

Selection of entomopathogenic fungi against the red spider mite *Tetranychus kanzawai* (Kishida) (Tetranychidae: Acarina)

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Abstract

The pathogenicity of three entomopathogenic fungal species to *Tetranychus kanzawai* was investigated. Seven isolates of *Metarhizium anisopliae*, six isolates of *Beauveria bassiana*, and an isolate of *Paecilomyces lilacinus* from the Philippines and Indonesia were evaluated. The following studies were undertaken: (1) screening of *M. anisopliae*, *B. bassiana* and *P. lilacinus* pathogenic to *T. kanzawai*, and (2) bioefficacy studies of the selected entomopathogenic fungi under greenhouse conditions. Conidia of each isolate were mass-produced on potato dextrose agar (PDA) at $26 \pm 1^\circ\text{C}$ and a 12-hour photophase for a maximum of 21 days. Preliminary screening for the most pathogenic isolate within the same species was determined using suspension with 10^4 to 10^8 conidia ml^{-1} . At 4 days after treatment (DAT), the pathogenicity within *M. anisopliae* isolates in decreasing order was Ma5>Ma6>Ma4>Ma2>Ma1>Ma3>Ma7 while for *B. bassiana*, was Bb6>Bb5>Bb4>Bb3>Bb1>Bb2. The top three most pathogenic isolates within the two species were subjected to further studies to determine the most virulent isolate against *T. kanzawai*. At 5 DAT, the LC_{50} values of *M. anisopliae* isolates ranged from 5.0×10^2 to 1.4×10^3 while for *B. bassiana* ranged from 1.2×10^3 to 2.4×10^3 conidia ml^{-1} . Based on LC_{50} , the virulence of the fungal isolates within the species in decreasing order was Ma6>Ma5>Ma4 and Bb6>Bb5>Bb4. However, the LC_{50} values are not significantly different from each other. Green house trials showed that the epizootic of entomopathogenic fungus can regulate the population of mites. The fungal isolates used in the study, although not originally isolated from mites were virulent to *T. kanzawai*, indicating their wide host range.

Keywords entomopathogenic fungi; mortality; selection; *Tetranychus kanzawai*; *Beauveria bassiana*; *Metarhizium anisopliae*; greenhouse.

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1 Introduction

The entomopathogenic fungi include those genera that are associated with insects and related arthropods in a variety of ways. These arthropod-fungi associations range from saprophytic, commensalistic, parasitic, or pathogenic. The pathogenicity of a fungal isolate is dependent on its ability to attach on and penetrate the host cuticle as well as to replicate within the host, usually in the hemocoel. The virulence of an entomopathogenic fungus is also associated with their ability to produce toxic substances, which interfere not only with the normal host development and metamorphosis but in some cases with the immune system until the host ultimately dies from fungal infection (Tanada and Kaya, 1993).

Spider mites, belonging to family Tetranychidae, is a cosmopolitan arthropod pest species, In Indonesia and the Philippines, *Tetranychus kanzawai*, is one of the most common mite pest species. It is a polyphagous species, infesting over a hundred species of plants including agricultural crops (Takafuji et al., 2005). Throughout East and South Asia, it commonly infests cassava and papaya plants (Gavarrá, 1981) as well as hundreds of plants including vegetables and food crops such as strawberries, peppers, tomatoes, potatoes, beans and corn. The mites severely damage the older leaves of papaya and sometimes, its seedlings. They often feed on chloroplasts on the under surface of the leaf, which causes the upper leaf surface to develop characteristic whitish or yellowish stippling. As mite feeding continues, the stippling coalesces to form brownish lesions (Cheng et al., 2009). Heavy damage eventually leads to wilting and defoliation, which further reduces plant growth due to reduced photosynthetic activity of the plant.

A substantial number of mycoinsecticides and mycoacaricides have been developed worldwide since the 1960s. At least 12 species or subspecies or varieties of fungi have been employed as active ingredients of mycoinsecticides and mycoacaricides for inundative and inoculative applications, although some are no longer in use. Products based on *B. bassiana* (33.9%), *M. anisopliae* (33.9%), *Isaria fumosorosea* (5.8%), and *B. brongniartii* (4.1%) are the most popular insecticides. For acaricide, twenty eight products are claimed to control mites such as *B. bassiana*, *Hirsutella thompsonii*, *I. fumosorosea*, *L. muscarium*, *Lecanicillium sp.* and *M. anisopliae* among the common 171 products (de Faria and Wraight, 2007).

