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Identification of an Entomopathogenic Fungus, *Pseudozyma flocculosa* (Traquair, Shaw & Jarvis), and Its Efficacy against *Tetranychus urticae* Koch

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Abstract: The two-spotted spider mite, *Tetranychus urticae* Koch, is one of the most important agricultural pests worldwide, with chemical application being the primary control method. However, frequent heavy use or misuse of insecticides has accelerated the development of varying degrees of resistance in *T. urticae*. This makes the chemical control of this mite more challenging. Biocontrol methods have attracted much attention due to their safety and environment-friendly impact. Based on previous observations that the population of *T. urticae* was infected by unknown pathogenic fungi, we isolated, identified, and evaluated the pathogenicity of the fungi from infected mites. Through available morphological and molecular identification, the fungus was identified as *Pseudozyma flocculosa*. The virulence activity of the strain was evaluated at different concentrations of spore suspension (10^6–10^9 conidia/mL) using a spraying method. The strain showed pathogenic activity against the *T. urticae* in adult females that varied with different concentrations and temperatures. Meanwhile, the *P. flocculosa* also had a significant toxic effect on the developmental stages of *T. urticae*. In the laboratory bioassay, the mortality rate of the tested mites reached 100% at 9 d after *P. flocculosa* treatment. Additionally, a wettable powder processed with *P. flocculosa* conidia was applied on the *T. urticae* in the greenhouse and the control efficacy reached up to 90% at 7 d after treatment. The results showed a high insecticidal activity of *P. flocculosa* against *T. urticae*, indicating that this fungus possesses great potential for use as a bio-insecticidal agent.

Keywords: fungus identification; two-spotted spider mite; virulence activity; biological control

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1. Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch, belonging to the Acariformes, Tetranychoidea, is one of the most destructive polyphagous pests, causing substantial losses to fruit and vegetable crops worldwide [1]. Currently, the main control measures for *T. urticae* are chemical agents. According to the Arthropod Pesticide Resistance Database (http://www.pesticideresistance.org, accessed on 1 January 2024), *T. urticae* has the highest number of pesticide resistance cases among the *Tetranychus* genus, limiting the efficient application of pesticides in global agricultural production. The frequent and extensive use of pesticides leads to the development of resistance to a wide range of active ingredients, including abamectin, pyrethroids, organophosphates, and even some newly developed pesticides such as bifenazate, chlorfenapyr, cyflumetofen, cyenopyrafen [2–4]. Therefore, it is necessary to determine some environment-friendly and sustainable control technologies, especially the biological control agents, because of their green, safe, and non-toxic advantages [5].

Most studies and development of biological control solutions for *T. urticae* have focused on predatory mites and microbial control [6–8]. Entomopathogenic fungi are widespread and are important biocontrol microorganisms for managing insect populations in nature

as part of integrated pest management programs [9]. More than 100 genera and over 1000 species of entomopathogenic fungi have been described [10,11]. Some developed biocontrol products are being used effectively to control a wide range of insect pests worldwide, for example, in India, Korea, Japan, Thailand, Ukraine, and the USA [10,12]. Some typical entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin [8,13,14] and *Metarhizium* spp., have been developed as fungal agents for controlling different insect pests, including *Cimex lectularius*, *Frankliniella occidentalis*, and *T. urticae*, in the field [15,16]. Among these, some fungal strains have been reported to have significant pathogenicity on *T. urticae*, by inhibiting egg hatching and reducing the fecundity of females [15]. *B. bassiana* and *Isaria fumosorosea* colonized the infected eggs by penetrating the egg shell and extending hyphae to feed on the eggs, causing the mite eggs to fail to hatch [17].

Aside from the above common fungi, the new genus infecting some pests is poorly studied or explored. Therefore, the screening and application of fungi with pesticidal activity are of significant theoretical and practical importance for the harmless prevention and control of phytophagous mites, including *T. urticae*. Here, we report the species identification of an unknown pathogenic fungus isolated from dead mites with fungus infestation reared in the laboratory. We determined the pathogenic potential of the pathogenic fungus toward *T. urticae* mites. The result will provide a scientific basis for developing biological control technology for *T. urticae* mites.

