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An entomopathogenic strain of *Beauveria bassiana* (hypocreales: Cordycipitaceae) against *Eotetranychus kankitus* (acarina: Tetranychidae) and its compatibility with *Neoseiulus barkeri* (acarina: Phytoseiidae)

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Abstract

Eotetranychus kankitus is an important pest on several agricultural crops, and its resistance to pesticides has promoted the exploration of biological control strategies. *Beauveria bassiana* and *Neoseiulus barkeri* have been identified as potential agents for suppressing spider mites. This study aimed to investigate the pathogenicity of *B. bassiana* on *E. kankitus* and its compatibility with *N. barkeri*. Results showed that among the five tested strains of *B. bassiana*, Bb025 exhibited the highest level of pathogenicity on *E. kankitus*. Higher application rates (1×10^8 conidia/mL) of Bb025 led to a higher mortality rate of *E. kankitus* (90.402%), but also resulted in a 15.036% mortality of *N. barkeri*. Furthermore, preference response tests indicated that both *E. kankitus* and *N. barkeri* actively avoided plants sprayed with Bb025 compared to the control group that was sprayed with Tween-80. In a no-choice test, we observed that *N. barkeri* actively attacked Bb025-treated *E. kankitus* with no adverse effect on its predatory capacities. Furthermore, *N. barkeri* laid more eggs when fed on Bb025-treated *E. kankitus* compared to Tween-80-treated *E. kankitus*, but the subsequent generation of surviving individuals fed on Bb025-treated *E. kankitus* was reduced. These findings demonstrate that the Bb025 strain of *B. bassiana* is highly virulent against *E. kankitus* while causing less harm to *N. barkeri*. Consequently, a promising strategy for controlling *E. kankitus* could involve the sequential utilisation of Bb025 and *N. barkeri* at appropriate intervals.

Introduction

Eotetranychus kankitus is a significant pest mite found in orchards primarily in the Oriental and Palearctic regions (Wang *et al.*, 2014). The mobile life stages of this mite feed on the surfaces of leaves and young terminal shoots by using their piercing-sucking mouthparts, causing mesophyll collapse and subsequent leaf drop (Zhou *et al.*, 1999). Although *E. kankitus* is not as widely distributed as other pest mites like *Panonychus citri* and *Phyllocoptruta oleivora*, it causes more serious damage when it does appear (Li *et al.*, 2017). Additionally, *E. kankitus* frequently co-occurs with *P. citri* or *P. oleivora*, forming in a pest complex that poses significant difficulties for orchard management (Li *et al.*, 2014; Zhou *et al.*, 1999). Traditionally, acaricides have been used to control *E. kankitus* (Chen *et al.*, 2023). However, the effectiveness of acaricides is limited due to the mite's quick development of resistance, short life cycle, high reproductive rate, and parthenogenesis. Therefore, the development of biological control strategies is necessary to manage this mite.

One promising approach to managing *E. kankitus* is the use of entomopathogenic fungi as biological control agents. These fungi can infect spider mites, leading to their mortality and ultimately reducing their population densities (Shah and Pell, 2003). *Beauveria bassiana* is distributed worldwide and can infect various pest species, including Lepidoptera, Hemiptera, Coleoptera, Diptera, and Acarina (Altinok *et al.*, 2019; Sohrabi *et al.*, 2019; Wu *et al.*, 2016a). According to earlier research, *B. bassiana* has potential in controlling pest mite species, such as *Tetranychus urticae* (Wu *et al.*, 2016a), *T. evansi* (Wekesa *et al.*, 2005), *P. oleivora* (Alves *et al.*, 2005), and *P. citri* (Shi and Feng, 2006). However, there is limited research on the use of *B. bassiana* for controlling *E. kankitus*.

The effectiveness of biological control may be increased by employing multiple natural enemies (Chandler *et al.*, 2005). Research suggests that using natural enemies in cooperation with *B. bassiana* shows potential in controlling pests (Baverstock *et al.*, 2010; Castillo-Ramírez *et al.*, 2020; De Freitas *et al.*, 2021; Lin *et al.*, 2017). For example, the combined use of *B. bassiana* and *Stratiolaelaps scimitus* increased control efficacy for *Frankliniella occidentalis* (Zhang *et al.*, 2021). Furthermore, natural enemies such as insects or mites, acting as vectors for *B. bassiana* conidia, also show potential in controlling pests. *Diaphorina citri* died after *B. bassiana* conidia were successfully delivered to it by *Amblyseius swirskii* or *Neoseiulus cucumeris* (Zhang *et al.*, 2015). Therefore, it is recommended to combine the use of entomopathogenic fungi with release of natural enemies as a potential strategy to improve the efficacy of controlling *E. kankitus*.

