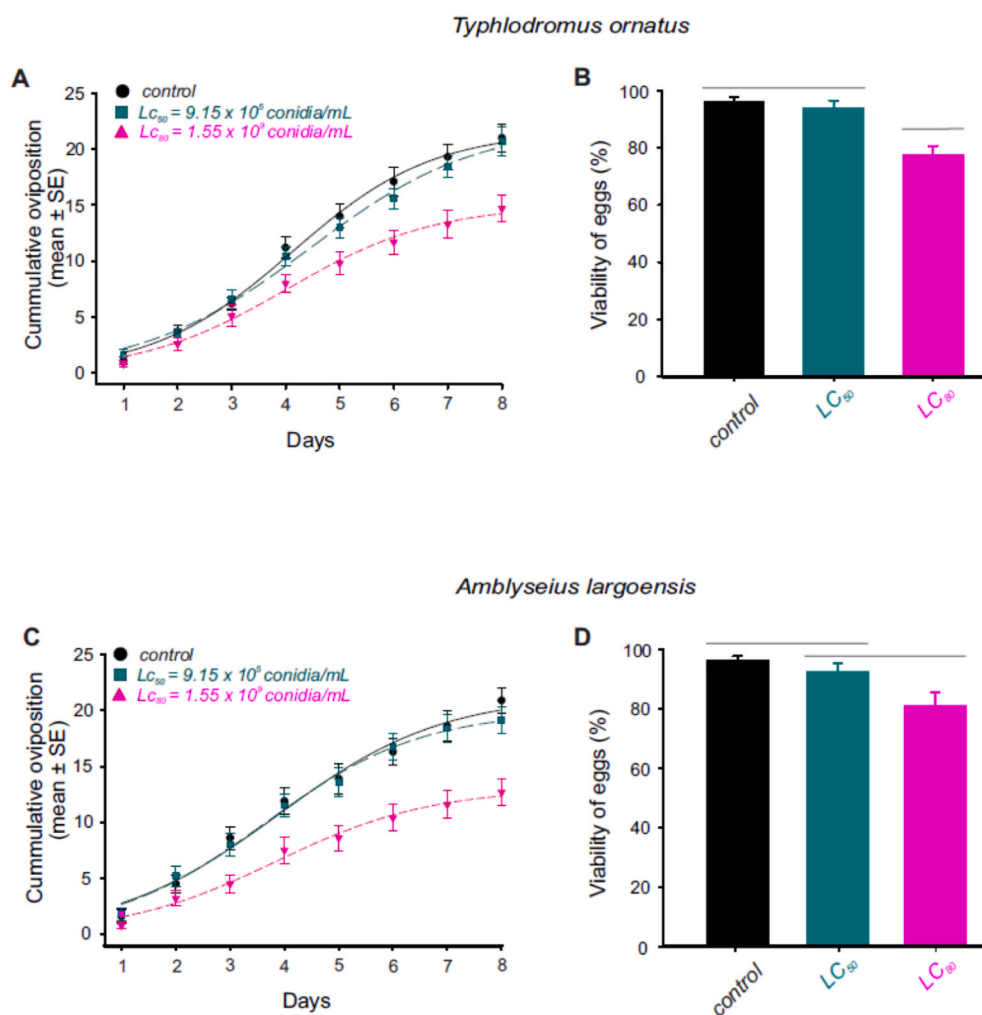


Fig. 3. Number and viability of eggs of *Typhlodromus ornatus* (A, B) and *Amblyseius largoensis* (C, D) exposed to LC_{50} and LC_{80} of *Beauveria bassiana* strain (Bb-032) as estimated for *Raoiella indica*. Symbols represent the mean of ten replicates and vertical bars denote the standard errors. Treatments for each species under the same horizontal line do not differ statistically ($P < 0.05$).

Table 2

Non-linear regression analyses of cumulative oviposition for *Typhlodromus ornatus* and *Amblyseius largoensis* exposed to *Beauveria bassiana* shown in Fig. 3 A, C.

