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ORIGINAL RESEARCH ARTICLE

Comparative pathogenicity of four entomopathogenic fungal species against nymphs and adults of citrus red mite on the citrus plantation

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Abstract

Panonychus citri (citrus red mite) is a devastating pest of citrus orchards. The conventional chemical acaricides have been strongly forbidden for the management of agricultural insect pests in China. Therefore, we evaluated the susceptibility of adult and nymphs *P. citri* in laboratory against eight isolates of four fungal species, *Akanthomyces lecanii*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Aschersonia aleyrodis*. Each citrus seedling having 40 adults (2-d-old) and nymphs (on separate plants) were sprayed with isolates at the concentration of $10^4 \sim 10^8$ conidia mL^{-1} whereas controlled seedlings were sprayed with 0.02% Tween-80. After 9 days of fungal exposure, the four fungal isolates caused more than 50% mortality of mites, such as; 85.6%, 87.9%, 64.6% and 79.7% by *A. lecanii* (V3450), *B. bassiana* (BFZ0409), *M. anisopliae* (MFZ0706) and *A. aleyrodis* (AsG0910), respectively. The nymphal mites were less susceptible to applied fungi compared to adults. The LC_{50}s of the tested isolates were determined by the fitted time-concentration-mortality relationships, which declined over days after spray. LT_{50}s were decreased with a high concentration of isolates. After the 9-d inoculation, two isolates of *B. bassiana* (BFZ0409 and D1344) and one isolate of *A. lecanii* (V3450) were highly effective at the minimal dose of LC_{50} of 10^4 conidia mL^{-1} and are promising candidates to control mites, as compared to other tested fungal isolates.

Keywords Citrus seedlings · Time-concentration-mortality · *Panonychus citri* · *Metarhizium anisopliae* · *Beauveria bassiana* · *Aschersonia aleyrodis*

Introduction

Mites belonging to Arachnida class (Ruppert et al. 2004) consist of four stages, i.e. egg, larva, nymph and adult. Nymphs (protonymph, deutonymph, and tritonymph) and adult are most feeding and damaging stages. There are more than 50,000 species of mites, which are predatory, parasitic, saprophagous, herbivores, necrophagous, fungivores, coprophagous as well as phoretic species (Dhooria 2016).

Mites that attack citrus plantations worldwide include *Panonychus citri* McGregor, *Eotetranychus kankitus* Ehara, *Polyphagotarsonemus latus* Banks, *Tetranychus kanzawai*

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Kishida, *Phyllocoptruta oleivora* Ashmead, as well as *Brevipalpus yothersi* Baker (Al-Azzazy 2016; Vechia et al. 2018; Zhou et al. 1999). Among them, *Panonychus citri* (citrus red mite) (CRM) is more harmful to fresh citrus shoots (Haiyuan 1996; Li 1990). Adult and nymph nurture and survive on the delicate plant leaves through sucking (Kranz et al. 1977), and develop lighter grey spots on leaves, that may hinder the process of photosynthesis (Kennett et al. 1999). High infestations cause early leaf dropping as well as shoot dieback, and weaken the plant vigor, along with feeding and damaging the fresh fruits (Jamieson et al. 2005; Kranz et al. 1977), and destroys the citrus plantation in China (Li et al. 1990; Yang et al. 2009).

A considerable number of acaricides have been used to control mites in China. Therefore, mite pests—including *P. citri*—become resistant to various insecticides due to long-term usage (Gerson and Cohen 1989; Meng et al. 2000). It is easy for CRM to become resistant to insecticides, and since the 1970s, citrus mites have become resistant to organophosphates and organochlorines, e.g. dipterex, dimethoate and chlorodifon (Gerson and Cohen 1989). Likewise, *P. citri* has developed resistance to pyridaben, abamectin and dicofol up to 11.2, 13.4 and 23 fold, respectively, in China (Meng et al. 2000). Therefore, conventional chemical acaricides have been strongly forbidden for the management of agricultural insect pests in China (Yu 2001). However, many other alternative control measures are being considered against mites in the world (Idrees et al. 2016). Predators and entomopathogens are being used as biological agents to control mites on broad-spectrum (Chandler et al. 2000; Jamieson et al. 2005; Paz et al. 2007; Poinar Jr and Poinar 1998; Shi and Feng 2004; Van der Geest et al. 2000). Therefore, entomopathogenic fungi are being applied as biopesticides for the management of mites (Chandler et al. 2000).

Entomopathogenic hyphomycetes infecting citrus mites have been described, especially on citrus plantation such as *Beauveria bassiana*, *Akanthomyces* (*Lecanicillium*) *lecanii* (Kepler et al. 2017), *Metarhizium anisopliae* and *Cordyceps* (*Isaria*) *fumosorosea* (Kepler et al. 2017) are well known microbial agents (Martins et al. 2016; Roberts and Leger 2004) and are being applied in pests control (Qasim et al. 2018; Wraight et al. 2000). These entomopathogens strongly respond against various mite pest across the globe in different ecosystems (Chandler et al. 2000). Recently, it has been observed that entomopathogenic fungi infect the various stages of spider mites (Alves et al. 2002; Shi et al. 2008a; Wekesa et al. 2005, 2006) as well as ectoparasitic mites (Shaw et al. 2002). Some other fungal isolates had been exploited against CRM, which caused significant mortality of mites. *Meira geulakonigii* (Boekhout, Scorzetti, Gerson & Sztejnberg) was applied on seedlings of sour orange at the rate of 2×10^8 conidia mL^{-1}, which caused 75% mortality of *P. citri* within 1 week (Sztejnberg et al. 2004). While Paz et al. (2007) claimed that *M. geulakonigii* caused 63% mortality of *P. citri* with the dose of 1×10^8 conidia mL^{-1} within a week. Furthermore, *M. argovae* (Boekhout, Scorzetti, Gerson & Sztejnberg) and *Acaromyces ingoldii* (Boekhout, Scorzetti, Gerson & Sztejnberg) resulted in the death of 59% and 58% CRM population, respectively. Whereas, Puspitarini et al. (2011) reported that more than 40% population of *P. citri* collected from natural citrus plantation was infected with *Hirsutella* sp. in Indonesia.

The present research was conducted to test the lethal response of *P. citri* to the four hypocrealean fungi with a complementary log-log (CLL) model, and this model was selected to confirm an apparent trend of fungal toxicity under the binary effect of concentration and time. Moreover, the comparative effectiveness of all isolates was assessed at different concentrations as well as fungal exposure.