The predatory mite, *N. barkeri*, has been successfully used to suppress *E. kankitus* (Li *et al.*, 2017). To optimise the efficiency of *E. kankitus* control, we explored the potential of combining the application of *B. bassiana* with the releases of *N. barkeri*. However, there is a potential risk that the fungus could harm the predatory mites, given that various insect and mite species are susceptible to *B. bassiana*. Previous research has shown that spraying *B. bassiana* on adult predatory mites resulted in approximately 43% mortality of predators (Numa Vergel *et al.*, 2011). Additionally, several researchers have found negative effects on predator life cycles and predation parameters when *B. bassiana* was sprayed (Ullah and Lim, 2017) or when predators fed on *B. bassiana*-infected prey (Seiedy, 2015; Seiedy *et al.*, 2012a; Wu *et al.*, 2015b). Therefore, evaluating the compatibility between *B. bassiana* and predatory mites is crucial for the success of integrated pest management (IPM) programs targeting the control of *E. kankitus*.

In this study, we evaluated the pathogenicity of five isolates of *B. bassiana* against *E. kankitus*. Subsequently, we assessed the direct lethal effects of different concentrations of the selected virulent isolates on both *E. kankitus* and *N. barkeri* by exposing the mites to *B. bassiana*. Additionally, we determined the habitat preference, predatory behaviour, fecundity, and offspring survival of *N. barkeri* in the presence of risks posed by *B. bassiana*.

Materials and methods

Rearing of entomopathogenic and mites

Five strains of *B. bassiana* (Bb02, Bb014, Bb025, Bb062, and Bb252) were obtained from the Biotechnology Centre of Southwest University and regularly cultivated on Potato Dextrose Agar plates at 25°C in the dark for 14 days. Conidia were collected from the agar plates for the tests by flooding them in a sterile 0.05% Tween-80 solution, and their concentration was measured using a haemocytometer. The citrus yellow mites, *E. kankitus*, were cultivated on *Citrus sinensis* leaf discs (7 cm in diameter). These leaf discs were placed in Petri dishes (9 cm in diameter, 2 cm in depth) on water-soaked polyurethane mats. To prevent mites from escaping, the edges of the leaf discs were surrounded with wet cotton wool. The predatory mites, *N. barkeri*, were maintained in a plastic cylindrical container (15 cm in diameter, 8 cm in depth). A plastic lid covered the container, with a 5 cm diameter opening in the centre covered with stainless steel wire netting to provide ventilation. Spider mites were swept into the container twice per day using a brush to rear the predatory mites. All mites were kept in a climate chamber at a temperature of 25 ± 1 °C, relative humidity of 80 ± 5%, and a photoperiod of L16: D8 hours.

Seection of B. bassiana strains on E. kankitus

The pathogenicity of five *B bassiana* isolates on female *E. kankitus* was tested. Thirty *E. kankitus* females (one-day-old) were placed onto leaf discs and then sprayed with 1 mL of a 1×10^7 conidia/mL fungal suspension using a spray tower. *Eotetranychus kankitus* sprayed with 0.05% Tween-80 solution served as the control. The mites treated with *B. bassiana* or Tween-80 were then transferred onto new leaf disc, respectively. Mortality was recorded daily for 9 days. Dead spider mites were transferred to a sterile Petri plate lined with wet filter paper at each observation. The plates were then covered with Parafilm® and maintained at 25°C in the dark. They were monitored daily for symptoms of mycosis. Spider mites showing visible mycelium growth on their body surface were considered to have died from fungal infection. Three replicates were performed for each *B. bassiana* isolate.

Efects of Bb025 on susceptibility of E. kankitus and N. barkeri

Based on the pathogenicity of five *B. bassiana* strains on *E. kankitus*, the strain Bb025 was selected for multiple concentration bioassays against both *E. kankitus* and *N. barkeri*. Thirty *E. kankitus* females (one-day-old) were sprayed with six different concentrations of Bb025 conidial suspension (1×10^3, $1 \times 10^{4,}$ 1×10^5, 1×10^6, 1×10^7, 1×10^8 conidia/mL), while thirty *N. barkeri* females (one-day-old) were sprayed with two different concentrations (1×10^7, 1×10^8 conidia/mL). Tween-80 solution (0.05%) was sprayed on *E. kankitus* and *N. barkeri* as the control. The spraying method of *B. bassiana* and the method for determining the number of dead mites are described above. Each concentration was replicated three times, and mortality was recorded daily for 9 days.

