Article

Pathogenicity of three entomopathogenic fungi, *Metarhizium anisopliae*, *Beauveria bassiana*, and *Paecilomyces lilacinus*, to *Tetranychus kanzawai* infesting papaya seedlings

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Abstract

Tetranychus kanzawai is an economically important polyphagous mite species in East and South Asia. In the Philippines, it commonly infests cassava and papaya plants. The mites attack and severely damage the older leaves of papaya trees as well as seedlings. Its serious damage causes the leaves to dry up, thus, reducing the photosynthetic activity of the plant. Three entomopathogenic fungal isolates were tested on mites under greenhouse conditions using treated papaya seedlings following a completely randomized design. The mites tested were examined under a dissecting scope to determine the causal agent and to confirm mortality. The LT_{50} of *Metarhizium anisopliae, Beauveria bassiana* and *Paecilomyces lilacinus* on *T. kanzawai* were estimated. Our results indicate that among these entomopathogenic fungi, the *Metarhizium anisopliae* Ma6 isolate (LT_{50}= 3.00 days) has potentiality for the control of *T. kanzawai.*

Keywords *Tetranychus kanzawai*; greenhouse; entomopathogenic fungi; papaya seedlings; *Metarhizium anisopliae*; *Beauveria bassiana*; *Paecilomyces lilacinus*.

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1 Introduction

The importance of entomopathogenic fungi in agriculture and household entomology has only been recognized recently, in spite of the fact that they have long been reported as one of the key regulatory factors in insect population. Entomopathogenic fungi provide the only practical microbial control of sucking insects and for the many acridid (grasshoppers and locusts) and coleopteran pests, which have no known viral or bacterial diseases. A major drawback on the development of entomopathogenic fungi as a biological control agent is its slower killing action compared to synthetic chemical insecticides. After entomopathogenic fungal infection, it usually takes 5 to 10 daysto kill an insect pest, during which, the infected insect can still cause serious damage to the crop (de Faria and Wraight, 2007).

Damage to crops is perhaps the most costly effect of mites, especially by the spider mites and their relatives (Tetranychoidea), earth mites (Penthaleidae), thread-footed mites (Tarsonemidae) and the gall and rust mites (Eriophyidae). De Angelis et al. (1982) estimated that spider mites account for as much as 5% loss in total agricultural productivity worldwide. They cause severe injury in a very short time. The spider mites feed by piercing the leaf surface with stylet-like chelicerae and extracting leaf cells and fluid. This manner of feeding causes tiny holes to be punched into the otherwise protective, waterproof leaf surface. Leaves that have been fed on by spider mites are usually dry and brittle and the usual green color is lost. Injured leaves are shed more quickly and eventually the whole plant may die. Even a minor spider mite infestation can have significant impact on a plant's health. Furthermore, Sivritepe (2009) stated that leaves normally control water loss through a system of stomates or valves that can be opened and closed. When the stomates are closed the surface of a leaf is highly resistant to water loss. Spider mite feeding disrupts this system by creating holes that allow water to escape. This uncontrolled water loss eventually dehydrates the leaf. Ironically, the water stress caused by spider mite feeding actually makes the leaves a better food source because stressed leaves have higher levels of sugars and soluble nitrogen - both of which are needed by spider mites.

Spider mite (Tetranychidae) is widely distributed worldwide, including in the Philippines and Indonesia. The most common Tetranychid mite as pest in the Philippines and Indonesia are the *Tetranychus kanzawai* and *T. urticae*. *T. kanzawai,* an important species throughout East and South Asia, is polyphagous. In the Philippines, it commonly infests cassava and papaya plants (Guavarra, 1981) as well as hundreds of plants that include vegetablesand food crops such as strawberries, peppers, tomatoes, potatoes, beans, and corn. The mites attack and severely damage the older leaves of papaya and sometimes, its seedlings. Its serious damage causes the leaves to dry up, thus, reducing the photosynthetic activity of the plant.

Some research have been carried out using entomopathogenic fungi against mites. Alves et al. (2004) reported the potential of using the entomopathogenic fungus, *B. bassiana* at its yeast phase, to control *T. urticae*. Furthermore, Barreto et al. (2004) conducted bioaasay studies on *B. bassiana* and *M. anisopliae* against the green mite, *Mononychellus tanajoa* (Bondar) on 3.5 cm diameter cassava leaf discs. In a study by Ihsan and Ibrahim (2004), bioassay experiments revealed that *B. bassiana, M. anisopliae* and *P. fumosoroseus* were effective in killing the adult female mite, *Polyphagotarsonemus latus* Banks. These findings thus suggest that entomopathogenic fungi could be promising microbiological control agents for *T. kanzawai.*

2 Materials and Methods

The isolates were tested on mites under green house condition using treated papaya seedlings following a completely randomized design. All the test mites were examined under a dissecting scope to verify the disease-causing agent and confirm mortality. The mortality values observed were used to estimate the LT_{50} of the entomopathogenic fungi using Polo PC program.

