Article

Infection process of entomopathogenic fungi *Beauveria bassiana* in the *Tetrancyhus kanzawai* (Kishida) (Tetranychidae: Acarina)

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Abstract

Characteristic of entomopathogenic fungus to *Tetranychus kanzawai* was investigated. Three selected isolates of *Beauveria bassiana,* from Philippines and Indonesia were evaluated. The following aspect was investigated: (1) Investigate infection process on each fungal against mite. In this experiment, adult mites exposed by spraying to 10^8 per ml concentrations of conidia observing by light microscope and Scanning Electron Microscopy (SEM). The result found *T. kanzawai* was very susceptible to three isolates *B. bassiana* which the end of the trials, fungal growth was detectable as early as 2 until 4 day observation. Infection process with microphotograph and SEM showed attachment, germination and penetration, extrusion and conidiogenesis fungal form.

Keywords infection; *Tetranychus kanzawai*; *Beauveria bassiana*; light microscopy; Scanning Electron Microspopy (SEM)

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1 Introduction

One of the earliest experiments in which a fungus was tested against a phytophagous mite was a field application of *B. bassiana* spores for the control of the two spotted spider mite *T. urticae* (Dresner, 1949). Mites were treated with a dust containing 0.5% spores of the fungus, resulting in a mortality of 71%. However, these experiments did not lead to the development of a microbial acaricide. Deuteromycetes have widely been studied for the control of insect (and in a few instances, of mite) pests. A number of these fungi (e.g. *Metarhizium, Beauveria*) have broad host spectrum and can easily be mass-produced on relatively simple culture media. In Brazil, research is being conducted to evaluate several Deuteromycetes as possible control agent of the two-spotted spider mite (Tamai et al., 1998). Furthermore fungal pathogens have an opportunity to control spider mites. Some fungi that have been observed on mites are Hyphomycetes, including *Beauveria, Metarhizium, Paecilomyces* and *Verticillium* (Chandler, 2000).

Alves et al. (2002) mentioned the potential to control *T. urticae* (Koch) (Tetranychidae: Acina), a major mite pest, using the entomopathogenic fungus of *B bassiana* (Bals) yeast phase spraying suspensions of 10^5, 10^6,10^7 and 10^8 conidia/ml caused mortality up to 77.8 %. This research has also been elucidated by Bareto et al. (2004) who experimented with *B. bassiana* and *M. anisopliae* which were bioassayed for their lethal effects on the green mite, *Mononychellus tanajoa* (Bondar) on 3.5 cm cassava leaf discs. The suspensions were prepared by adding 20 ml of sterilized distilled water plus Tween 80 at 0.01% to the conidia. The suspensions were filtered through sterilized gauze; counts were made in a Neubauer chamber. Suspensions standardized at 10^8 conidia $ml^{-1.}$ were inoculated with the pathogen by immersion in 20 mL of the suspension for 5 seconds. The result showed that lethal concentrations (LC_{50}) of 3.93×10^6 conidia ml^{-1} and 7.44×10^8 conidia ml^{-1} were determined for *B. bassiana* and *M. anisopliae*, respectively. *B. bassiana* isolate 645 was the most efficient, being an alternative for use in biological control programs against the cassava green mite.

Shi and Feng (2004), on the other hand, experimented with *B. bassiana* and *P. fumosoroseus* which were bioassayed for their lethal effects on the eggs of the carmine spider mite, *T. cinnabarinus* Boisduval. According to Ihsan and Ibrahim (2004), *B. bassiana* and *M. anisopliae* are effective to kill the adult female mite, *Polyphagotarsonemus latus* Banks. In other studies, *B. bassiana* caused mortality on the adult female mites *T. evansi* Baker & Pritchrad which had caused damage to tobacco and tomato plants by 74.3% (Wekesa, 2003).

This very polyphagous pest species thrives under warm and humid conditions, and this seemed to be a good reason to study the feasibility of using fungi as control agents. Three fungi, namely: *B.bassiana, H. thompsonii* and *P. fumosoroseus* were tested under controlled temperature and humidity conditions in the laboratory and in the greenhouse. All fungi were capable to infect the mites. Higher doses resulted in a faster death of the mites, while density of the mites also affected disease incidence. The authors concluded from their experiments that the fungus selected should cause epizootics within 2–3 days following application. Promising results for the control of the broadmite on mulberry have been obtained with *M. anisopliae* by Maketon et al. (2008).