2 Materials and Methods

2.1 Maintenance of host plants

Papaya plants of the solo variety were used as the host plant for laboratory rearing of red spider mite, *Tetranychus kanzawai*. The seeds were collected, dried, planted in nursery plots using sterilized soil, fertilized using urea, and watered every day. When the plants reached 5 cm in height, they were transferred to plastic pots, which contained soil and cattle manure (2:1). These papaya plants were used as rearing medium for mites once they reached 10 cm high. The plants were maintained at the Crop Protection Cluster (CPC) greenhouse, College of Agriculture, U.P. Los Baños.

2.2 Mass-rearing of *Tetranychus kanzawai*

A number of female adults of *T. kanzawai*, aged 24 to 36 hours after emergence from deutonymph stage collected from papaya plants in the Institute of Plant Breeding, Crop Science Cluster, College of Agriculture, U.P. Los Baños, were introduced into the papaya plants at the CPC greenhouse. The papaya plants were watered regularly and were also observed daily for population growth of mites. Heavily damaged papaya plants were replaced with new healthy plants. Adult mites from previously reared generations were maintained and used for different experiment assays. Papaya leaves containing mites were continuously transferred to other papaya seedlings.

2.3 Test fungi

Fourteen isolates (Table 1) from the Philippines and Indonesia comprised of six (6) *Beauveria bassiana*, seven

(7) *Metarhizium anisopliae*, and one (1) *Paecilomyces lilacinus* isolates were evaluated in this study. The isolates were plated onto potato dextrose agar (PDA) in flat bottle flasks and incubated at $26 \pm 1^\circ\text{C}$ with 12-hour photophase for a maximum of 21 days. The isolates were then stored in glass vials containing PDA culture medium.

Table 1 Fourteen fungal isolates from the Philippines and Indonesia used in the study.

FUNGAL SPECIES	CODE	COLLECTION SITE	INSECT HOST
<i>Beauveria bassiana</i>	Bb1	Jember, Indonesia	<i>Hypotenemus hampei</i>
	Bb2	Jombang, Indonesia	<i>H. hampei</i>
	Bb3	Balitsa, Indonesia	<i>Bemisia tabaci</i>
	Bb4	Cipanas, Indonesia	<i>Plutella xylostella</i>
	Bb5	Lembang, Indonesia	<i>Spodoptera litura</i>
	Bb6	Los Baños, Philippines	Unidentified insect
<i>Metarhizium anisopliae</i>	Ma1	Cirebon, Indonesia	<i>Oryctes rhinoceros</i>
	Ma2	Lembang, Indonesia	<i>Helicoverpa armigera</i>
	Ma3	Lembang, Indonesia	<i>S. exigua</i>
	Ma4	Cipanas, Indonesia	<i>Agrotis ipsilon</i>
	Ma5	Cikampek, Indonesia	<i>S. exigua</i>
	Ma6	Los Baños, Philippines	Unidentified insect
	Ma7	Los Baños, Philippines	<i>H. armigera</i>
<i>Paecilomyces lilacinus</i>	Pl	Los Baños, Philippines	Unidentified insect

2.4 Bioassay tests

2.4.1 Laboratory screening for the virulent strains

Preliminary screening. Ten adult female mites, aged 24 to 36 hours after emergence from the stock colony, were transferred on to abaxial surface of a 1.5 cm leaf disc in 2.5 cm petri dish with a brush. The females were classified visually by observation of the shape of the opisthosoma, which is round in females and funnel-shaped opisthosoma in males.

A series of five dilutions was prepared for each of the fungal suspensions with 10^4 , 10^5 , 10^6 , 10^7 , and 10^8 conidia ml^{-1} . Phosphate buffered saline (PBS) served as control. These suspensions were transferred into a plastic sprayer. Spraying was done first on the control mites using 0.5 ml of the suspension and then followed by the fungal suspensions starting with the lowest up to the highest concentration. Six trials were conducted using 10 mites/concentration, with 3 replications. Percent mortality was observed at 5 days post infection (DPI).