Material and methods

Source of fungal isolates and conidia preparation

Eight isolates of four species were used in this study, i.e. *A.* (*L.*) *lecanii* (Vl6063, V3450, Vp28 and V09); *B. bassiana* (D1344, BFZ0409); *M. anisopliae* (MFZ0706) and *A. aleyrodis* (AsG0910), and all of the isolates were procured from different labs (Table 1). All fungal isolates were cultured on the Petri plates of Sabouraud dextrose agar (SDA), under the conditions of 25 ± 1 °C, 80 ± 5% R.H., and 14:10 Light: Dark (L:D) photoperiod. Conidia of all isolates were collected separately according to the method of Ye et al. (2005). Then the suspension was prepared into universal flasks, having 3 mm glass beads, by using 10 mL deionized water as well as 0.02% (*v*/v) Tween®-80 (Fluka). After that, suspensions were homogenized by shaking tubes on vortex for 5 min, and concentration was determined by using a Neubauer hemocytometer (model 1103) (Goettel and Inglis 1997). However, conidial viability of all isolates was observed before each bioassay, according to Wang et al. (2004). The conidial viability of suspensions was consistently high in all bioassays being greater than 98.3 ± 0.52%.

CRM rearing

Colonies of *P. citri* were established from individuals collected from citrus seedlings (*Citrus sinesis* Osbeck) in a greenhouse (25 ± 2 °C) of Fujian Agriculture and Forestry University, Fuzhou, China and reared on citrus plants. After that, bioassays were done against both stages of mite, nymph and adult. For this purpose, 20 healthy adult females were collected from the population, and kept separately on fresh seedlings for 24 h, to get a homogeneous batch of eggs, and at last uniform aged nymphs. The vigorous nymphs (24 h after

Table 1 Geographical location and original host of fungi in present study

Species	Fungal isolate	Geographical location	Original host
Akanthomyces lecanii	Vl6063	Halifax, Canada	*Trialeurodes vaporarioum*
A. lecanii	V3450	Guangzhou, China	*Bemisia tabaci*
A. lecanii	Vp28	Guangzhou, China	*Pseudococcus* sp.
A. lecanii	V09	Hefei, China	*Noplophora chinesis*
Beauveria bassiana	BFZ0409	Fuzhou, China	*Plutella xylostella*
B. bassiana	D1344	DSMZ, Germany	Unknown
Aschersonia aleyrodis	AsG0910	Guangzhou, China	*Dialeurodes citri*
Metarhizium anisopliae	MFZ0706	Fuzhou, China	*Blattella germanica*

hatching from eggs of uniform age in the same growth chamber) were transferred onto detached citrus twigs with two leaves on a sponge containing a rhizocaline (100 g mL^{-1}) for bioassays. To collect particular aged adults for further experiment, 50 quiescent deutonymphs (second stage of nymph of mite) were taken from the seedlings and transferred onto detached citrus twigs with two leaves on a sponge containing a rhizocaline of 100 g mL^{-1}, under the conditions of 25 ± 1 °C, 12:12 L: D as well as 80 ± 5% RH. After that, nymphs and adult females were collected under a stereo-microscope (Nikon SX-45-TR) and then transferred onto new twigs of healthy citrus leaves, and these twigs were maintained the glass pot (12 × 9 cm).

Bioassays

We assayed for the biocontrol potential of eight isolates from four fungal species mentioned above against the nymphs and adults of *P. citri* in a lamp-chimney-caged seedling bioassay system. A 3.0 mL spore suspension (1.0×10^4 to 1.0×10^8 conidia mL^{-1}) (of each fungal species) was sprayed into a chamber having infested twigs with a gas sprayer (Preval Sprayer, NY, USA, Vapor Pressure 4.018, Vapor Density 1.8) as fungal treatment whereas 3.0 mL 0.02% (*v*/v) Tween®-80 was sprayed on controlled twigs. After that, the top of lamp-chimney-cage was covered with a water-proof mesh film up to initial 24 h to maintain relative humidity, for the conidial germination. While inner wet filter paper lined on the Petri dish was changed daily (surviving mites on the filter paper dropping from the twigs were transferred onto the leaves of the twigs again). All observations (counting of dead and alive for nymph and adult) were done daily for 9 days with the help of a 10-fold hand magnifier. Later on, these mite cadavers were transferred into moist Petri dishes for fungal growths up to 2–3 days and followed by the verification of fungal infection, under the stereomicroscope (Nikon SX-45-TR) at 50X magnifications. Then the particular individuals, having fungal outgrowths, were counted as dead as a consequence of the tested isolates. All bioassays were repeated for five times with 40 female adult or nymphal mites for every treatment or blank control in each repeat.

Data analysis

The serial time-concentration-mortality was designed according to the description of Preisler and Robertson (1989) as well as Robertson and Preisler (1992). Data were analyzed by using the complementary log-log model (CLL model), and cumulative mortality was estimated (Nowierski et al. 1996; Wang et al. 2004). Controls were adjusted according to the model, described by Christensen and Chen (1985), and Robertson and Preisler (1992). Similarly, the particular formulae of Robertson and Preisler (1992) were used for the values of LC_{50} (or LC_{90}) whereas LT_{50} values were estimated by linear interpolation (Feng et al. 1998; Nowierski et al. 1996). Mortality was adjusted according to the method of Nowierski et al. (1996).

The procedures, including modeling, estimation of time and concentration-effect parameters for the CLL models, test for goodness of fit, and estimation of virulence indices (LC_{50}) using the parameters were analyzed using DPS data processing system software (Feng et al. 1998; Tang and Feng 2007).

Results

CRM infected by fungal pathogens

Early mycosis-caused deaths of CRM (nymphs and adults) began from 3-d inoculation, and the infected nymph and female adults became lethargic before death. The mycosed dead bodies indicated subtle fungal out-growths on the confined citrus leaves, perhaps, due to less humidity. However, all nymphs and adults became well infected after being transferred into moist petri dishes, within 3 days. All fungal infected mites (adult and nymphs) produced proper mycelia and conidia in the petri dishes, and, which mean all of eight strains, were capable of infecting both stages of the mites.