2. Materials and Methods

2.1. Tetranychus urticae Mites

The *T. urticae* laboratory strain (named IPP-SS), susceptible to most commonly used pesticides, was provided by the Institute of Plant Protection, Chinese Academy of Agricultural Sciences. After the introduction, the mite species was fed on the clean and insect-free "Bifeng" bean plants in a greenhouse at a temperature of 26.5 ± 3 °C, 75% relative humidity and 16 h (light):8 h (dark).

2.2. Preparation of Eggs, Larvae, and Protonymphs of T. urticae

Active female adults were randomly selected from the IPP-SS population using a fine brush and placed on the bean leaf discs (approximately 5 cm diameter) on 6-cm-diameter filter paper in a 10-cm-diameter glass dish to obtain *T. urticae* eggs of the same age. The leaf petiole was wrapped in water-absorbent cotton. The female adults were allowed to oviposit freely for 24 h and then removed, leaving the eggs for use. The eggs were kept in an artificial climate chamber at 26.5 ± 1 °C and 75% relative humidity. After approximately 3–4 d, the larvae hatched from the above eggs and were used for the bioassay. Similarly, protonymphs were obtained 6–7 d after the egg stage. The mites were observed daily under a microscope (Olympus SZX7) to ensure that the individuals in each developmental stage were healthy and kept in the same condition.

2.3. Fungus and Fungal Suspensions

The tissue separation method was adopted to isolate and culture pathogens, with slight modifications [16,18]. Dead adult mites having been infected with fungi were collected from the laboratory, rinsed five times with sterile water, inoculated with a needle onto a potato dextrose agar (PDA, BD-Pharmingen, Becton, Dickinson and Company, New York, NY, USA) plate, and cultured in a 28 °C incubator (ZQZY-88BN, Zhichu Instrument Co., LTD, Shanghai, China) for 2–3 d. The grown mycelium was plated on a new PDA plate three times for repeated purification. The pure colony was inoculated onto the bevel of a PDA test tube when the mycelium had grown over the bevel and was stored at 4 °C.

The fungus was cultured in a potato dextrose broth (PDB, BD-Pharmingen, Becton, Dickinson and Company, New York, NY, USA) liquid medium at 28 °C for 7 d in a shaking incubator to obtain a concentration gradient fungal suspension. The conidia to be

measured for viability were obtained by double filtration with blotting paper to separate the propagule. They were randomly transferred to the counting plate and counted under a microscope (Olympus CX31, 40× magnification) using a Neubauer hemocytometer (Qiujing Biochemical Instrument Co., LTD., Shanghai, China) and expressed as conidia/mL.

The conidial concentrations used in the laboratory bioassay were 10^9, 10^8, 10^7, and 10^6 submerged conidia/mL diluted with sterile water, respectively. The 50% wettable powder (WP) used in the field trial was adsorbed from the conidial solution onto the talc (Solaibao Technology Co., LTD., Beijing, China) and dried at 37°, then mixed with the auxiliary agent: disodium methylenebisnaphthalenesulphonate (Yuanye Bio-Technology Co., Ltd., Shanghai, China), sodium dodecyl sulfate (Solaibao Technology Co., LTD., Beijing, China), hydrated silica (Macklin Biochemical Co., Ltd., Shanghai, China), diatomaceous earth (Yuanye Bio-Technology Co., Ltd., Shanghai, China), and monobasic potassium phosphate (Yuanye Bio-Technology Co., Ltd., Shanghai, China) in proportion and ground into a powder using a grinder. Then, the 50% wettable powder diluted 50-, 100-, and 200-fold was sprayed on the *T. urticae* infesting on strawberry plants in the greenhouse.