Efect of Bb025 on habitat preference in E. kankitus and N. barkeri

Two leaflets of the same size (4 cm in diameter) were used in the experiment and placed upside down on a foam cube (14 cm in diameter, 1 cm in depth). The foam cube was then placed in Petri dishes (15 cm in diameter, 2.5 cm in depth) filled halfway with water. A wax bridge (4 × 0.5 cm) connected the leaflets (Walzer *et al.*, 2006). One of the leaflets was sprayed with 1 mL of a 1×10^8 conidia/mL Bb025 conidial suspension, serving as the treatment group. The other leaflet was treated with 1 mL Tween-80 solution (0.05%), serving as the control group. For each choice, a single female was randomly selected and placed in the middle of the bridge. Patch selection was observed at 0, 15, 30, 45, 60, 90, and 120 minutes. One hundred individuals for each mite species (*E. kankitus* or *N. barkeri*) were tested, with each experimental unit and mite being used only once.

Efect of Bb025 on predatory behaviour of N. barkeri

Predatory mites may invest a significant amount of time and energy in self-grooming behaviours following treatment with *B. bassiana*, potentially reducing their ability to search for and feed on prey. Thus, we conducted an experiment to observe the movement and self-grooming behaviours of *N. barkeri* when inhabiting Bb025-treated citrus leaves and feeding on Bb025-treated *E. kankitus*. The experiment involved spraying leaf discs containing thirty *E. kankitus* eggs with a 1 mL Bb025 conidial suspension (1×10^8 conidia/mL) as the treatment group, while leaf discs sprayed with Tween-80 (0.05%) served as the control group. After 10 minutes, a single *N. barkeri* female (starved for 24 hours) was introduced into each leaf disc, and their movement, self-grooming behaviour, and predatory tendencies towards *E. kankitus* eggs were observed and recorded over a 10-minute period. Additionally, the number of *E. kankitus* eggs consumed by *N. barkeri* was recorded after 2 hours of exposure to the leaf disc. A new leaf disc was used for each test, with nine mites tested individually.

Effect of Bb025 on fecundity and offspring survival of N. barkeri

To evaluate the safety of predators feeding on prey infected with Bb025, we determined the predatory capacity, fecundity, and offspring survival of *N. barkeri*. Females of *N. barkeri* (starved for 24 hours) were placed on a leaf disc. Then, thirty *E. kankitus* females, previously sprayed with a concentration of 1×10^8 conidia/mL of Bb025, were offered to *N. barker* individuals daily at 3.5 days post-inoculation (corresponding to the LT$_{50}$ value of *E. kankitus*). As a control group, thirty *E. kankitus* females sprayed with Tween-80 (0.05%) were provided to *N. barker* individuals at 3.5 days post-spray. The number of consumed prey and the number of eggs laid by *N. barkeri* were counted daily for 7 days. The eggs laid by *N. barkeri* were transferred daily to a new leaf disc, where both Bb025 and Tween-80 sprayed *E. kankitus* females were provided daily. The individuals were monitored daily, and the number of live individuals was recorded after 7 days. Each treatment was replicated six times.

Data analyses

All statistical analyses were performed using SPSS 26.0. Mortality data were corrected for natural mortality (Abbott, 1925), and then normalised using arcsine-transformed before conducting a one-way ANOVA. Probit analysis was used to estimate the lethal time to 50% mortality (LT$_{50}$) and the lethal concentration causing 50% mortality (LC$_{50}$). Preference data were analysed using a chi-squared test. The frequency of *N. barkeri* self-grooming, grooming time, moving time, frequency of predation tendencies, *E. kankitus* egg and female consumption by *N. barkeri*, total number of eggs laid by *N. barkeri*, and the total number of new generation individuals were analysed using an independent-sample *t*-test between the Bb025 and Tween-80-treated groups.

Results

Section of B. bassiana strains on E. kankitus

The pathogenicity of five *B. bassiana* isolates (1×10^7 conidia/mL) was evaluated against the female of *E. kankitus* (Fig. 1 and Table 1). The five isolates were highly effective against *E. kankitus*, with mortality rates increasing over time. After 9 days, the cumulative corrected mortality rates of *E. kankitus* varied significantly among the five isolates ($F = 26.720$; $df = 4, 10$; $P < 0.001$), ranging from 53.611% to 81.899% (Fig. 1). The LT$_{50}$ values against female *E. kankitus* for the five isolates ranged from 4.414 to 7.324 days (Table 1). Notably, the Bb025 isolate exhibited the highest effectiveness (Fig. 1), with an LT$_{50}$ value of 4.414 days, which lower than that of the other *B. bassiana* isolates (Table 1).