A test due to fungal attack process is important. The importance of fungal attack development related to the effectivity of this entompathogenis fungi.This study aimed to observed the infection process of *B. bassiana* selected from Indonesia and from the Philippines (collected from the Insect Pathology Laboratory of the Crop Protection Cluster, UPLB).

2 Materials and Methods

2.1 Light microscope

The process of adhesion, germination and colonization of *Tetranychus kanzawai* on 10^8 PIB/ml was observed by by light microscope and microcamera under 1000 x magnification from a small amount of colony taken from the flask.

2.2 Scanning Electron Microscope (SEM)

Specimens of four-day old infected female adult mites were examined by SEM. Infected mites were immersed overnight in 2.5 % glutaraldehyde in PBS, pH 7.0 and washed 3 times with PBS at 10 minute intervals for each wash. Washed samples were immersed in 1% osmium tetroxide (diluted in PBS) for 30 to 45 min. The osmium tetraoxide treatment was followed by 3 washes of sterile water at 10-minute interval per wash and dehydrated at room temperature in a graded series of 25 %, 50%, 75% and 95% ethanol with 30-minute interval for each step. The final step was followed by 3 changes of absolute grade ethanol, with two changes at 30 minute interval and overnight for the last 100% ethanol change. After dehydration, the samples were transferred into mesh microcontainers flooded with 100% ethanol for critical point drying. Critical point

drying was done for 45 minutes. The dried samples were mounted onto pin stubs with double-sided tape in different orientations and spitter coated with gold coating. Samples were examined and images were taken using Hitachi variable pressure SEM in high vacuum mode.

3 Results and Discussion

Adhesion process of conidia to the cuticle is the first step is the development of mycosis in insect. The attachment of conidia of *M. anisopliae* was passive and non-specific in conia adhered readily to both host and non-host cuticle preparations. The attachment was mediated by strong binding force and hydrophobicity of the conidial wall and the insect epicuticle seemed to the adhesion process. Entomopathogenic fungi invade their hosts by direct penetration of the host cuticle. There are different for the three isolates of *B. bassiana.* After application of *B. bassiana*, the conidia stick to the integument on conidial germination processes (Figs 1a, 3a, and 6a).