2.4. Morphological and Molecular Identification

Morphological identification was performed as follows: After purification, individual spores of the fungal strain were inoculated into a sterile PDB triangular flask and cultured on a shaking table at 28 ± 1 °C for 72 h. The fresh cultures were used to prepare the water-impregnated sheets, observed under a light microscope (Olympus IX53, 400× magnification), and the characteristic structures were photographed.

Fungus with obvious 7 d sporulation, having been cultured at 28 °C, was scraped from the PDA plate, and total DNA was extracted according to the instructions of the Genomic DNA Extraction Kit (Tiangen Biochemical Technology, Beijing, China). PCR amplification was then performed using ITS1 5′-TCCGTAGGTGAACCTGCGG-3′ and ITS2 5′-GCTGCGTTCTTCATCGATGC-3′ universal primers for fungal molecular identification [19]. The reaction system contained 0.1 U Taq (E/mL), 2 × Es mix at 10 μL, ITS primers forward/reverse primers (10 mM) at 1 μL each, DNA at 2 μL, and ddH_2O at 6 μL. The PCR reaction conditions were performed as follows: initial denaturing for 4 min at 94 °C; followed by 35 cycles of 45 s at 94 °C, 45 s at 55 °C, and 1 min at 72 °C; and a final extension of 10 min at 72 °C. PCR products were detected by agarose gel electrophoresis and sequenced by Sangon Biotech Co., Ltd. (Shanghai, China). Sequences were submitted to the NCBI database for BLAST comparison with other GenBank sequences to determine their homogeneous counterparts (BLAST, https://blast.ncbi.nlm.nih.gov/BlastAlign.cgi, accessed on 8 January 2021). The rDNA-ITS region sequences of the experimental strain were compared using DNAMAN 7.0 software, and a phylogenetic analysis was performed using MEGA7.0 software with *Photorhabdus luminescens* as an outgroup of the measured strain. The clade stability of the resulting phylogenetic tree was constructed using neighbor joining (NJ) with 1000 replicates.

2.5. Laboratory Bioassays

The pathogenicity of the pathogenic fungi against *T. urticae* was determined according to Koch's postulates (https://www.britannica.com/biography/Robert-Koch, accessed on 1 October 2021), and conidia treated with the optimized formulation were used to assess mortality and hatchability. All bioassays were performed on a glass platform consisting of circular filter paper and a piece of sponge filled with water. The mites are female-biased in the field, so the adult females of *T. urticae* were selected on fresh leaves, and the petiole was covered with absorbent cotton in a glass dish. The tested mites were sprayed with the suspension of the fungal strain in four concentrations (10^6, 10^7, 10^8, and 10^9) and reared in an artificial climate chamber at 26.5 °C and 75% R.H. The control was treated with sterile water, and three replicates for each treatment were performed.

Newly hatched eggs, one-day-old larvae, and protonymph mites were used for infestation experiments for different developmental stages of *T. urticae*. In the above experiments, four concentrations were evaluated, namely 10^6, 10^7, 10^8, and 10^9, for each tested stage. Two concentrations (10^7 and 10^9) were selected as representatives for the fungal infestation assays at different temperatures, and each treatment containing approximately 20–30 adult female mites was maintained in an artificial climate chamber (MLR-352H-PC, Panasonic, Sanyo Co., LTD., Ikeda City, Osaka Prefecture, Japan) at 24 °C, 26 °C, 28 °C, 30 °C, 34 °C, and 36 °C, respectively. After inoculation, the infection and death of the mites were observed daily under a microscope, and the control was treated with sterile water. Three replicates were performed for each treatment.

The mortality rate associated with the assessment of the toxic effect of fungus was corrected by adjusting treated mortality below control mortality as follows [20]:

$$\text{Corrected mortality rate } (\%) = \left(\frac{\text{treatment mortality} - \text{control mortality}}{1 - \text{control mortality}} \right) \times 100.$$

2.6. Field Trials

The field trial was conducted on the *T. urticae* populations on the strawberry plants in the greenhouse, located in the Agricultural Technology Extension Station, Yanqing, Beijing (40°27′29″ N 115°58′5″ E), in the winter of 2023. The greenhouse covers an area of 667 m^2 and the temperature was approximately 10 °C (night) to 26 °C (day) during the trial period. The 50% WP fungal concentration diluted 50-, 100-, and 200-fold was sprayed on the mite-infested leaves separately using a mini manual sprayer, in the afternoon. The control was treated with water. Three replicates were performed for each treatment. The original number of mites was counted before spraying, and the mortality of mites was checked and recorded on the 3rd and 7th d after treatment through hand-held magnifying glass. The control efficacy in the field trial was calculated using the same method as the corrected mortality rate.