Mortality of CRM by fungal isolates

The cumulative mite mortalities by different concentrations of all fungal isolates are presented in Figs. 1 and 2. The trends of the observed mite mortalities were depended on both concentration and time. After 4 days of fungal application, there was considerable mortality of both stages (nymph and adults). After the ninth day of fungal application at the concentration of 10^8 against nymphal mite population, maximum mortality was 70.7% by BFZ0409 and D1344 (*B. bassiana*), whereas least mortality was 46.5% by AsG0910 (*A. aerlodis*) (Fig. 2). Similarly, after 9-d fungal exposure at the highest concentration against adult mites, BFZ0409 (*B. bassiana*) caused maximum mortality (87.9%), followed by V3450 (*A. lecanii*) with 85.6% mortality. The least mortality (51.9%) was presented by Vl6063 (*A. lecanii*). On the other hand, after 9-d of observation in the controlled treatment, the maximum mortality was 5.4% and 6.9% for nymphal and adult mites, respectively (Fig. 1).

The results of time-concentration-mortality modeling of nymph and adult female mites infected by eight isolates simulated by CLL model are shown in Figs. 1 and 2. The *t*-tests

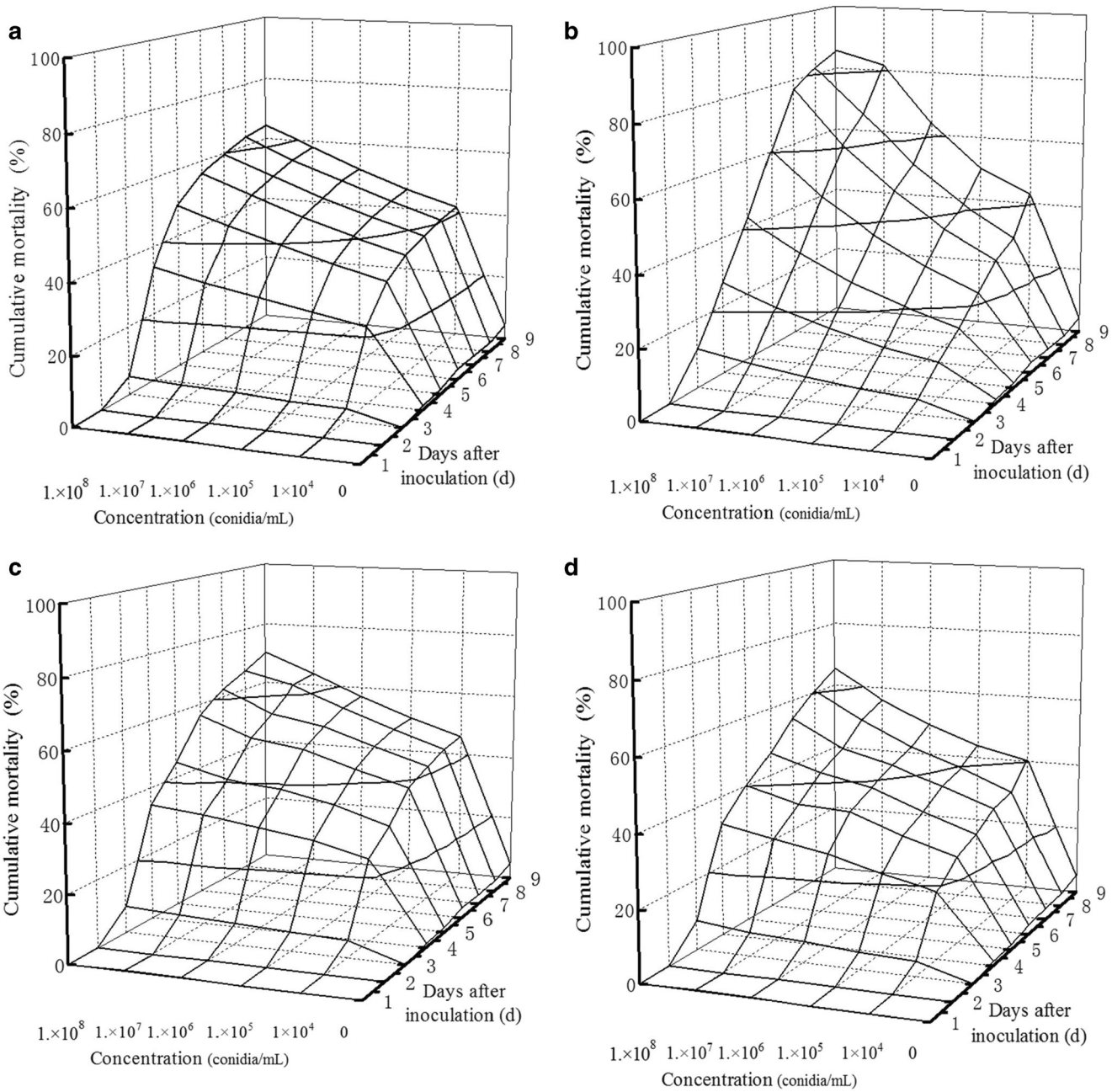

Fig. 1 Relationship between percentage mortality, spores concentration, and time for the female mites of *Panonychus citri*. **a** Vl6063; **b** V3450; **c** Vp28; **d** V09; **e** BFZ0409; **f** D1344; **g** AsG0910; **h** MFZ0706