Efects of Bb025 on susceptibility of E. kankitus and N. barkeri

The mortality rates of *E. kankitus* varied among different conidial concentrations of Bb025 and increased with conidial concentrations. The 1×10^8 conidia/mL Bb025 treatment consistently resulted in the highest mortality of *E. kankitus* during the test period, with a corrected mortality rate of 90.402% on the 9th day (Fig. 2a). The mortality of *N. barkeri* treated with 1×10^8 conidia/mL Bb025 was comparable to that of the 1×10^7 conidia/mL Bb025 treatment for the first eight days (Fig. 2b). However, after exposure to 1×10^7 and 1×10^8 conidia/mL Bb025 conidial suspension, the predatory mite's corrected mortality rates were 8.385% and 15.036%, respectively, on the 9th day.

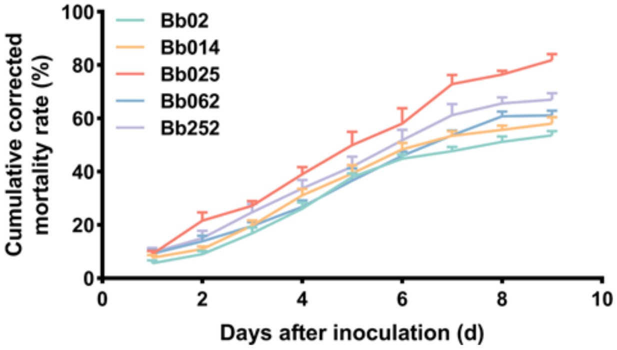

Figure 1. Cumulative corrected mortality rate (mean ± SE) of *Eotetranychus kankitus* females caused by five isolates of *B. bassiana*, including Bb02, Bb014, Bb025, Bb062, and Bb252.

The Bb025 strain had a lower LC$_{50}$ value of 3.488×10^5 conidia/mL for *E. kankitus*. However, the LC$_{50}$ value for the predatory mites could not be calculated because the mortality rate of *N. barkeri* remained low, even when exposed to higher concentrations of Bb025 (Table 2). Furthermore, *E. kankitus* had a lower LT$_{50}$ value (3.581 days) compared to *N. barkeri* (22.773 days) when treated with a concentration of 1×10^8 conidia/mL of Bb025 (Table 3).

Efect of Bb025 on habitat preference in E. kankitus and N. barkeri

The citrus yellow mites, *E. kankitus*, exhibited a habitat preference for Tween-80-treated leaflets at different time points: 0, 15, 30, 45, 60, 90, and 120 minutes after treatment (Fig. 3a). A similar preference for Tween-80-treated leaflets was also observed in *N. barkeri* (Fig. 3b).

Efect of Bb025 on predatory behaviour of N. barkeri

The predatory mites, *N. barkeri*, displayed various behaviours such as movement, remaining stationary, and self-grooming when exposed to citrus leaves sprayed with a concentration of 1×10^8 conidia/mL of Bb025 or 0.05% Tween-80. No significant differences were observed in the frequency of self-grooming between *N. barkeri* on leaf discs sprayed with Bb025 and Tween-80 ($t = 1.682$, $df = 16$, $P = 0.112$) (Fig. 4a). However, *N. barkeri* spent significantly more time self-grooming on citrus leaves sprayed with Bb025 (109.889 seconds) compared to those sprayed with Tween-80 (45.111 seconds) ($t = 2.631$, $df = 16$, $P = 0.018$) (Fig. 4b). Furthermore, a significant difference was found in the time spent moving on citrus leaves sprayed with Bb025 and Tween-80 (460.111 seconds vs. 535.667 seconds) for *N. barkeri* ($t = -2.497$, $df = 16$, $P = 0.024$) (Fig. 4c). Conversely, no significant differences were noted in the frequency of predatory tendencies (10 minutes, $t = -0.985$, $df = 16$, $P = 0.339$) or in the number of *E. kankitus* eggs consumed by *N. barkeri* (2 hours, $t = -0.483$, $df = 16$, $P = 0.636$) when preying on spider mites sprayed with Bb025 and Tween-80 (Fig. 5).