Model	Species	treatment	Estimated parameters (\pm SD)			df _{error}	F	P	R ²
			a	b	x0				
$f = a/[1 + \exp(-(x-x_0)/b)]$	<i>Typhlodromus ornatus</i>	Control	21.6 (19.7–23.6) a	1.3 (1.0–1.6)	4.1 (3.7–4.5)	5	522.2	<0.0001	0.99
		LC_{50}	22.2 (19.0–25.4) a	1.5 (1.1–1.9)	4.4 (3.8–5.1)	5	398.9	<0.0001	0.99
		LC_{80}	15.0 (13.2–16.9) b	1.4 (0.9–1.8)	4.0 (3.5–4.6)	5	313.1	<0.0001	0.99
	<i>Amblyseius largoensis</i>	Control	21.4 (17.6–25.2) a	1.5 (0.9–2.1)	3.9 (3.1–4.7)	5	163.1	<0.0001	0.98
		LC_{50}	19.9 (18.0–21.9) a	1.4 (1.1–1.8)	3.7 (3.2–4.1)	5	395.8	<0.0001	0.99
		LC_{80}	13.0 (11.1–14.9) b	1.4 (0.9–1.9)	3.8 (3.2–4.5)	5	201.9	<0.0001	0.98

Different letters within a parameter column indicate statistical differences between treatments for each predatory mite.

($F_{3,39} = 6.072$, $P < 0.001$) ($LC_{50} = 9.15 \times 10^5$ conidia/mL and $LC_{80} = 1.55 \times 10^9$ conidia/mL) (Fig. 1 and Table 1) and therefore selected for further studies.

The survival of *R. indica* drastically decreased when exposed to *B. bassiana* strain Bb-032 at both LC_{50} ($\chi^2 = 548.21$, $df = 5$, $P < 0.001$) and LC_{80} ($\chi^2 = 617.23$, $df = 5$, $P < 0.001$) in comparison with the control (Fig. 2A). Similarly, there was a sharp reduction in the mean survival time of *R. indica* exposed to LC_{50} (4.17 ± 0.22 days) and LC_{80} (3.80 ± 0.16 days) compared with the control (7.42 ± 0.11 days) (Fig. 2B). In contrast, neither survival (Fig. 2A) nor mean survival time (Fig. 2B) of the predatory mites *A. largoensis* and *T. ornatus* were affected by the exposure to LC_{50} and LC_{80} of *B. bassiana*, as estimated for *R. indica*.

Likewise, the LC_{50} of *B. bassiana* did not reduce the oviposition of either of the predatory mites (Fig. 3A, C). However, adult females of *A. largoensis* and *T. ornatus* laid the least number of eggs when exposed to LC_{80} of *B. bassiana* (Table 2). Egg viability was also reduced when predators were exposed to LC_{80} of *B. bassiana* ($F_{2,27} = 5.82$; $P < 0.05$) (Fig. 3B, D).

The *B. bassiana* strain Bb-032 drastically reduced the instantaneous rate of increase (r_i) of *R. indica* in a concentration-dependent manner. Less drastic reductions in r_i occurred when predatory mites were exposed to either LC_{50} or LC_{80} of *B. bassiana* (Fig. 4).

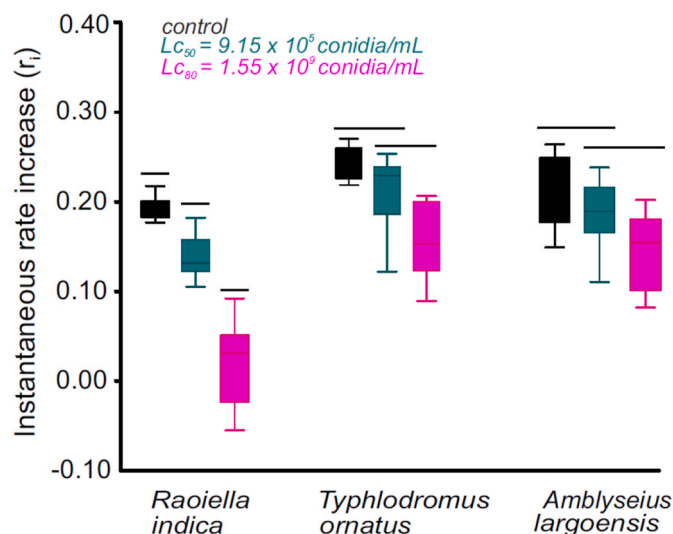


Fig. 4. Effect of *Beauveria bassiana* on the instantaneous rate of increase (r_i) (means \pm SE) of *Raoiella indica*, *Typhlodromus ornatus* and *Amblyseius largoensis*. Treatments for each species under the same horizontal line do not differ statistically ($P < 0.05$).