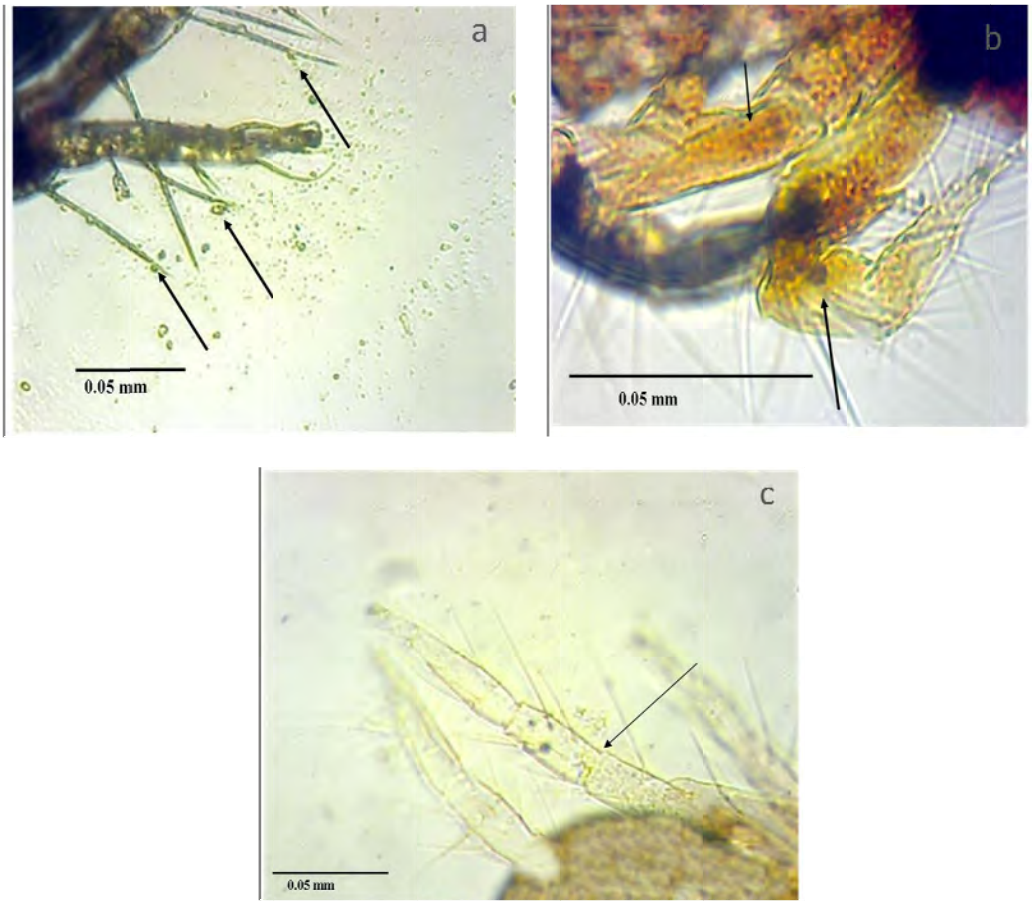

Fig. 1 Infection process of *Beauveria bassiana* Bb4 isolates to *Tetranychus kanzawai* (1000 x) (a) adhesion after 24 hours (b) germination after 48 hours and (c) colonization on intersegmental after 72 hours. Arrows indicate the location of the fungus.

Following attachment, the next step is the adhesion of dry spores to the cuticle was suggested to be due to non-specific hydrophobic forces exerted by the rodlets (Boucias et al., 1998) As shown in Figs 1 b, 3b and 5). In addition, lectins, a kind of carbohydrate binding glycoproteins, have been detected on the conidial surface of *B. bassiana*. It was also suggested that lectins could be involved in binding between conidia and the insect cuticle. The exact mechanisms responsible for the interaction between fungal spores and the cuticle remain to

be determined (Holder and Kehyani 2005). After the pathogen reaches and adheres to the host surface, it proceeds with rapid germination. There are some classes of enzymes produced by *B. bassiana* during germination including protease, chitinases and lipases whose function is to break down the host cuticle. The spores fungal growth are profoundly influenced by the availability of nutrients, oxygen, water, as well as pH, and temperature, and by the effects of toxic host-surface compound. Generally, fungi with a broad host range germinate in culture in response to a wide range of nonspecific carbon and nitrogen sources. Entomopathogenic fungi with restricted host range appear to have more specific requirements for germination (St. Leger et al., 1993).

Conidia germinate in response to a range of exogenous carbon and nitrogen sources (St. Leger et al., 1993). Conidia growth were found on the mite gnathosoma and anal regions, but some conidia were also saw on the leg. Germination tubes on the mite's cuticle was growth observed during the penetration process (Figs 1b, 3b and 5 b). It assumed due to production of enzymes by on the infection process.

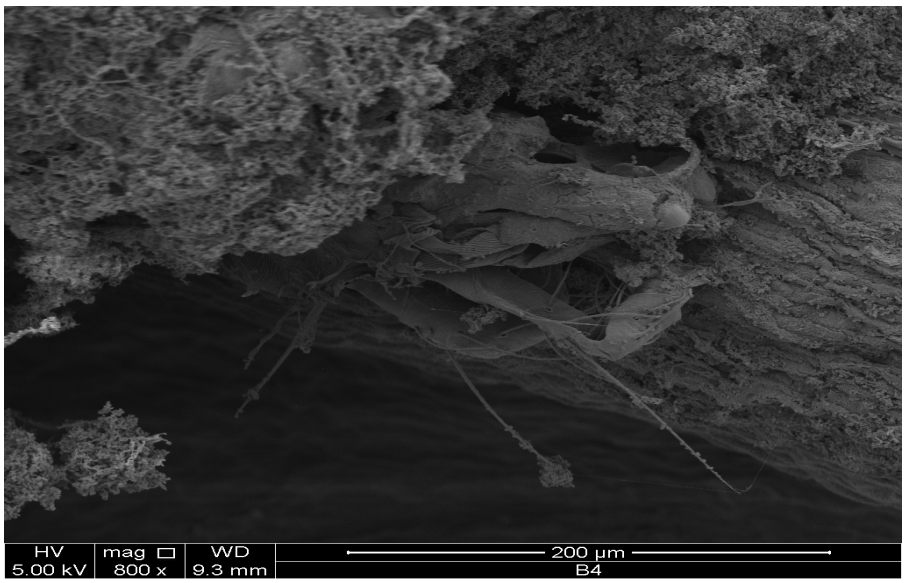

Fig. 2 Scanning electron micrograph of *Beauveria bassiana* Bb4 isolates infecting *Tetranychus kanzawai* 4 days after infection (800 x).