Final screening. The three most virulent isolates within the *B. bassiana* and *M. anisopliae* species identified from the preliminary study including the lone *P. lilacinus* were further subjected to LC_{50} determination using the same procedure but using eight concentrations from 10^1 to 10^7 conidia ml^{-1} . Mortality data obtained at 5 DPI were subjected to probit analysis. Six trials were conducted using 10 mites per concentration with 3 replications per treatment.

2.4.2 Greenhouse bioassay

The selected isolates were tested on mites under greenhouse condition using 60-days old Solo papaya seedlings in a completely randomized design. Ten adult mites were introduced in each papaya seedling using a fine brush. The seedlings were then hand sprayed with fungal suspensions with 10^8 conidia ml^{-1} . Three trials were done with three replications for each isolate. Adult mortality was recorded daily with 10x handheld magnifying glass until 100% mortality was observed. All the test mites were examined under a dissecting

scope to verify that fungal infection caused the mortality. The LT_{50} values were calculated using Polo Plus (Le Ora Software, Petaluma, California, USA).

3 Results and Discussion

3.1 Preliminary screening

All isolates of *B. bassiana*, *M. anisopliae* and *P. lilacinus* infected the mite *T. kanzawai* (Figs 1a-1n). They demonstrated rapid external hyphal development and sporulation under moist condition. Initially, hyphal strands emerged from the anal region of the mite cadaver and then quickly covered the cadaver with profused hyphal growth followed by sporulation within 4 to 6 days. *B. bassiana* showed a whitish colour when sporulation occurred, *M. anisopliae* appeared greenish in colour, while *P. lilacinus* appeared pinkish upon sporulation. Their pathogenicity to *T. kanzawai* was established using Koch's postulate test (Sanjaya et al, 2013a and 2013b).