2.7. Statistical Analysis

For the bioassay data, the slope ± SE, LT_{50} values, and 95% fiducial limits were calculated by corrected mortality using a probit analysis with the *ecotox* package in R statistical software (version 4.3.1) [21]. A one-way analysis of variance (ANOVA) was followed by the Tukey–Kramer test with a significant difference at $p = 0.05$ in SPSS (version 19.0) that was used to determine the differences between the different treatments of *T. urticae*. The hatchability and mortality figures for pathogenic experiments were graphed using GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA). All values are represented with means ± standard error (SE).

3. Results

3.1. Conidia Isolation and Identification

The fungal strain was isolated from naturally dying mites and named PFTu-X. The colony was white and round, with a villous projection and a wavy edge (Figure 1). Strain PFTu-X grew quickly without rhizomes. The sporophore was spherical-oval or columnar with appendicular filaments, which was inferred to be *Pseudozyma flocculosa*, compared to the description in previous reports [22].

The PFTu-X sequences were amplified with general primers and were submitted to the NCBI database for sequence comparison. The results of the BLASTn analysis showed that the rDNA-ITS sequences of PFTu-X had 99.85% similarity to those of *Pseudozyma flocculosa*. The result of the similarity comparison was consistent with that of morphological identification. Furthermore, the phylogenetic analysis showed that strain PFTu-X was clustered

into the taxon clade with *P. flocculosa* (Figure 2), according to the similarity analysis of ITS sequences. After identification, the strain was stored at the General Microbiology Center of the China Microbiological Culture Preservation Administration Commission (CGMCC No. 23345).

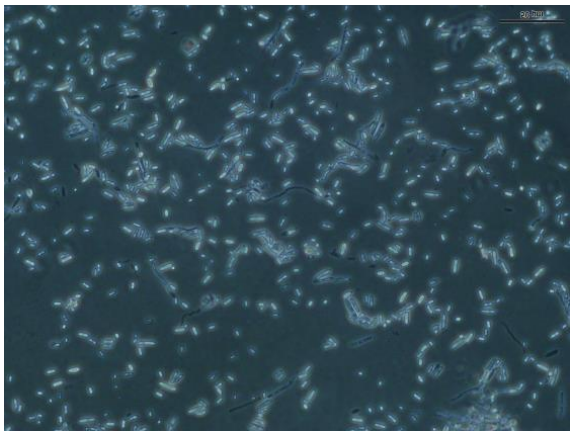

Figure 1. Colony image of the PFTu-X strain isolated from *T. urticae*.

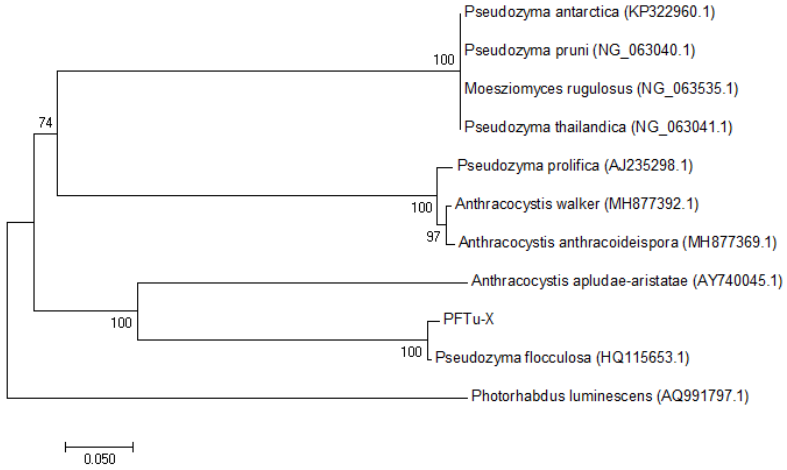

Figure 2. Phylogenetic tree of PFTu-X based on ITS sequences by the NJ method.