Table 1 Lethal time (LT_{50}) estimations of five *B. bassiana* in *E. kankitus*

		95% confidence intervals				
Isolates	LT_{50} (days)	Lower bound	Upper bound	Intercept	Slope $\pm$ SE	χ^2 ($df = 7$)
Bb02	7.324	6.054	9.735	−1.835	2.122 $\pm$ 0.358	1.275
Bb014	6.564	5.466	8.441	−1.691	2.070 $\pm$ 0.337	1.462
Bb025	4.414	3.763	5.155	−1.614	2.503 $\pm$ 0.336	3.154
Bb062	6.511	5.387	8.457	−1.657	2.037 $\pm$ 0.338	2.531
Bb252	5.529	4.664	6.761	−1.587	2.137 $\pm$ 0.321	1.949

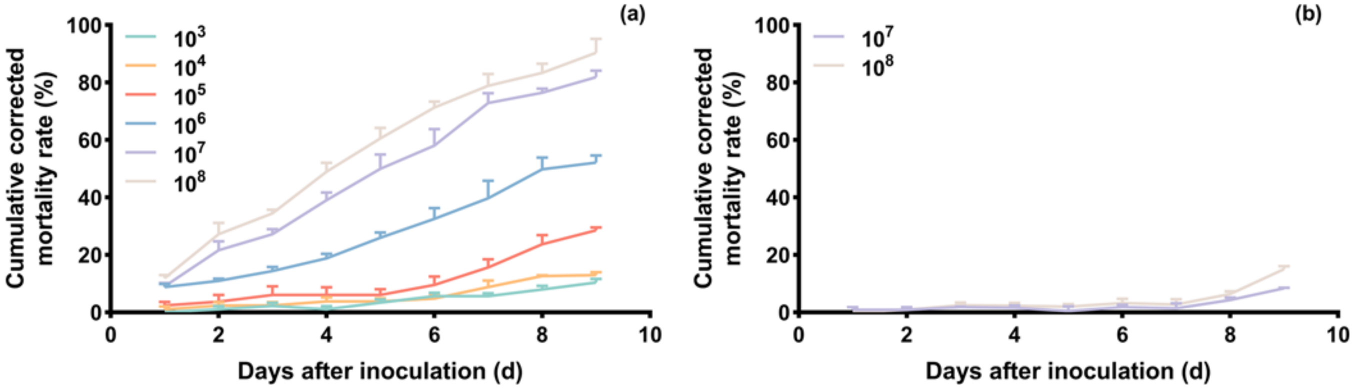

Figure 2. Cumulative corrected mortality rate (mean $\pm$ SE) of *Eotetranychus kankitus* (a) and *Neoseiulus barkeri* (b) females caused by Bb025 at different concentrations of conidia.

Table 2 Lethal concentration (LC_{50}) estimations of *B. bassiana* isolate Bb025 on the *E. kankitus* and *N. barkeri*

		95% confidence intervals				
Species	LC_{50} (conidia/mL^{-1})	Lower bound	Upper bound	Intercept	Slope $\pm$ SE	χ^2 ($df = 4$)
E. k[1]	3.488×10^5	1.317×10^5	9.685×10^5	−2.853	0.515 $\pm$ 0.073	2.733
N. b[2]	–[3]	–	–	–	–	–

[1] E. k: E. kankitus.
[2] N. b: N. barkeri.
[3] The mortality rate of *N. barkeri* remained low when treated with higher concentrations of Bb025, making it impossible to calculate the LC_{50} value for the predatory mites in this study.

Table 3 Lethal time (LT_{50}) estimations of *B. bassiana* isolate Bb025 on the *E. kankitus* and *N. barkeri*

		95% confidence intervals				
Species	LT_{50}(days)	Lower bound	Upper bound	Intercept	Slope $\pm$ SE	χ^2 ($df = 7$)
E. k[1]	3.581	2.931	4.243	−1.444	2.606 $\pm$ 0.378	2.343
N. b[2]	22.773	13.841	118.701	−3.063	2.257 $\pm$ 0.636	1.068

[1] E. k: E. kankitus.
[2] N. b: N. barkeri.

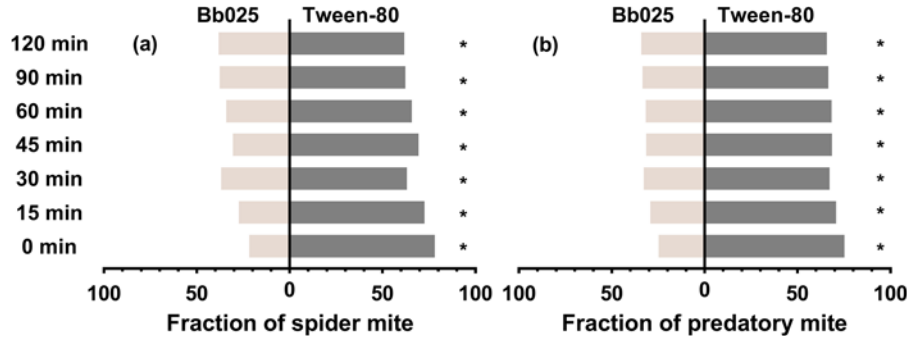

Figure 3. Preference of *Eoteranchus kankitus* (a) and *Neoseiulus barkeri* (b) to citrus leaflets inoculated with Bb025 (1×10^8 conidia/mL) and 0.05% Tween-80. Data were analysed using a chi-squared test to evaluate differences in each choice experiment ($P < 0.05$). The asterisk indicates significant differences between Bb025 and Tween-80 treatments.