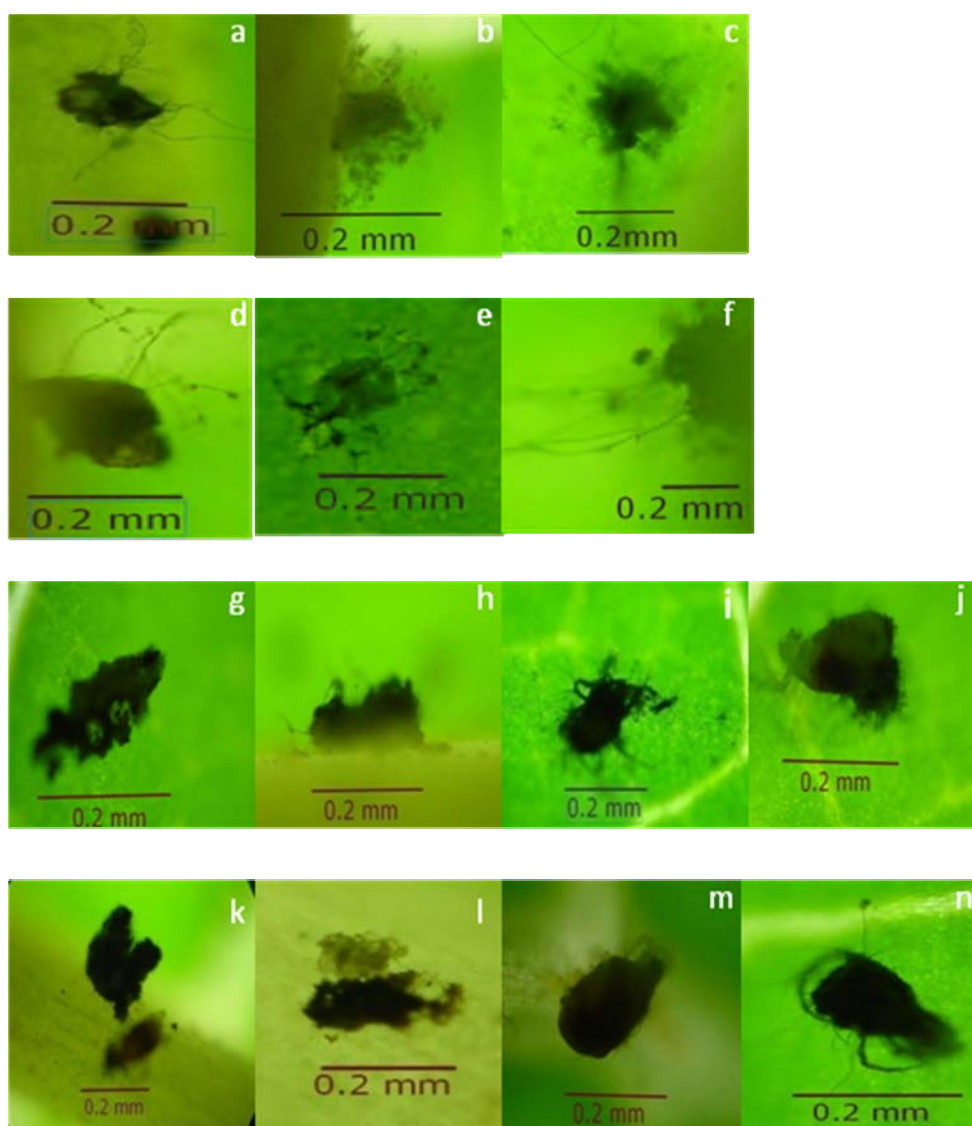


Fig. 1 *Tetranychus kanzawai* infected with entomopathogenic fungi isolates of *Metarhizium anisopliae*, *Beauveria bassiana* and *Paecilomyces lilacinus* (400 x magnification). (a) *B. bassiana* Bb1, (b) *B. bassiana* Bb2, (c) *B. bassiana* Bb3, (d) *B. bassiana* Bb4, (e) *B. bassiana* Bb5, (f) *B. bassiana* Bb6, (g) *M. anisopliae* Ma1, (h) *M. anisopliae* Ma2, (i) *M. anisopliae* Ma3, (j) *M. anisopliae* Ma4, (k) *M. anisopliae* Ma5, (l) *M. anisopliae* Ma6, (m) *M. anisopliae* Ma7, and (n) *P. lilacinus* Pl.

Six trials were conducted to determine the most pathogenic isolates that were subjected to further characterization. Mortality rates of mites infected the fourteen fungal isolates at five conidial concentrations used at 5 days post infection (DPI) are summarized in Fig. 2. In general, the five concentrations caused 50.00% to 100.00% mortality.

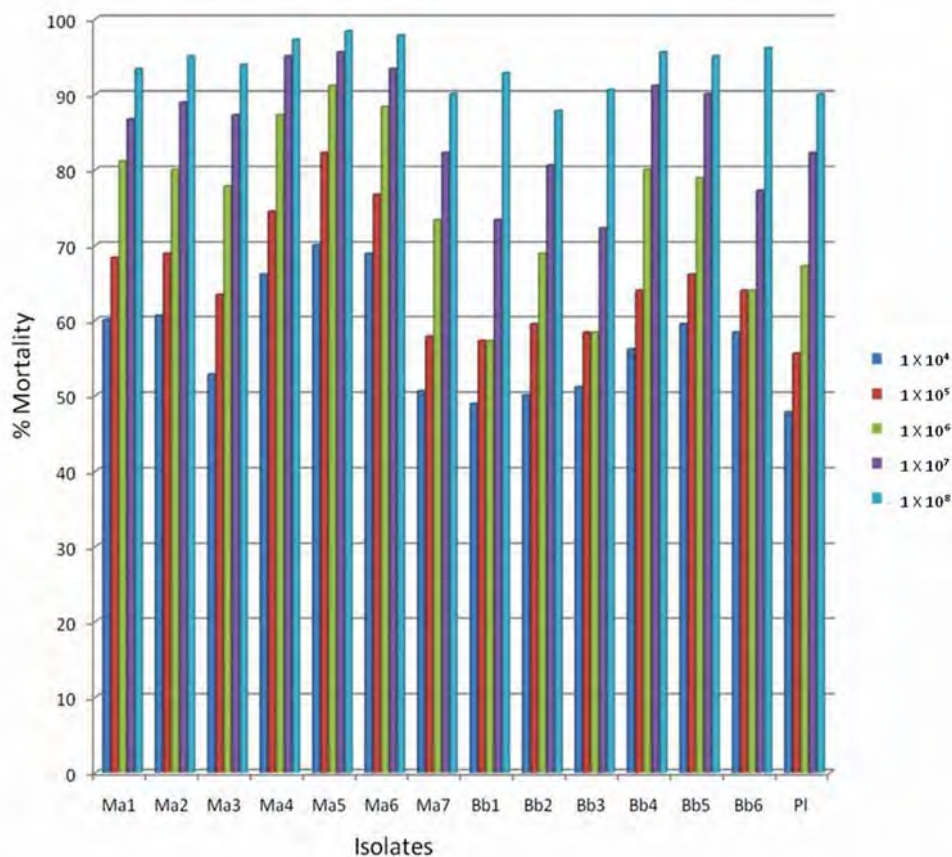


Fig. 2 Percent mortality of female adults of *Tetranychus kanzawai* at 5 days post infection infected with 14 isolates of entomopathogenic fungi.