Entomopathogenic fungi invade their hosts by direct penetration of the host cuticle. The cuticle has two layers, the outer epicuticle and the procuticle. The epicuticle is a very complex thin structure that lacks chitin but contains phenol-stabilized proteins and is covered by a waxy layer containing fatty acids, lipids and sterols. The procuticle forms the majority of the cuticle and contains chitin fibrils embedded into a protein matrix together with lipids and quinones (Holder and Kehyani, 2005).

Protein may account for up to 70% of the cuticle. In many areas of the cuticle the chitin is organized helically giving rise to a laminate structure. In common with many entomopathogenic fungi, *B. bassiana* conidia germinate on the host surface and differentiate an infection structure termed appressorium (St Leger et al., 1993a).

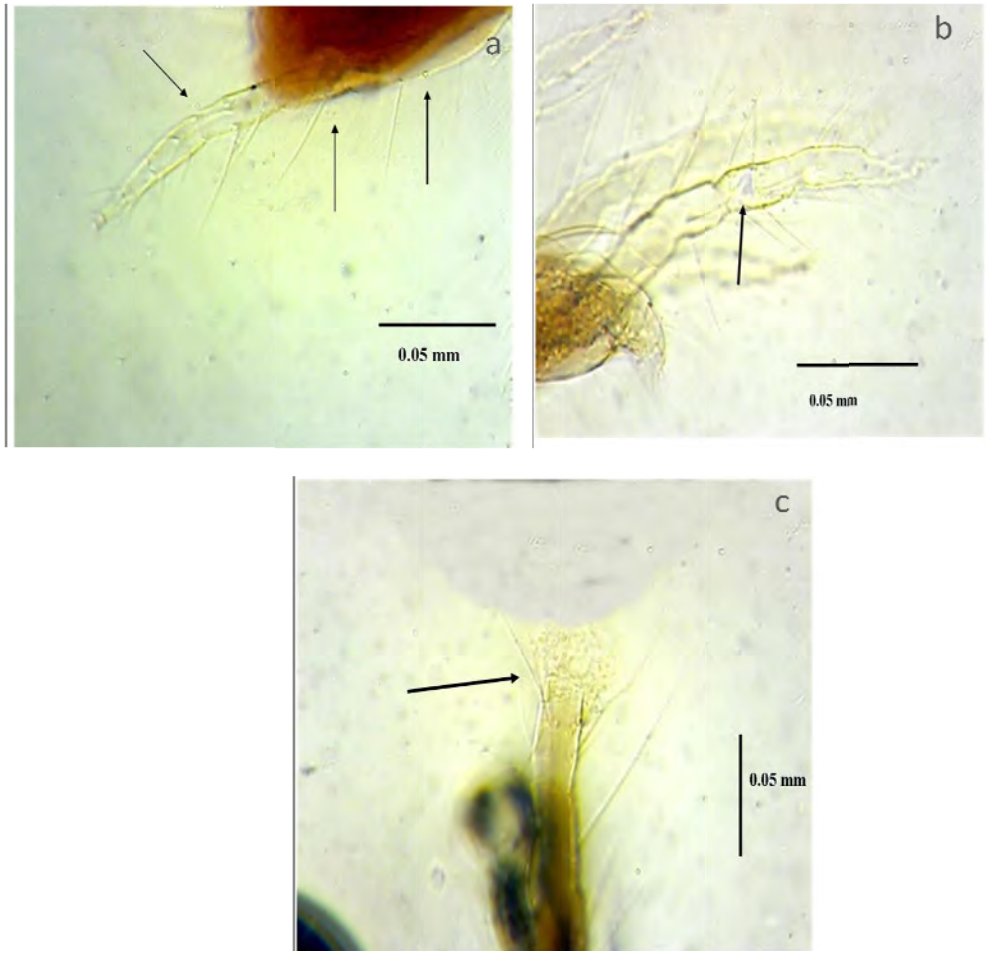

Fig. 3 Infection process of *Beauveria bassiana* Bb5 isolate to *Tetranychus kanzawai* (1000 x) (a) adhesion after 24 h (b) germination after 48 h and (c) colonization and extrusion from 48 to 72 h. Arrow indicates the location of the fungus.