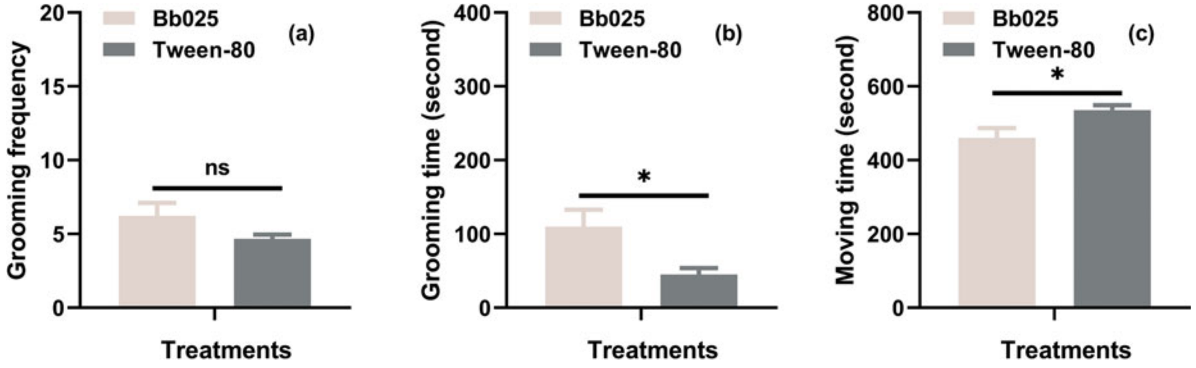

Figure 4. Grooming frequency (a), grooming time (b) and moving time (c) of *Neoseiulus barkeri* on citrus leaflets inoculated with Bb025 (1 × 10^8 conidia/mL) and 0.05% Tween-80. The 'ns' and asterisk indicate no significant and significant differences, respectively, between Bb025 and Tween-80 treatments, based on an independent-samples *t*-test ($P < 0.05$).

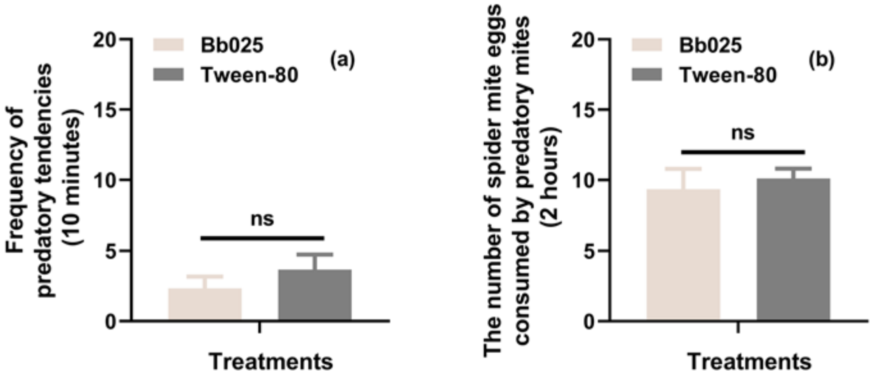

Figure 5. Frequency of predatory tendencies of *Neoseiulus barkeri* towards *Eoteranchus kankitus* eggs over a 10-minute period (a) and the number of *E. kankitus* eggs consumed by *N. barkeri* within 2 hours (b) on citrus leaflets inoculated with Bb025 (1 × 10^8 conidia/mL) and 0.05% Tween-80. The 'ns' indicates no significant differences between Bb025 and Tween-80 treatments, based on an independent-samples *t*-test ($P < 0.05$).

Efect of Bb025 on fecundity and offspring survival of N. barkeri

A higher number of *E. kankitus* females sprayed with Bb025 were consumed by *N. barkeri* compared to those treated with Tween-80 ($t = -2.818$, $df = 10$, $P = 0.018$) (Fig. 6a). Additionally, the total number of eggs laid by *N. barkeri* was greater when fed on *E. kankitus* females sprayed with Bb025 compared to those sprayed with Tween-80 (18.833 vs. 13.000; $t = -2.637$, $df = 10$, $P = 0.025$) (Fig. 6b). However, the total number of new generation individuals was 10.333 and 14.833 for *N. barkeri* female fed on *E. kankitus* sprayed with Bb025 and Tween-80, respectively, and these values were not significantly different ($t = -2.212$, $df = 10$, $P = 0.051$) (Fig. 6c).