The bioassay experiments were done using 60-day old papaya seedlings (Solo variety) infested with 10 adult mites. After sticking the mites onto the papaya leaves using tangle foot, the papaya seedlings were covered with a mylar cage. Conidial suspension of the seven entomopathogenic fungal isolates tested, which belong to three species namely, *B. bassiana, M. anisopliae* and *P. lilacinus,* were prepared in 20 ml distilled water with Triton X-100 as spreader. The papaya seedlings were hand-sprayed with the conidial suspension using the conidia concentrations of 1×10^6, 1×10^7, 1×10^8 conidia ml^{-1}. Each fungal isolate was tested using 3 replications. Adult mortality was observed daily with a 10x hand-held magnifying glass until 10 days after treatment when100% mortality achieved as observed in the previous laboratory experiments (Sanjaya et al., 2013a; 2013b).

The percentage mortality of adult female *T. kanzawai* was calculated using the following formula:

$$\text{percent of mortality} = \frac{\text{total mites mortality}}{\text{total mites tested}} \text{x } 100$$

Percentage mortality was corrected when below 10 % mortality in the control group was observed using the Abbot's formula (1925):

$$\text{Pt} = \frac{\text{Po} - \text{Pc}}{100 - \text{Pc}} \text{x } 100$$

where: Pt = % mortality after correction
Po = % mortality of adult mites after treatment
Pc = % mortality of adult mites on control

The significant differences on the percentage mortality was determined using analysis of variance (F test) and the means were compared by Duncan multiple range test (P<0.05) using the SAS software package.

3 Result and Discussion

The LT_{50} estimates of the entomopathogenic fungi against *T. kanzawai* were presented in Tables 1. The results showed that spray suspension of 10^8conidia/ml of the fungus gave superior control of the mite. Furthermore, LT_{50} values indicated that there was no significant difference on the pathogenicity among the *M. anisopliae* isolates was observed based on the overlapping fiducial limit. On the other hand, *B. bassiana* Bb4 has significantly longer killing time than Bb5 and Bb6. *P. lilacinus* (Pl) exhibited the longest LT_{50} thus was. the least virulent fungus. Based on the LT_{50}, Ma6 was the most virulent among the entomopathogenic fungi tested based since it has the shortest $LT_{50.}$

Table 1 Median lethal time, LT_{50} in days of spray suspension of the entomopathogenic fungi *Beauveria bassiana* (Bb4, Bb5 and Bb6), *Metarhizium anisopliae* (Ma4, Ma5 and Ma6) and *Paecilomyces lilacinus* (Pl) at 10^8 conidia ml^{-1} to *Tetranychus kanzawai* on 60-day old papaya seedlings under greenhouse conditions.

FUNGAL SPECIES	LT_{50} (DAYS)	95% FIDUCIAL LIMIT
Bb4	5.48	5.082 to 5.915
Bb5	4.53	4.202 to 4.866
Bb6	3.98	3.697 to 4.261
Ma4	4.23	3.898 to 4.567
Ma5	3.66	3.341 to 3.913
Ma6	3.00	3.728 to 3.805
Pl	6.33	5.847 to 6.889

The same trend on the virulence of Ma6 and Pl to *T. kanzawai* was observed based on theirLT_{90}values.The longest LT_{90} (19.34 days) was observed on *T. kanzawai* treated with *P. lilacinus* while the shortest was on Ma6 (8.57 days). Our results showed that *B. bassiana, M. anisopliae* and *P. lilacinus* are capable of causing epizootics on this mite species. Although these entomopathogenic fungi were not originally isolated from *T. kanzawai*, these fungi can be pathogenic to *T. kanzawai* (Fig. 1). All the entomopathogenic fungal isolates invaded, developed and produced conidia in the test mites. The same phenomenon was observed by Shaw et al. (2002), on fungal isolates from non-acarine hosts that were pathogenic to *Varroa destructor* Anderson and Trueman. In another study, Maketon et al. (2008) reported that fungal isolates originally isolated 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 *Tetranychus evansi* (Wakesa, 2003; Shi and Feng, 2004).