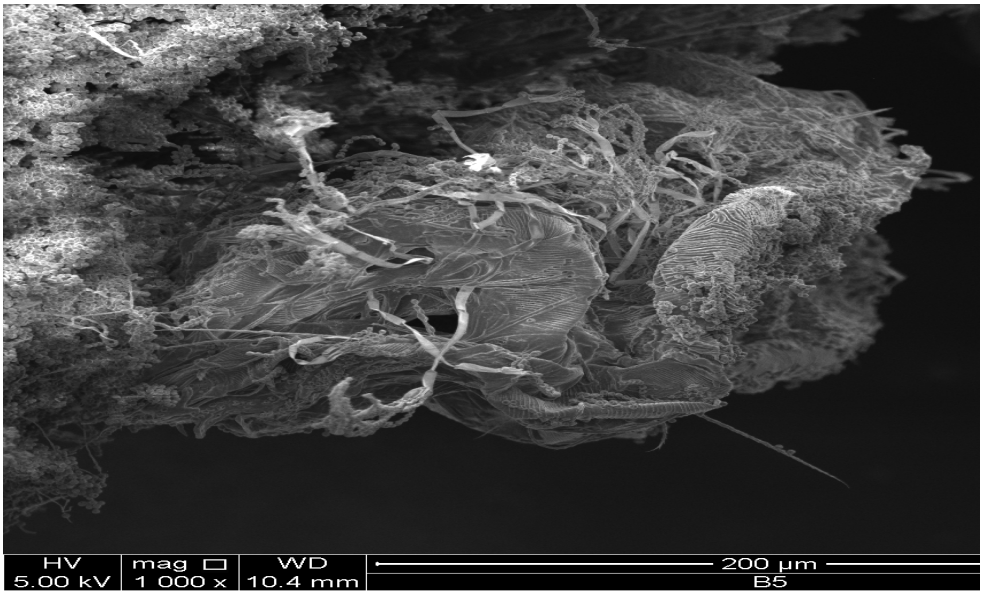

Fig. 4 Scanning electron micrograph of *Beauveria bassina* Bb5 isolate infecting *Tetranychus kanzawai* at 4 days after infection (1000 x).

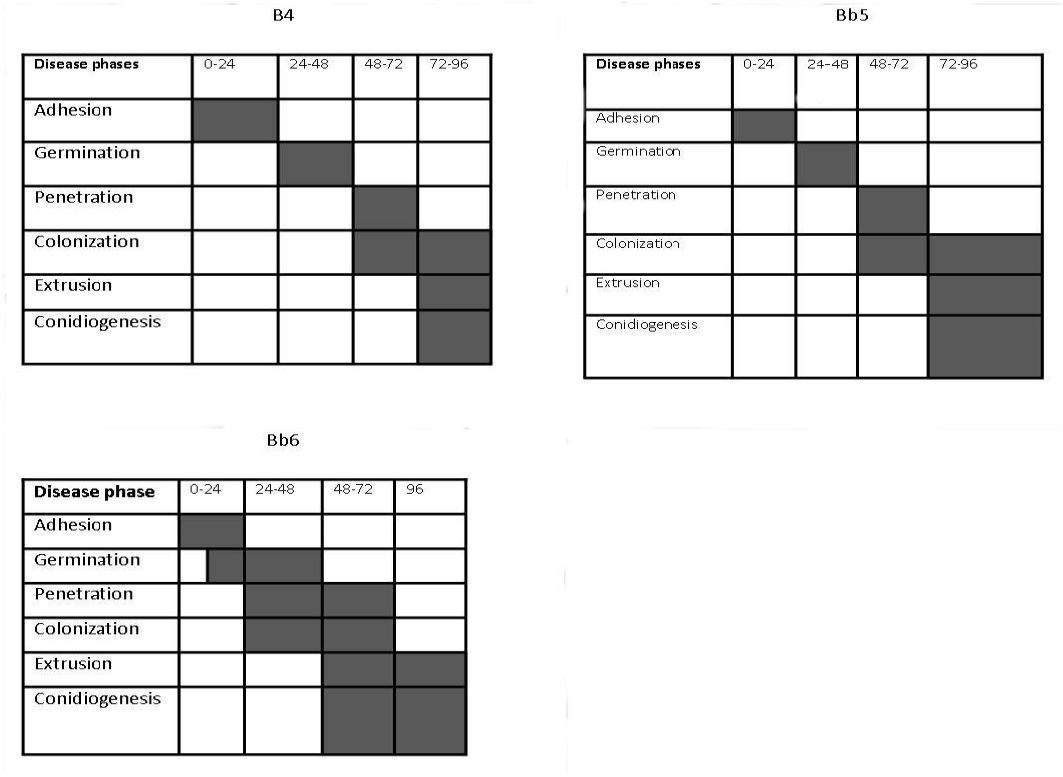

Fig. 5 Infection process of three isolates of *Beauveria bassiana* from Indonesia (Bb4 and Bb5) and from Philippines (Bb6).