Discussion

In this study, a strain of *B. bassiana* (Bb025) was selected for its high virulence against *E. kankitus* and lower pathogenicity towards *N. barkeri*. Both *E. kankitus* and *N. barkeri* exhibited avoidance behaviour towards leaves infected with Bb025. Additionally, the predatory capacity of *N. barkeri* female was not influenced by the presence of Bb025, although *N. barkeri* spent significantly more time engaging in self-grooming behaviour on leaf discs sprayed with Bb025 conidia. Notably, the number of eggs laid by *N. barkeri* feeding on infected *E. kankitus* increased, but subsequent generation individuals of *N. barkeri* feeding on treated *E. kankitus* were affected. These findings provide the potential of a combined approach using Bb025 and *N. barkeri* for effective biological control of *E. kankitus* at appropriate intervals.

Lower LT_{50} values and higher mortality rates indicate that the pests were rapidly infected by the fungus, which are important characteristics for choosing fungal strains as potential biocontrol agents (Geroh *et al.*, 2015; Wekesa *et al.*, 2005). In our study, the isolate of Bb025 showed lower LT_{50} values and a higher corrected mortality rate when targeting *E. kankitus*. These findings indicate that the fungal strain Bb025 has the potential to effectively control the population of *E. kankitus*. The fungal pathogenicity on pest populations is mainly influenced by dosage, and the mortality of pests often varies with conidial concentration (Krishnan *et al.*, 2012; Sarasan *et al.*, 2011). Our results also found that the application of Bb025 to control *E. kankitus* exhibited a clear concentration-dependent relationship, with higher fungal concentrations leading to increased mortality rates of *E. kankitus*. The maximum mortality rate observed was 90.402% in *E. kankitus* sprayed with 1 × 10^8 conidia/mL Bb025. However, it is important to note that using higher concentrations of fungi can also result in the direct mortality of non-target natural enemies (Flexner *et al.*, 1986). Castillo-Ramírez *et al.* (2020) reported that over 20% of *N. californicus* and *Phytoseiulus persimilis* died when infected with 1 × 10^8 conidia/mL Bb88 during the 9-day experiment. In our study, the mortality rate of *N. barkeri* was found to be 15.036% when infected with 1 × 10^8 conidia/mL Bb025, indicating that *N. barkeri* was susceptible to Bb025.

The pathogenicity of a fungus to a target insect is primarily caused by the penetration of the insect's cuticle by conidia (Wu *et al.*, 2018a). Previous study demonstrated that germinated

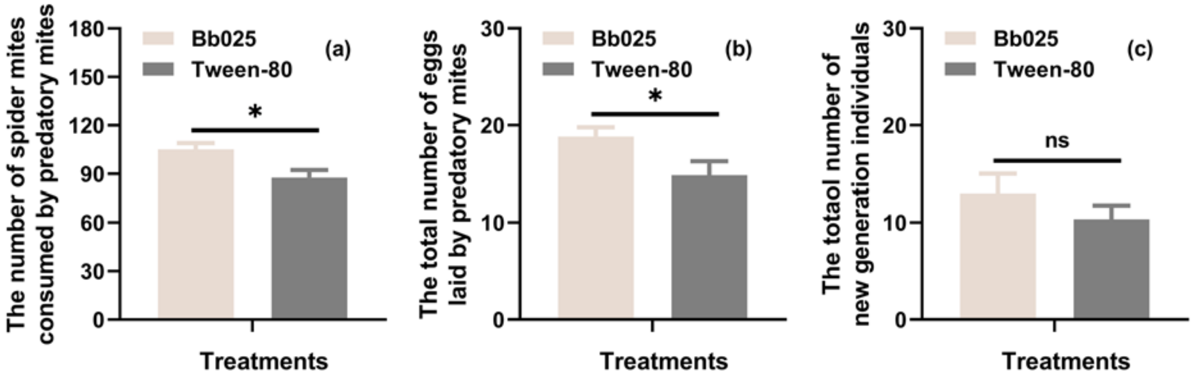

Figure 6. The number of *Eoteranchus kankitus* inoculated with Bb025 (1×10^8 conidia/mL) and 0.05% Tween-80 that were consumed by *Neoseiulus barkeri* (a), along with the number of eggs laid by *N. barkeri* (b) and the number of new generation individuals of *N. barkeri* (c) after feeding on these treated *E. kankitus*. The 'ns' and asterisk indicate no significant and significant differences, respectively, between Bb025 and Tween-80 treatments, based on an independent-samples *t*-test ($P < 0.05$).