Among the six *B. bassiana* isolates, the three most virulent isolates at 5 DPI were Bb4, Bb5 and Bb6 with mortality rates of 95.55%, 94.99% and 96.10%, respectively. While among the seven *M. anisopliae* isolates, the three most virulent isolates of *Metarhizium anisopliae* were Ma4, Ma5 and Ma6, with mortality rates of 97.20%, 98.31% and 97.78%, respectively (Fig. 2). Although the mortality caused by the lone *P. lilacinus* ranked second from the least virulent strain, it was still included from the selection of fungal pathogens for further study.

The results obtained in the present study are comparable with those of other researches that used fungal pathogens against mites. In a study by Tamai (1997) on the mite *T. urticae* using *Beauveria* spp. isolates at a concentration of 5×10^8 conidia ml⁻¹ mortality ranged from 5.50% to 100% were observed. Similarly, de Oliveira et al. (2002), worked with *B. bassiana* isolates at 10^8 conidia ml⁻¹ and the red mite *Oligonychus yothersi* (McGregor), recorded 77.00% to 98.00% mortality. On the other hand isolates of *M. anisopliae*, caused 12.00% to 45.00%, and LT₅₀ values that ranged from 8.6 to 18.4 days.

The variation in virulence among the different fungal isolates to *T. kanzawai* may be associated with the enzymes produced by each isolate. A high enzymatic activity of cuticle-degrading enzymes was observed Sosa

Gómez and Alves (1983) in the more virulent isolates of *M. anisopliae* isolated from several Brazilian regions. St. Leger et al., (1993 and 1996a) and de la Rosa et al. (1997) suggested that the differences in virulence of entomopathogenic fungal strains are probably associated with the presence of enzymes that influence the penetration process of the fungus. Secondary metabolites production for instance, toxins such as destruxins and beauvericin, present in *M. anisopliae* and *B. bassiana*, respectively, which vary in may also contribute to the observed variation in virulence (Roberts and St. Leger 2004). However, compared with chemical insecticides, fungal infection takes 4 to 6 days after application to kill a mite (St. Leger et al., 1996b).

3.2 LC₅₀ values of the selected isolates

The mortality of *T. kanzawai*, at 5 DPI, caused by the seven most pathogenic *B. bassiana* and *M. anisopliae* isolates at various concentrations is presented on Table 2. The approximate LC₅₀ values (Table 2) for the selected fungus isolates were estimated for *T. kanzawai* at 5 DPI. A change in virulence ranking of the isolates within the *Metarhizium* and *Beauveria* species was observed as Ma6>Ma5>Ma4 and Bb6>Bb5>Bb4. Among the entomopathogenic fungal species, *M. anisopliae* was more pathogenic than *B. bassiana* with *P. lilacinus*, a distant third.

Table 2 LC₅₀ values (conidia/ml) of the entomopathogenic fungi isolates of *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces lilacinus* at 5 days post infection to adult females of *Tetranychus kanzawai*.

SPECIES	ISOLATE	LC ₅₀	95% FIDUCIAL LIMITS
<i>B. bassiana</i>	Bb4	2.4 x 10 ³	9.2 x 10 ² – 3.5 x 10 ³
	Bb5	2.0 x 10 ³	1.1 x 10 ³ – 5.1 x 10 ³
	Bb6	1.2 x 10 ³	9.0 x 10 ² – 4.0 x 10 ³
<i>M. anisopliae</i>	Ma4	1.4 x 10 ³	6.6 x 10 ² – 4.0 x 10 ³
	Ma5	7.2 x 10 ²	3.1 x 10 ² – 1.5 x 10 ²
	Ma6	5.0 x 10 ²	2.3 x 10 ² – 1.0 x 10 ³
<i>P. lilacinus</i>	Pl	1.8 x 10 ⁴	9.2 x 10 ³ – 3.5 x 10 ⁴