Table 2 LT_{90}in days of spray suspension of the entomopathogenic fungi *Beauveria bassiana* (Bb4, Bb5 and Bb6), *Metarhizium anisopliae* (Ma4, Ma5 and Ma6) and *Paecilomyces lilacinus* (Pl) at 10^8 conidia ml^{-1} to *Tetranychus kanzawai* on 60-day old papaya seedlings under green house conditions

FUNGAL SPECIES	LT_{90} (DAYS)	95% FIDUCIAL LIMIT
Bb4	15.78	13.533 to 19.269
Bb5	12.13	10.732 to 14.140
Bb6	9.58	8.683 to 10.792
Ma4	12.22	10.734 to 14.394
Ma5	10.24	9.125 to 11.796
Ma6	8.57	7.485 to 10.150
Pl	19.34	16.091 to 24.721

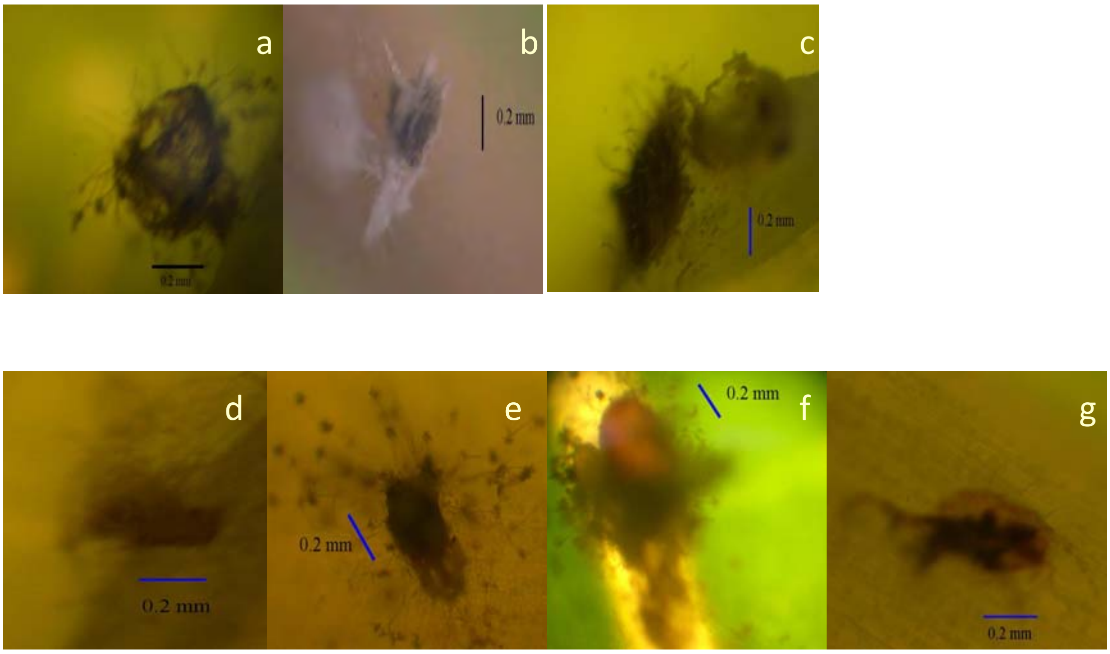

Fig. 1 Entomopathogenic fungal infection in *Tetranychus kanzawai*. Mites were infected with the entomopathogenic fungi by contact on leaf surface contaminated with 10^8 conidia $ml^{-1}$of *Beauveria bassiana*, *Metarhizium anisopliae*, or *Paecilomyces lilacinus* (400 x) (a) *B. bassiana* Bb4; (b) *B. bassiana* Bb5; (c) *B. bassiana* Bb6; (d) *M. anisopliae* Ma4; (f) *M. anisopliae* Ma5; (f) *M. anisopliae* Ma6; (g) *P. lilacinus* Pl.

The utilization of entomopathogenic fungi is an important component of Integrated Pest Management (IPM) programs. There are some advantages on using entomopathogenic fungi; such as, they can be applied as contact bioinsecticides since they are capable of infecting arthropods directly through the integument (Chandler et al., 2000); cultivation of those fungi and production of infective conidia are easy and fairly cheap (Roberts and Krasnoff, 1998); and finally, entomopathogenic fungi can be found under different ecological conditions (Kaaya and Hassan, 2000).

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