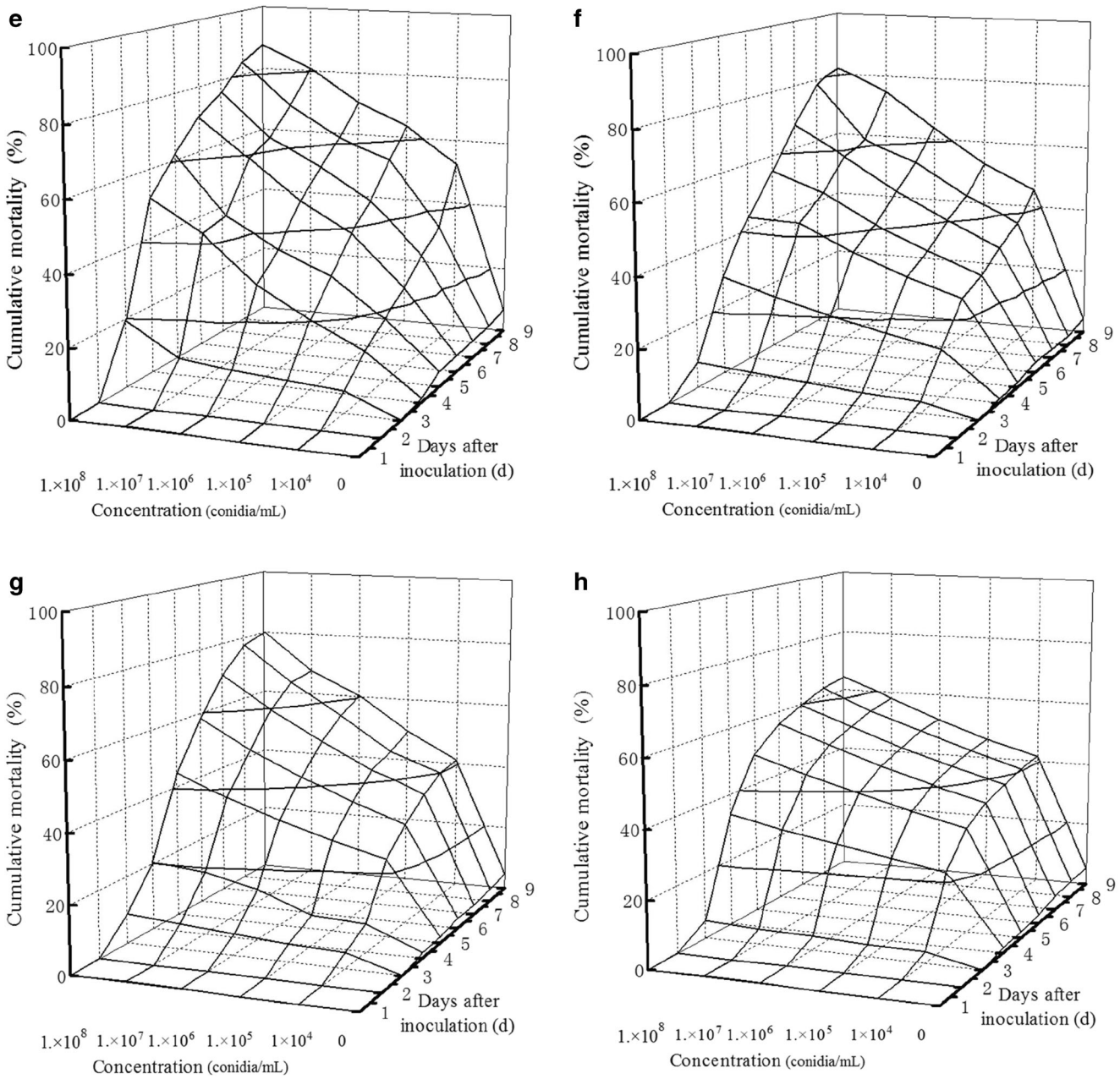

Fig. 1 (continued)

for all parameters estimated were significant ($P < 0.01$). Hosmer–Lemeshow statistic $\hat{C}$ (a grouped Pearson's χ^2, i.e. modified Pearson's χ^2 by Nowierski et al. (1996) for the heterogeneity of the goodness of fit were non-significant for all eight fungal isolates ($P < 0.05$, Table 2). The data of the eight fungal isolates against the nymphs and females was fitted well to the CLL model. The slope values (β), the parameters from the maximum likelihood estimation in the CLL model indicated the rate of the proportion of CRM mortality as a function of log (spores concentrations of each suspension). Significant relationships between proportion mortality and log dose were found in all eight fungal isolates considered ($P < 0.01$, Table 2). The mortality and treatment had a strong relationship, as illustrated by the magnitude of the slope values ($P = 0.05$ by DMRT, Table 3). The fitted parameter β represented the slope values of the fitted curve, with the range from 0.15 to 0.49 and 0.16 to 0.36 against nymphal and adult mites, respectively. Isolates with a larger magnitude of slope values caused CRM mortality at a faster rate. Two isolate of *A. lecanii* (Vp28 ($\beta = 0.49$) and V3450 ($\beta = 0.36$)) were found to have the most substantial magnitude of slope value among all strains, examined for the impact on nymphal and adult mites, respectively (Table 2). The fitted parameters indicated that the concentration and time affected the efficiency of the tested isolates. The