3.2. Pathogenicity of Multiple Conidium Concentrations on Adult T. urticae

The pathogenicity of the strain was evaluated according to Koch's postulates, the results showed that PFTu-X was highly virulent to adult female mites at 10^6~10^9 conidia/mL, and the fiducial limits and regression slopes are shown in Table 1. The tested mites died on the first day after treatment, with the highest corrected mortality (16.60%) in the 8×10^9 conidia/mL treatment. Mortality increased rapidly from the third to the ninth days after treatment, with corrected mortality rising from 30.61% to 100.00%. The lowest LT_{50} value of the strain was 2.26 d. In addition, significant differences were observed between the 8×10^7 conidia/mL and 8×10^8 conidia/mL and 8×10^9 conidia/mL treatments, based on the 95% fiducial limits of lethal time to 50% mortality. After one week of treatment, the mortality rate in all treatments was over 80%. After 9 d of treatment, all the tested mites died due to *P. flocculosa* infestation.

Table 1. Mortality of *Tetranychus urticae* adult females exposed to *Pseudozyma flocculosa* at different concentrations.

Concentration (Conidia/mL)	N [1]	Corrected Mortality Rate									LT_{50} (95% FL [2]) [3]	Slope (±SE) [4]	Chi-Square (*df*)	Heterogeneity
		1d	2d	3d	4d	5d	6d	7d	8d	9d				
8×10^6	102	7.97%	6.04%	30.61%	68.34%	75.13%	78.27%	85.94%	95.20%	100.00%	3.15 (2.47–3.77) ab	3.73 ± 0.22	39.54 (7)	5.65
8×10^7	92	3.57%	13.38%	51.48%	74.58%	78.69%	88.59%	92.21%	97.34%	100.00%	3.05 (2.81–3.28) b	3.43 ± 0.21	6.62 (7)	1.00
8×10^8	100	14.57%	19.23%	62.57%	77.38%	91.89%	91.80%	92.53%	96.17%	100.00%	2.39 (1.99–2.77) a	3.78 ± 0.22	19.32 (7)	2.76
8×10^9	94	16.60%	18.66%	64.09%	82.23%	88.98%	92.55%	93.64%	98.70%	100.00%	2.26 (1.79–2.69) a	3.59 ± 0.22	24.05 (7)	3.44

[1] Numbers of adult mites assayed. [2] FL = fiducial limits. [3] Different lowercase letters represent a significant difference among different concentrations. [4] SE = standard error.

3.3. Mortality at Different Stages of *T. urticae* after Exposure to *P. flocculosa* Concentrations

The strain *P. flocculosa* in different concentrations significantly affected the developmental stage of *T. urticae*. Most of the exposed eggs were infected, and the hatching rate was reduced considerably (<78.62% at all doses, compared to 95.73% in the control, F = 22.01, df = 4.14, $p < 0.0001$) (Figure 3A). The larva was infected 24 h after exposure to conidia. The larva failed to complete ecdysis successfully or become a nymph after 4 d, which was confirmed to be completely dead, with mortality ranging from 30.77% to 57.36% (F = 106.26, df = 4.14, $p < 0.0001$) (Figure 3B). Similarly, *P. flocculosa* caused fungal disease in most tested nymphs 24 h after exposure. The highest mortality rate (39.13%) was observed in the 5×10^9 conidia/mL treatment group (F = 90.59, df = 4.14, $p < 0.0001$) after fungal application (Figure 3C).