For LC₅₀ determination, a total of eight concentrations was used that resulted to mortality rates of 10 to 100%, indicating a reliable data set. The results are similar with Maketon et al. (2008) using *M. anisopliae* CKM-048, *M. anisopliae* was the most virulent fungal strain, hence the most promising candidate for controlling broad mite immature and adult of *Polyphagotarsoemus latus*. Also, *B. bassiana* CKB-048 showed good efficacy against immatures of broad mites with 95.00 % mortality and it was also effective against adults (ca. 50% mortality), so this may be considered another effective microorganism against broad mites. The concentrations of *M. anisopliae* CKM-048 needed for killing 50% of broad mite larvae and adults (8.71 x 10⁶ and 1.32 x 10⁷ conidia/ml, respectively), were not far apart. Yet, the time needed for killing 50% (when treated with 2.00 x 10⁸ conidia/ml) of adults (3.4 days) was clearly longer than the time needed for killing half of the larvae (2.4 days).

3.3 Efficacy of selected entomopathogenic fungi under greenhouse conditions

The LT₅₀ values of the seven isolates were estimated from data obtained from three trials Table 3. The results showed that spray suspension with 10⁸ conidia ml⁻¹ of the fungal isolates gave superior controls of mite. Furthermore, LT₅₀ values indicated that there was no difference between *B. bassiana* and *M. anisopliae* based on overlapping fiducial limits, while *P. lilacinus* was statistically different from the previous two fungal species.

Table 3 LT₅₀ values (days) of spray suspension of 7 entomopathogenic fungi with 10⁸ conidia ml⁻¹ to *Tetranychus kanzawai* on papaya seedlings under greenhouse conditions.

SPECIES	ISOLATES	LT ₅₀ (DAYS)	95% FIDUCIAL LIMIT
<i>Beauveria bassiana</i>	Bb4	5.48	5.082 to 5.915
	Bb5	4.53	4.202 to 4.866
	Bb6	3.98	3.697 to 4.261
<i>Metarhizium anisopliae</i>	Ma4	4.23	3.898 to 4.567
	Ma5	3.66	3.341 to 3.913
	Ma6	3.00	2.728 to 3.305
<i>Paecilomyces lilacinus</i>	Pl	6.33	5.847 to 6.889

The findings of the three trials showed that the epizootic of entomopathogenic fungus can regulate the population of red spider mites. Although the fungi used in the study were not originally isolated from mites, the fungi showed pathogenicity to *T. kanzawai*. The same was observed by Shaw et al. (2002) in another study where he reported fungal isolates infecting non-acarine hosts were pathogenic *Varroa destructor* Anderson and Trueman. Peña et al. (1996) found that fungal isolates originating from *Polyphagotarsonemus latus* Banks (Tarsonomidae) were more pathogenic than those isolated from other hosts. Strict adaptation of *M. anisopliae* strains to the original host, though, has been likewise reported in the case of scarabaeid beetles (Ferron et al., 1972).

In addition, greenhouse data indicate that pathogenic fungi have great potential for control of *T. kanzawai*. The LT₉₀ of *T. kanzawai* infected with all isolates ranged from 8.57 to 19.34 days among experimental units. These data show that the LT₉₀ was more or less the same prior to the trial except on *P. lilacinus*. The longest LT₉₀ (19.34 days) was observed on plots treated with *P. lilacinus* while the shortest was found on Ma6 (8.57 days).

4 Summary and Conclusions

Fourteen entomopathogenic fungi isolated from different insect species were evaluated against the spider mite, *Tetranychus kanzawai*. The three most pathogenic isolates of *Metarhizium anisopliae* (Ma4, Ma5, Ma6) from among seven in the original list, and the three most pathogenic isolates of *Beauveria bassiana* (Bb4, Bb5, Bb6) from among the seven in the original list and the lone *Paecilomyces lilacinus* isolate (PI) were chosen based on percent mortality to the mites at five days after exposure by contact spraying. *M. anisopliae* Ma6 was the most virulent isolate based on LC₅₀ (5.0 x10²) and LT₅₀ (3.00 days). In general, all *Metarhizium* and *Beauveria* isolates tested in this study showed potential for management of *T. kanzawai*.

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