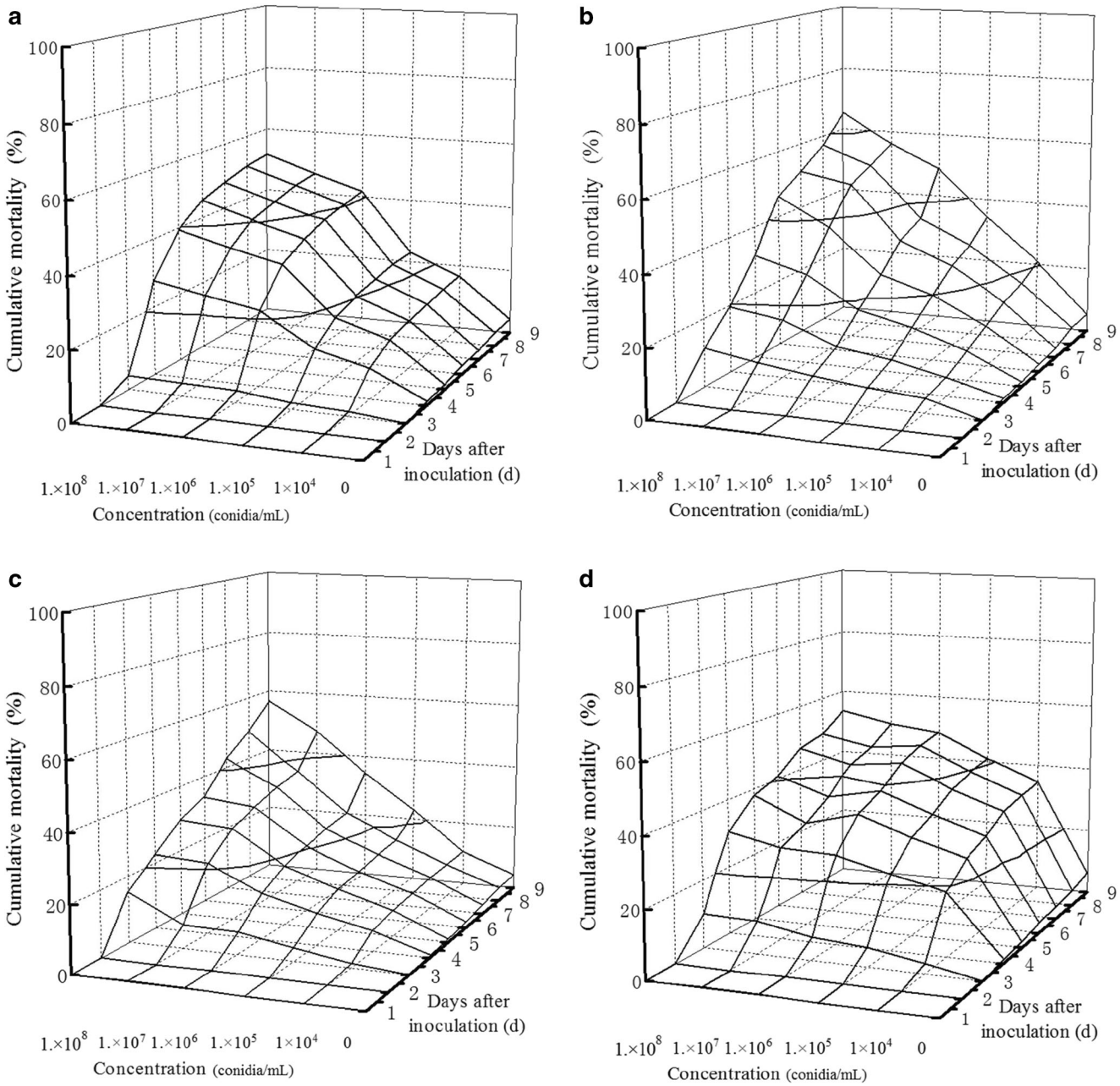

Fig. 2 Relationship between percentage mortality, spores concentration, and time for the nymphal mites of *Panonychus citri*. **a** Vl6063; **b** V3450; **c** Vp28; **d** V09; **e** BFZ0409; **f** D1344; **g** AsG0910; **h** MFZ0706

estimated parameters of 4-d spaying (γ_4) were the largest for most tested isolates (Vl6063, Vp28, V09, BFZ009), indicating the estimate of latent periods for these microbial agent tested (Christensen and Chen 1985).

Based on the cumulative relationships of the fungal isolates against the mites determined by the fitted β and γ_j, the values of LC_{50}s and associated confidence 95% intervals were computed as a function of the post-spray days (Tables 3 and 4). After 9 days of fungal exposure to adult mites, two isolates of *B. bassiana* (BFZ0409 and D1344) and one isolate of *A. lecanii* (V3450) showed the highest virulence at the least LC_{50} value (10^4 conidia mL^{-1}). On the other hand, two isolates of *A. lecanii* (Vl6063 and V09) showed the least virulence at higher LC_{50}s of 10^6 conidia mL^{-1}, respectively. However, at the same fungal and time exposure, the LC_{50}s values were higher for all isolates against nymphal mites. For example, least LC_{50} value was 3.25×10^5 conidia mL^{-1} for BFZ0409 (*B. bassiana*), and higher LC_{50} value was 6.59×10^8 conidia mL^{-1} for MFZ0706 (*M. anisopliae*).

The estimated LT_{50}s were reduced with the increment of concentrations of fungal concentration (Tables 3 and 4). For

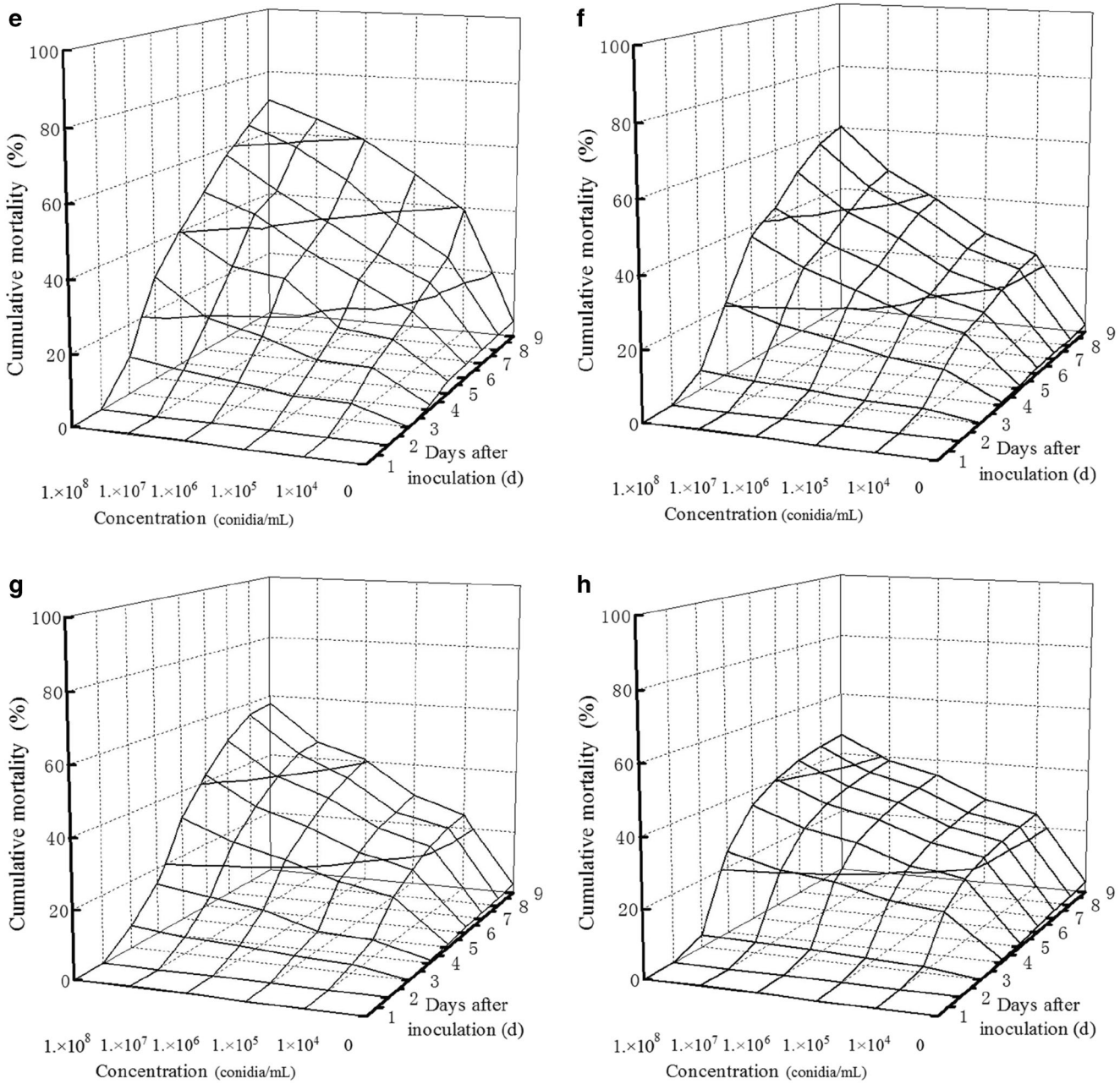

Fig. 2 (continued)

example, the LT_{50}s were computable at the concentration of 10^5 conidia mL^{-1}, only for BFZ0409. The two *B. bassiana* isolates showed rapid mortality of 4.4d-5.0d at the high concentration of 10^8 conidia mL^{-1}. For four tested *A. lecanii* isolates, the estimates ranged from 5.3d-6.5d, V09 exhibited the slowest mortality with 6.5d at the concentration of 10^8 conidia mL^{-1}. The isolates of *A. aleyrodis* (AsG0910) and *M. anisopliae* (MFZ0706) showed intermediate mortality.