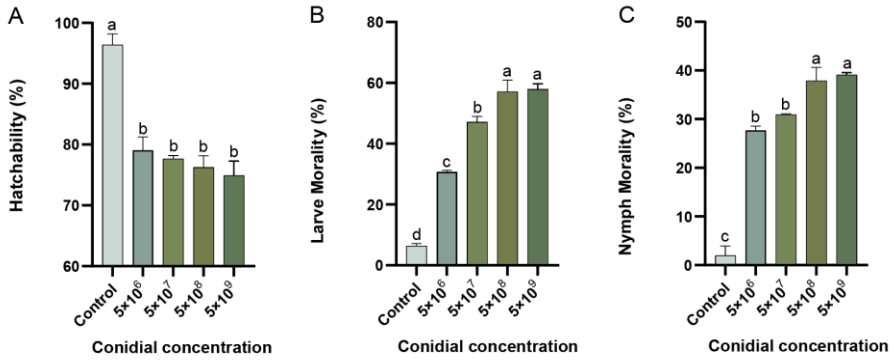

Figure 3. Effects of *Pseudozyma flocculosa* concentrations on the different stages of *Tetranychus urticae*. (**A**) Hatchability of *Tetranychus urticae* eggs; (**B**) mortality of *Tetranychus urticae* larvae; (**C**) mortality of *Tetranychus urticae* nymphs. Significance ($p < 0.05$) is indicated by different letters above the bars after the Tukey–Kramer test.

3.4. Differential Pathogenicity of *P. flocculosa* against *T. urticae* at Different Temperatures

The pathogenicity of *P. flocculosa* infesting *T. urticae* differed with different temperatures. All treatments had a significantly lethal effect compared to the control groups (24 °C: F = 4057.33, df = 2.9, $p < 0.0001$; 26 °C: F = 1102.51, df = 2.9, $p < 0.0001$; 28 °C: F = 3327.60, df = 2.9, $p < 0.0001$; 30 °C: F = 6289.35, df = 2.9, $p < 0.0001$; 34 °C: F = 655.10, df = 2.9, $p < 0.0001$; 36 °C: F = 337.54, df = 2.9, $p < 0.0001$) (Figure 4). With the temperature increase, the mortality in different treatments increased rapidly, with higher mortality occurring in about 2 d at 36 °C, 34 °C, and 30 °C (>80.31% for all treatments). In addition, no significant differences were observed between 36 °C, 34 °C, and 30 °C for the LT_{50} values (Table 2). Furthermore, equally efficient lethal effects were found at 24 °C, 26 °C, and 28 °C after fungal exposure.

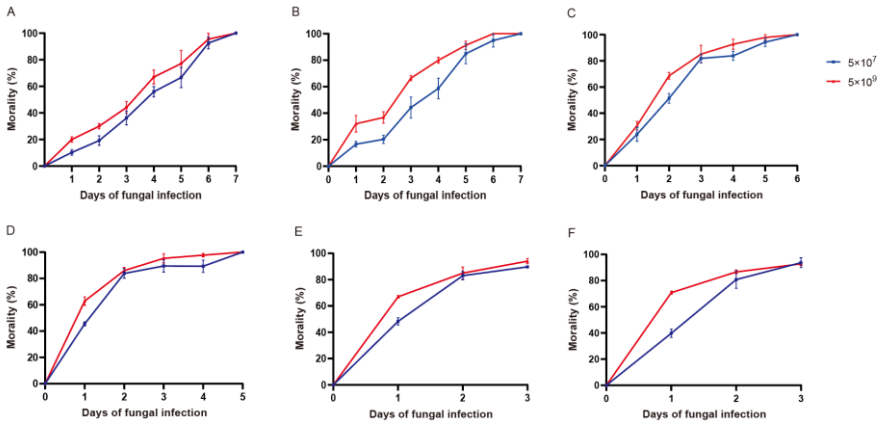

Figure 4. Corrected mortality (mean ± SE) of *Tetranychus urticae* adults exposed to *Pseudozyma flocculosa* at different temperatures. (**A**) 24 °C; (**B**) 26 °C; (**C**) 28 °C; (**D**) 30 °C; (**E**) 34 °C; (**F**) 36 °C.