conidia of *B. bassiana* SZ-26 were unable to penetrate the cuticle of *N. barkeri* (Wu *et al.*, 2014), and transmission electronic microscopy revealed that most SZ-26 *B. bassiana* conidia in the gut of the predatory mite dissolved within 24 h post-ingestion (Wu *et al.*, 2016b). However, Niu *et al.* (2023) reported that Bb025 can invade through the depressions and pores of the *N. barkeri* body wall. Additionally, Bb025 was detected in *N. barkeri* tissue using a specific nested PCR technique (Supplementary Fig. S1). These findings further confirm that the strain Bb025 has lower pathogenicity for the predatory mites *N. barkeri.*

Citrus yellow mites, *E. kankitus*, significantly avoided leaves sprayed with Bb025, suggesting that the presence of Bb025 decreased the consumption of citrus leaves by *E. kankitus*. This finding is consistent with the research by Rondot and Reineke (2017), who found that the presence of endophytic entomopathogenic fungi influenced the host choice behaviour of adult black vine weevils, leading to the avoidance of colonised plants and a consequent decrease in pest consumption. Seiedy (2014) reported that the presence of fungi in the prey or its habitat can affect the behaviour of predators, such as preference, activity, and feeding. This response implies that predators can recognise dangerous conditions through odour (Wu *et al.*, 2015b), and adjust their behaviour (Baverstock *et al.*, 2005; Faraji *et al.*, 2001). In our study, the predator *N. barkeri* also showed avoidance behaviour towards plants treated with Bb025. Additionally, *N. barkeri* invested more time in self-grooming on leaf discs with Bb025 conidia, which reduced the time spent searching for prey. Surprisingly, this self-grooming behaviour did not influence the predation capacity of *N. barkeri* on *E. kankitus*. This indicates that *N. barkeri* may be capable of recognising the presence of Bb025 and responding with avoidance behaviour or post-contact responses. If contact with spores is unavoidable, the self-grooming behaviour may effectively remove most spores attached to *N. barkeri*. This selection and self-grooming behaviour potentially enhances the survival rate of the predator (Seiedy *et al.*, 2012b).

Insects possess a selective advantage through their ability to detect and avoid fungal pathogens. According to Ríos-Moreno *et al.* (2018), *Chrysoperla carnea* larvae exhibited a preference for consuming healthy prey over those treated with *Metarhizium brunneum*. However, females of *N. barkeri* consumed more *E. kankitus* females infected with Bb025, laying more eggs compared to prey sprayed with Tween-80 in a no-choice test. This could be due to the decreased vitality of *E. kankitus* caused by fungal penetration, making them more vulnerable to predation by *N. barkeri*. These results are consistent with the findings of Wu *et al.*. (2015a). Furthermore, the population of subsequent generation individuals that fed on infected *E. kankitus* was reduced, presumably due to the weakly sclerotised cuticle of *N. barkeri* juveniles (Koehler, 1999; Shipp *et al.*, 2003). Thus, applying Bb025 and *N. barkeri* at short intervals may negatively impact the number of predatory mite offspring and hinder the establishment of predatory mites in the field.

Based on our findings, we suggest a combined approach using Bb025 and *N. barkeri* for *E. kankitus* control. Initially, spraying plants with Bb025 will decrease the density of *E. kankitus* due to the direct lethal effect of the Bb025 on *E. kankitus* in the sprayed areas. Additionally, the repelling effect of Bb025 on spider mites can help prevent the spread of *E. kankitus* from unsprayed to sprayed areas. Subsequently, releasing *N. barkeri* at appropriate intervals after spraying the fungus is recommended. These predatory mites can move to areas where Bb025 has not been sprayed, effectively controlling *E. kankitus*. Although *N. barkeri* may come into contact with the fungus while moving around and handling prey on the leaves, their grooming behaviour can help remove the conidia from their bodies (Wekesa *et al.*, 2007; Wu *et al.*, 2018b). Meanwhile, *N. barkeri* consumed more infected *E. kankitus* adults, resulting in lower population density of prey. It was found that *B. bassiana* had limited efficacy in suppressing the immature stages of spider mites (Wu *et al.*, 2020), indicating that *N. barkeri* and its offspring could provide continuous control for the immature stage of *E. kankitus* where *B. bassiana* failed to control. In conclusion, these findings suggest that using Bb025 strains of *B. bassiana* in combination with *N. barkeri* may be an effective approach for controlling *E. kankitus* on plants.

This integrated pest management strategy takes advantage of the strengths of both the entomopathogenic fungus and the predatory mites to address the pest problem more effectively. However, there is insufficient evidence to accurately forecast how the combined application of the two biocontrol agents will contribute to an additive suppression of the pest mite population. It will also be necessary to evaluate the combined effect of the two predators on *E. kankitus* in a more natural environment.

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