The equivalent slopes for concentration effects, the virulence indices (LC_{50}s) and LT_{50}s indicated that two isolates of *B. bassiana* (BFZ0409 and D1344) were the most virulent and high efficient isolates against CRM, followed by one isolate of *A. lecanii* (V3450). The others were the intermediate in virulence and efficacy.

Discussion

In the current study, we assessed the potential of eight isolates from four fungal species against CRM on citrus seedlings. Our results proved the effectiveness of all tested isolates against CRM females, but LC_{50}s and LT_{50}s determined by the fitted

Table 2 Parameters estimated from the simulation of bioassay of eight isolates against *Panonychus citi* by the CLL model

Isolates/ adults	Parameters(Mean ± SE) [a]							Slope	$\hat{C}$ test [b]		
	γ_3	γ_4	γ_5	γ_6	γ_7	γ_8	γ_9	($\beta \pm$ SE)	$\hat{C}$	d.f	*P* value
Vl6063	−4.18 ± 0.37	−2.31 ± 0.30	−2.69 ± 0.31	−3.61 ± 0.36	−3.88 ± 0.38	−4.06 ± .41	−7.75 ± 2.03	0.16 ± 0.05	0.234	8	0.999
V3450	−4.86 ± 0.32	−4.54 ± 0.32	−4.23 ± 0.31	−4.02 ± 0.30	−3.76 ± 0.30	−4.26 ± 0.32	−3.54 ± 0.29	0.36 ± 0.04	1.641	8	0.990
Vp28	−3.76 ± 0.33	−2.27 ± 0.28	−3.13 ± 0.31	−2.88 ± 0.31	−3.36 ± 0.34	−3.47 ± 0.35	−4.72 ± 0.53	0.16 ± 0.04	1.292	8	0.996
V09	−3.99 ± 0.35	−2.66 ± 0.31	−3.43 ± 0.33	−4.02 ± 0.37	−3.69 ± 0.35	−3.97 ± 0.38	−3.63 ± 0.35	0.19 ± 0.05	2.185	8	0.975
BFZ0409	−4.18 ± 0.30	−3.28 ± 0.28	−4.50 ± 0.33	−3.63 ± 0.29	−3.94 ± 0.30	−3.58 ± 0.29	−3.04 ± 0.27	0.31 ± 0.04	4.438	8	0.816
D1344	−4.64 ± 0.33	−3.45 ± 0.29	−3.24 ± 0.29	−4.09 ± 0.33	−3.52 ± 0.30	−3.64 ± 0.31	−3.22 ± 0.30	0.27 ± 0.04	3.957	8	0.861
AsG0910	−4.40 ± 0.33	−4.28 ± 0.33	−3.08 ± 0.29	−3.48 ± 0.31	−3.42 ± 0.31	−3.81 ± 0.33	−4.52 ± 0.40	0.27 ± 0.04	2.498	8	0.962
MFZ0706	−4.38 ± 0.32	−4.63 ± 0.33	−3.72 ± 0.30	−3.55 ± 0.30	−3.45 ± 0.30	−3.75 ± 0.32	−4.53 ± 0.38	0.31 ± 0.04	0.720	8	0.999
Isolates/ nymph											
Vl6063	−5.91 ± 0.48	−4.07 ± 0.41	−4.50 ± 0.42	−5.38 ± 0.47	−5.87 ± 0.51	−6.49 ± 0.61	−6.89 ± 0.69	0.37 ± 0.06	2.488	8	0.962
V3450	−5.03 ± 0.39	−5.59 ± 0.43	−4.85 ± 0.39	−4.59 ± 0.38	−4.85 ± 0.39	−5.68 ± 0.46	−4.46 ± 0.38	0.38 ± 0.05	6.337	8	0.61
Vp28	−5.81 ± 0.48	−5.68 ± 0.47	−5.99 ± 0.49	−6.86 ± 0.55	−6.44 ± 0.53	−7.03 ± 0.61	−5.31 ± 0.47	0.49 ± 0.07	6.740	8	0.565
V09	−3.57 ± 0.36	−2.55 ± 0.33	−3.37 ± 0.35	−4.09 ± 0.40	−3.49 ± 0.37	−4.53 ± 0.46	−4.30 ± 0.46	0.15 ± 0.05	2.877	8	0.942
BFZ0409	−4.25 ± 0.33	−3.91 ± 0.33	−3.96 ± 0.33	−3.76 ± 0.32	−4.01 ± 0.34	−3.57 ± 0.32	−3.16 ± 0.30	0.26 ± 0.04	12.680	8	0.123
D1344	−5.42 ± 0.43	−4.28 ± 0.38	−4.04 ± 0.38	−5.23 ± 0.44	−4.59 ± 0.40	−4.66 ± 0.40	−5.71 ± 0.51	0.33 ± 0.05	0.256	8	0.999
AsG0910	−4.96 ± 0.41	−4.84 ± 0.41	−3.75 ± 0.37	−4.24 ± 0.39	−4.26 ± 0.39	−4.75 ± 0.43	−5.60 ± 0.52	0.29 ± 0.05	2.979	8	0.936
MFZ0706	−4.94 ± 0.46	−3.12 ± 0.38	−3.60 ± 0.39	−4.34 ± 0.43	−5.20 ± 0.52	−5.35 ± 0.55	−5.83 ± 0.64	0.21 ± 0.06	0.427	8	0.999

[a] A subscript associated with γ denotes the specific day after spray. All *t*-test were significant at $P < 0.01$ in each modeling for estimated parameters

[b] Goodness-of-fit statistic ($\hat{C}$ test) $\chi^2 < \chi^2_{0.05} = 15.51$ with *d.f.* =8 in each CLL modeling regression

Table 3 Time-specific LC_{50}s and concentration-specific LT_{50}s of isolates against the female adult of *Panonychus citri*