Table 2. Mortality (mean ± SE) of *Tetranychus urticae* adults exposed to *Pseudozyma flocculosa* at different temperatures.

Temperature	5×10^7			5×10^9		
	N [1]	LT50 (95% FL [2]) [3]	Slope (±SE) [4]	N [1]	LT50 (95% FL [2]) [3]	Slope (±SE) [4]
24 °C	78	3.12 (2.14–4.07) c	3.75 ± 0.29	80	2.52 (1.60–3.35) c	3.22 ± 0.25
26 °C	91	2.69 (1.68–3.60) bc	3.62 ± 0.26	91	1.87 (1.11–2.51) bc	3.18 ± 0.24
28 °C	71	1.78 (1.57–1.98) b	3.51 ± 0.31	106	1.44 (1.28–1.58) b	3.61 ± 0.28
30 °C	69	1.06 (0.32–1.56) a	2.87 ± 0.36	96	0.81 (0.61–0.97) a	3.03 ± 0.37
34 °C	80	1.00 (0.75–1.19) a	2.90 ± 0.48	103	0.65 (0.36–0.87) a	2.28 ± 0.45
36 °C	78	1.17 (0.99–1.33) a	3.74 ± 0.51	85	0.53 (0.17–0.80) a	1.95 ± 0.49

[1] Numbers of adult mites assayed. [2] FL = fiducial limits. [3] Different lowercase letters represent a significant difference among different temperatures. [4] SE = standard error.

3.5. Field Efficacy of P. flocculosa

After spraying the *P. flocculosa* solution, the control efficacy of *T. urticae* in the field gradually increased with the time and concentration. The highest control efficacy was 65.06% at the 3rd d after spraying 50% *P. flocculosa* WP at 50-fold (Figure 5), significantly higher than that (52.09%) at 200-fold ($p < 0.05$). At the 7th d after spraying the *P. flocculosa* solution, the control efficiency was more than 79.28%, and the highest was 90.24% with 50% *P. flocculosa* WP at 50-fold (Figure 5).

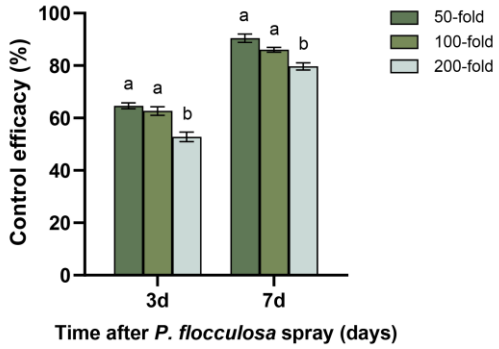

Figure 5. Field efficiency of 50% *Pseudozyma flocculosa* WP on *Tetranychus urticae* in greenhouse. Significance ($p < 0.05$) is indicated by different letters above the bars after the Tukey–Kramer test.

4. Discussion

The entomopathogenic fungi PFTu-X strain was isolated from an infected *T. urticae*. It was identified as *P. flocculosa* based on morphological and ITS gene sequence comparisons. The virulence activity assays of *P. flocculosa* at different concentrations of the spore suspension (10^6–10^9 conidia/mL) by the spraying method showed that the PFTu-X strain had pathogenic activity against *T. urticae* adult females and other different developmental stages. As previously reported, the fungus *P. flocculosa* is an epiphytic fungus isolated from clover leaves infected with powdery mildew (*Erysiphe polygoni*) [23]. Later, *P. flocculosa* received significant attention as a functional and highly efficient biocontrol agent against powdery mildew [24,25]. A recent study revealed that the effector pf2826 plays an essential role in the biocontrol activity of *P. flocculosa* by interacting with pathogenesis-related proteins in plants and a powdery mildew effector [26]. Therefore, this PFTu-X strain would have great potential for developing bioinsecticide agents for *T. urticae* mites and powdery mildew.