4. Discussion

Our results indicate that the entomopathogenic fungi *B. bassiana* has potential as a biological control agent of the red palm mite *R. indica* and appears compatible with predatory mites. In our study, we selected a highly-pathogenic strain of *B. bassiana* Bb-032 to *R. indica* that also presented considerable selectivity to the predatory mites *A. largoensis* and *T. ornatus*. Differences in susceptibility of mites possibly stem from species-specific physiological characteristics, foraging ability, and the larger size of predators compared to the pest mite (Cloyd et al., 2006; Lima et al., 2012). Chavan et al. (2019) showed that adults of *R. indica* are more susceptible to fungal infection than the immature stages, which may be related to the presence of the integument and ecdysis. Ecdysis is an important factor in arthropod resistance to fungal infection (Wu et al., 2014; Butt et al., 2016).

It is noteworthy that the virulence of fungal strains is related to their ability to produce compounds that surpass the well-developed physical structure and immune response of the target organisms. The entomopathogenic fungi *B. bassiana* produces several hydrophilic enzymes, and once in the host, its insecticidal effect is attributed to the production of non-ribosomal peptide-synthetase (NRPS), beauvericin, bassianolide, bassiacridin, dipicolinic acid and oxalic acid (Bidochka and Khachatourians, 1991; Wang and Xu, 2012; Xiao et al., 2012). The production of these compounds is also dependent on the type of isolation (Draganova and Simova, 2010), abiotic factors (Vega et al., 2012), application methods (Fernandes et al., 2012), and the developmental stage of the target organism (Shrestha et al., 2015).

Although *R. indica* lived for 8 days after fungal infection, which is corroborated by other studies (Draganova and Simova, 2010; Immediato et al., 2015), the moribund mites have their physiological activity impaired, which could possibly make them susceptible to natural enemies in the field. In addition, survival of *R. indica* also decreased with exposure as shown for the instantaneous rates of increase that were reduced at both LC_{50} and LC_{80} of *B. bassiana*. Similarly, Seyed-Talebi et al. (2012) also found that growth rate of *Tetranychus urticae* Koch (Acari: Tetranychidae) was reduced when exposed to sublethal concentrations of *B. bassiana*. Our results clearly indicate that the population growth of *R. indica* was susceptible to *B. bassiana* infection by direct exposure.

The tolerance of the predatory mites *A. largoensis* and *T. ornatus* to *B. bassiana* was observed in survival curves of these organisms when

exposed to LC_{50} and LC_{80} of this fungus, as estimated for *R. indica*. However, *B. bassiana* reduced oviposition, egg hatching and growth rate of these predators. The reduction in predator's population increase may be related to costly energy processes as it reduces the rate of feeding and food conversion (Roy et al., 2006). Our results are in accordance with other studies showing that *B. bassiana* is generally selective to predatory mites (Midthassel et al., 2016; Wu et al., 2016).

This study demonstrates that *B. bassiana* was effective at controlling *R. indica* at the two lethal concentrations tested (LC_{50} : 9.15×10^5 conidia/mL; LC_{80} : 1.55×10^9 conidia/mL), and showed considerable selectivity (compatibility) to the predatory mites *A. largoensis* and *T. ornatus*. The compatibility of natural enemies with entomopathogenic fungi suggests a viable alternative to acaricides to suppress *R. indica* populations. These findings reinforce the potential of *B. bassiana* as a relevant and safer tool to control *R. indica*. However, further studies are needed to determine the efficiency of *B. bassiana* to *R. indica* under field conditions.

CRediT authorship contribution statement

Giselle Santos de Freitas: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. **Vanessa de Araújo Lira:** Investigation, Writing – review & editing. **Luis Oswaldo Viteri Jumbo:** Data curation, Formal analysis, Writing – review & editing. **Francisco José dos Santos:** Methodology, Writing – review & editing. **Adriano Soares Rêgo:** Formal analysis, Writing – original draft, Writing – review & editing. **Adenir Vieira Teodoro:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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