Fungal isolates	LC_{50} / conidia mL^{-1} (95% CI / conidia mL^{-1})						LT_{50} (Days) [a]			
	day 4	day 5	day 6	day 7	day 8	day 9	1×10^5	1×10^6	1×10^7	1×10^8
Vl6063	1.53×10^{11} (4.39×10^{10}–5.30×10^{11})	1.96×10^8 (1.06×10^8–3.61×10^8)	2.70×10^7 (1.72×10^7–4.25×10^7)	7.00×10^6 (4.82×10^6–1.02×10^7)	2.50×10^6 (1.78×10^6–3.50×10^6	2.43×10^6 (1.74×10^6–3.41×10^6)	–	–	6.7	5.3
V3450	1.30×10^{10} (3.92×10^9–4.33×10^{10})	3.12×10^8 (1.44×10^8–6.76×10^8)	1.94×10^7 (1.15×10^7–3.27×10^7)	1.71×10^6 (1.11×10^6–2.64×10^6)	5.61×10^5 (3.58×10^5–8.79×10^5)	9.03×10^4 (5.28×10^4–1.54×10^5)	8.9	7.6	6.3	5.4
Vp28	2.51×10^{10} (1.56×10^9–4.03×10^{11})	3.79×10^8 (6.53×10^7–2.22×10^9)	6.94×10^6 (2.51×10^6–1.92×10^7)	9.35×10^5 (3.73×10^5–2.34×10^6)	1.93×10^5 (6.77×10^4–5.48×10^5)	1.26×10^5 (4.16×10^4–3.83×10^5)	–	7.0	5.9	5.3
V09	6.74×10^{10} (4.42×10^9–1.03×10^{12})	1.56×10^9 (2.41×10^8–1.02×10^{10})	2.89×10^8 (6.44×10^7–1.30×10^9)	3.82×10^7 (1.25×10^7–1.16×10^8)	1.02×10^7 (4.01×10^6--2.54×10^7)	1.97×10^6 (8.94×10^5–4.33×10^6)	–	–	8.0	6.5
BFZ0409	1.77×10^8 (4.00×10^7–7.79×10^8)	4.34×10^7 (1.24×10^7–1.51×10^8)	3.32×10^6 (1.28×10^6–8.64×10^6)	7.87×10^5 (3.09×10^5–2.00×10^6)	1.49×10^5 (5.21×10^4–4.28×10^5)	1.67×10^4 (4.33×10^3–6.46×10^4)	8.2	6.8	5.6	4.4
D1344	3.00×10^{10} (2.89×10^9–3.11×10^{11}	1.03×10^8 (3.02×10^7–3.49×10^8)	2.02×10^7 (7.67×10^6–5.34×10^7)	2.09×10^6 (9.48×10^5–4.61×10^6)	4.17×10^5 (1.81×10^5–9.60×10^5)	5.71×10^4 (2.02×10^4–1.61×10^5)	8.7	7.5	6.3	5.0
AsG0910	1.40×10^{12} (8.79×10^{10}–2.24×10^{13})	2.46×10^8 (7.47×10^7–8.13×10^8)	1.18×10^7 (5.41×10^6–2.57×10^7)	1.11×10^6 (5.68×10^5–2.18×10^6)	3.04×10^5 (1.46×10^5–6.35×10^5)	1.72×10^5 (7.85×10^4–3.77×10^5)	–	7.4	6.1	5.3
MFZ0706	1.14×10^{11} (2.96×10^{10}–4.39×10^1)	4.90×10^8 (2.30×10^8-1.05×10^9)	1.40×10^7 (8.78×10^6–2.22×10	1.02×10^6 (6.85×10^5–1.52×10^6)	2.33×10^5 (1.49×10^5–3.64×10^5)	1.29×10^5 (8.02×10^4–2.09×10^5)	–	7.0	6.1	5.4

[a] No computable LT_{50}s for the given concentrations (conidia mL^{-1}) if not given

Table 4 Time-specific LC_{50}s and concentration-specific LT_{50}s of isolates against the nymph of *Panonychus citri*

Fungal isolates	LC_{50} / conidia mL^{-1} (95% CI / conidia mL^{-1})						LT_{50}(Days [a])			
	day 4	day 5	day 6	day 7	day 8	day 9	1×10^5	1×10^6	1×10^7	1×10^8
Vl6063	5.17×10^9 (1.51×10^9–1.77×10^{10})	3.12×10^8 (1.36×10^8–7.16×10^8)	1.30×10^8 (6.38×1007–2.65×10^8)	8.02×10^7 (4.18×10^7–1.54×10^8)	6.28×10^7 (3.38×10^7–1.17×10^8)	5.35×10^7 (2.93×10^7–9.77×10^7)	–	–	–	6.5
V3450	9.34×10^{10} (9.77×10^9–8.93×10^{11})	3.16×10^9 (6.58×10^8–1.52×10^{10})	2.18×10^8 (7.44×10^7–6.37×10^8)	4.99×10^7 (2.14×10^7–1.16×10^8)	2.91×10^7 (1.35×10^7–6.27×10^7)	6.23×10^6 (3.36×10^6–1.15×10^7)	–	–	8.7	6.5
Vp28	3.68×10^9 (5.42×108–2.50×10^{10})	7.77×10^8 (1.70×10^8–3.55×10^9)	4.60×10^8 (1.15×10^8–1.85×10^9)	2.28×10^8 (6.79×10^7–7.66×10^8)	1.61×10^8 (5.23×10^7–4.96×10^8)	3.33×10^7 (1.45×10^7–7.62×10^7)	–	–	–	8.3
V09	1.29×10^{12} (3.66×10^9–4.55×10^{14})	1.97×10^{10} (2.99×10^8–1.29×10^{12})	3.70×10^9 (1.09×10^8–1.26×10^{11})	2.66×10^8 (2.13×10^7–3.32×10^9)	1.16×10^8 (1.25×10^7–1.08×10^9)	4.38×10^7 (6.59×10^6–2.91×10^8)	–	–	–	8.2
BFZ0409	3.59×10^{11} (1.01×10^{10}–1.28×10^{13})	7.24×10^9 (5.84×10^8–8.98×10^{10})	2.96×10^8 (5.25×10^7–1.67×10^9)	4.50×10^7 (1.19×10^7–1.69×10^8)	4.51×10^6 (1.68×10^6–1.22×10^7)	3.25×10^5 (1.18×10^5–8.92×10^5)	–	8.6	7.4	6.6
D1344	1.55×10^{11} (6.56×10^{10}–3.66×10^{11})	1.29×10^9 (7.72×10^8–2.16×10^9)	4.80×10^8 (3.07×10^8–7.51×10^8)	1.00×10^8 (7.07×10^7–1.42×10^8)	3.03×10^7 (2.28×10^7–4.02×10^7)	2.08×10^7 (1.59×10^7–2.71×10^7)	–	–	–	7.0
AsG0910	1.64×10^{13} (9.22×10^{11}–2.92×10^{14})	8.99×10^9 (1.88×10^9–4.31×10^{10})	7.21×10^8 (2.29×10^8–2.27×10^9)	1.10×10^8 ($4.67 \times 10^7 2.62 \times 10^8$)	4.21×10^7 (2.03×10^7–8.72×10^7)	2.88×10^7 (1.46×10^7–5.70×10^7)	–	–	–	7.1
MFZ0706	1.45×10^{12} (4.90×10^{11}–4.27×10^{12})	1.47×10^{10} (7.05×10^9–3.05×10^{10})	2.80×10^9 (1.52×10^9–5.15×10^9)	1.49×10^9 (8.50×10^8–2.62×10^9)	8.96×10^8 (5.30×10^8–1.52×10^9)	6.59×10^8 (3.98×10^8–1.09×10^9)	–	–	–	–