In this present study, different concentrations of *P. flocculosa* significantly affected the mortality of *T. urticae* mites. The mortality increased with the concentration increase, with the strongest pathogenicity against *T. urticae* at 8×10^9 conidia/mL. This is in agreement with the field trial, i.e., higher control efficacy was observed when the higher concentration was sprayed, with the highest control efficacy up to 90% at 7 d after *P. flocculosa* spraying. A similar finding was also reported in a previous study [27], showing the efficacy of

the fungal species *Metarhizium brunneum* in controlling adult *T. urticae* individuals at a concentration of 10^7 conidia/mL. Also, *Beauveria bassiana* is pathogenic to *T. urticae* females, with high mortality at 10^9 conidia/mL [28]. Except for *T. urticae*, some reports also focus on the insecticidal activities of other pests. De Souza et al. [29] reported that the efficacy of the fungal species *Aspergillus brunneoviolaceus* and *Clonostachys chloroleuca* affected adult *T. urticae* females but did have little pathogenicity on *Polyphagotarsonemus latus*. This differs from the results of our study. We found that *P. flocculosa* had high acaricidal activity against *T. urticae* and other insects, including aphids, *Frankliniella occidentalis*, and *P. latus* [30]. This may be related to the specific characteristics of different fungal strains.

Female adults, nymphs, larvae, and eggs have been evaluated in laboratory bioassays, as these different stages are the primary targets for control in the field. In this study, the viability of the eggs varied with the concentration of conidia. The higher the concentration, the lower the hatching rate. In *P. flocculosa* with 10^9 conidia/mL, the lowest hatching rate of 74.88%, the highest larval mortality of 56.96%, and nymph mortality of 39.13% were observed. Comparable results have been obtained with *B. bassiana* against *T. urticae* [31] and both *B. bassiana* and *Metarhizium anisopliae* against different developmental stages of *Tetranychus evansi* [32]. However, previous studies reported that the *Isaria cateniannulata* strain 08XS-1 had high virulence on *T. urticae*, causing 100% mortality of mite eggs and larvae at 10^7 conidia/mL [33]. Such differences are possibly related to the strain, experimental conditions, etc.

Meanwhile, we found that the different developmental stages of *T. urticae* differed in their susceptibility to infection by *P. flocculosa*, with a higher mortality rate in adult *T. urticae* than in immature stages. This is in accordance with the findings when the field trial was performed in this study, with more adults infected than immature stages after spraying the *P. flocculosa* solution (personal observation). Similar studies concluded that adults are more susceptible to fungal infection than other developmental stages of *T. urticae* [31,34]. The possible reasons are predicted, including the shell surface being physiologically unsuitable for conidium formation, and molting has been reported to be an essential factor in arthropod resistance to fungal infection, especially in arthropods with short ecdysis intervals [35]. Additionally, fungal infection can occur through direct contact with the cuticle of mites, especially adult mites, which are more active than eggs and have more opportunities to touch spores.

Environmental temperature can significantly affect the pathogen virulence, growth rate, and host capacity [36]. The population of *T. urticae* is easy to develop and explodes at high temperatures in the field [37]. Regarding the effect of temperature on the ability of the fungus to infect the *T. urticae* mites, the temperature tolerance of fungi should be considered in biological control if fungal isolates are to be used in pest management programs. In this study, the mortality of *T. urticae* infected with *P. flocculosa* could reach 100% at all tested temperatures. This is possible due to the cuticle touch susceptibility. As reported, *Drosophila melanogaster* has a light cuticle and increased susceptibility to pathogens in warmer environments [38]. Therefore, it is concluded that *P. flocculosa* can adapt to different temperatures, supporting its potential to control *T. urticae* [39].

5. Conclusions

The *Pseudozyma flocculosa* strain was identified and confirmed to have high virulence on *T. urticae* through a laboratory bioassay and field trial in this study. This indicates that the *P. flocculosa* strain could be developed as a potential fungal agent to control *T. urticae*. Nonetheless, further studies are necessary to determine how to make this biocontrol strain effective in preventing and controlling *T. urticae* populations in strawberry cultivation.

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