[a] No computable LT_{50}s for the given concentrations (conidia mL^{-1}) if not given

cumulative relationships varied considerably. All isolates were sufficiently virulent to both stages of CRM, although, all isolates presented significantly different mortality ranges. Adult females were more susceptible to all isolates as compared to nymphs. The reason may be some conidial spores drop off with ecdyses as molting. Therefore, with the time, nymphicidal activities of these isolates varied considerably with the complicated situation for their variation of enzymes and germination potency. All fungal entomopathogens could be applied in the greenhouse and field conditions, through various application methods. These entomopathogens could be spread directly in the target area, as spore coatings, spore bags, spore containing media or indirectly by spreading the fungal-infected insect bodies (Farenhorst and Knols 2010; Pilz et al. 2011; Stafford and Allan 2014).

Numerous entomopathogenic fungi, including *B. bassiana*, have been assessed for the management of several mites, such as *Tetranychus urticae* (Bugeme et al. 2014; Ullah and Lim 2015) and *T. cinnabarinus* (Erler et al. 2013). Tehri et al. (2015) described that *B. bassiana* reduced more than 60% population of *T. urticae* on okra in the field conditions. Likewise, the efficiency of *B. bassiana* was also evaluated against *T. urticae* at the rate of 1×10^8 conidia mL^{-1} on bean and cucumber, which caused mortality of 50% mite population in the greenhouse (Seyed-Talebi et al. 2014). Moreover, the pathogenicity of *B. bassiana* was explored against eggs and adults of *T. urticae* on Okra by Krishna and Bhaskar (2013), which caused mortality only 7 % of both stages. Moreover, *B. bassiana* was also presented 92% mortality against CRM adults in laboratory bioassays within 5 days at the rate of 1×10^8 conidia mL^{-1} (Alves et al. 2005). Variation in the potency of *B. bassiana* may be affected by several factors, such as temperature, humidity, experimental conditions, the concentration of used dose as well as plant variety. The pathogenicity of *B. bassiana* (ARSEF 2860) against *P. citri* has been documented up to 90% after 20 days of fungal application with a high dose of 1.2×10^{13} conidia ha^{-1} in the field conditions (Shi and Feng 2006). But according to our findings, *B. bassiana* (BFZ0409) caused quick mortality of CRM, and killed half population within 5 days with a dose of 1×10^8 conidia mL^{-1} on citrus seedlings in controlled conditions.

The susceptibility of different mites to various fungal species is much attractive for the management of the mites in a diverse environment. In the current study, we observed that both stages of CRM were significantly susceptible to all tested fungal isolates. Likewise, Aguirre and Krugg (2014) described that adults of CRM were more susceptible to *A. lecanii* as well as *B. bassiana* at the concentration of 10^6 conidia mL^{-1}, as both fungal species significantly reduced the adult population of mites by 71% within 2 weeks. Therefore, the results of the current study were in accordance with the findings of Aguirre and Krugg (2014), because two isolates (Vp28 and V3450) of *A. lecanii* killed more than half populations of nymphs and adults within 9 days by 10^8 conidia mL^{-1}. Similarly, *A. lecanii* has much potential to inhibit the growth of different mites, like *Tetranychus urticae* Koch (Amjad et al. 2012) and *Dendrolaelaps* sp. (Bałazy et al. 2008).

Similarly, the lethal potential of *M. anisopliae* was also appealing against several insect pests, as well as mite pests. *M. anisopliae* is much effective against several pest mites, like *Brevipalpus phoenicis* (Magalhães et al. 2005), *Mononychellus tanajoa* (Barreto et al. 2004), *T. urticae* (Bugeme et al. 2015), *T. evansi* (Maniania et al. 2016), *T. truncates* and *T. turkestani* (Shi et al. 2008a, 2008b). Likewise, it was observed in our current work that *P. citri* had faced 63% and 44% mortality of adult and nymphs, respectively, by *M. anisopliae* at 10^8 conidia mL^{-1} within 9 days of exposure, which shows that our findings are in accordance with the reports of García and Krugg (2015), who described that *M. anisopliae* caused up to 83% mortality of CRM in lab conditions under the fungal exposure for 2 weeks. Moreover, *Aschersonia aleyrodis* also has a significant potential to control various insect pest, like whitefly (Zhang et al. 2017). However, there is one report by Tamai et al. (2002) who described that *A. aleyrodis* had presented minute potential to inhibit the growth of *T. urticae*. Beyond all fungal explorations against CRM, *A. aleyrodis* still uncovered for its pathogenicity. We observed in the current research that *A. aleyrodis* (AsG0910) caused 70% and 52% mortality of adults and nymphs of CRM within a week, respectively, after application of 10^8 conidia mL^{-1}.

Conclusion

In conclusion, two isolates of *B. bassiana* (BFZ0409, D1344) and one isolate of *A. lecanii* (V3450) were highly virulent against both stages of CRM at the lowest doses, and therefore, these isolates could be recommended as promising candidates for the management of CRM. Whereas, other five isolates were less effective against both stages of CRM. Thus, employing these isolates into integrated management of mites could assist synthetic acaricides in the citrus orchards and avoid some predator mites susceptible to mycosis. However, the potency of these isolates is still needed to be evaluated in field conditions, especially, the compatibilities with some phytoseiid predators before field application.

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Compliance with ethical standards

Conflict of interest All authors declare no conflict of interest, and are agree to proceed the article in the International J. Tropical Insect Science.

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