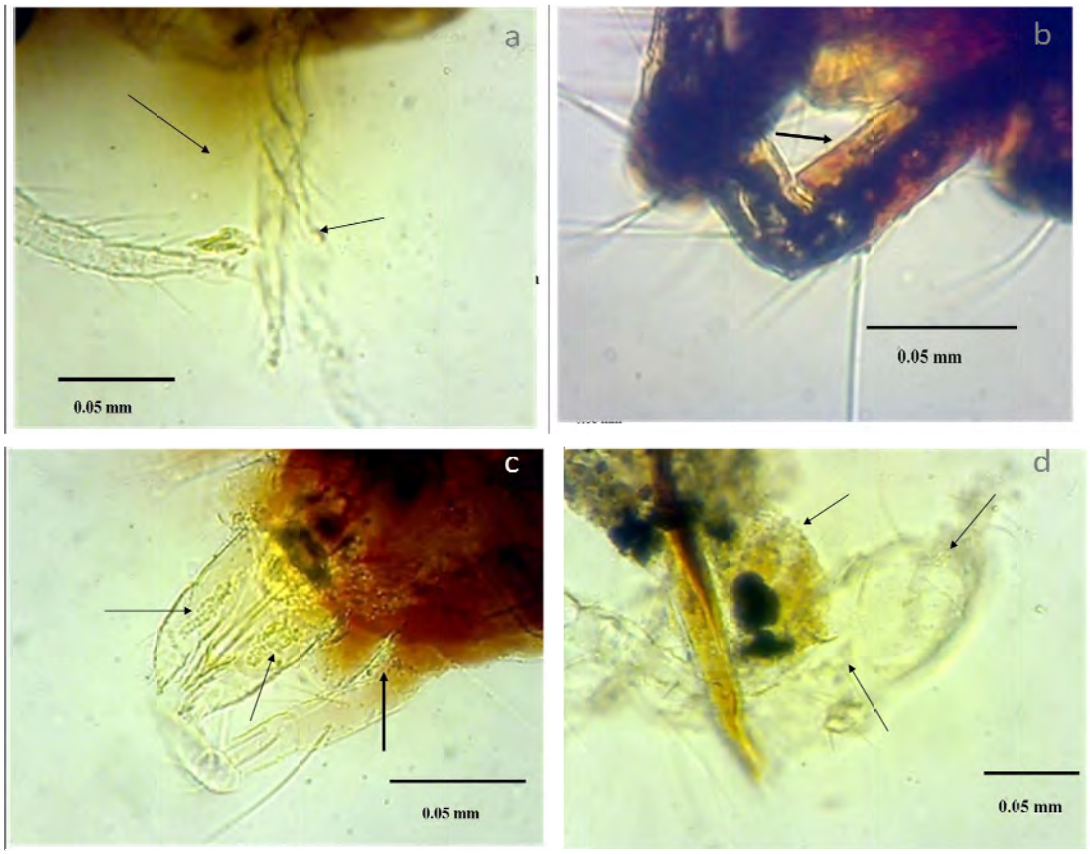

Fig. 6 Infection process of *Beauveria bassiana* Bb6 isolate to *Tetranychus kanzawai* (1000 x) (a) adhesion after 24 hours (b) germination af after 24 hours (c) penetration, colonization and extrusion from 24 to 48 hours, and (d) extrusion and conidiogenesis from 48 to 72 hours in hypostome. Arrows indicate the location of the fungus.

Host colonization was observed to occur after 48 hours (Figs 1c, 3c and 6c). The mites killed by *B. bassiana* had a white coloration which is a characteristic of oosporein activity common in insects infected by this fungus. It seems Bb6 was the most potential since had colonization on 24 hours. Physical penetration has been shown during host invasion by some entomopathogen (St. Leger et al., 1993).

Mycelial extrusion on the cadavers occurred between 96 and 120 hours after inoculation, mainly in the intersegmental areas and other area for complete cuticle degradation. The process of conidiogenesis occurred between 96 and 120 hours after inoculation.

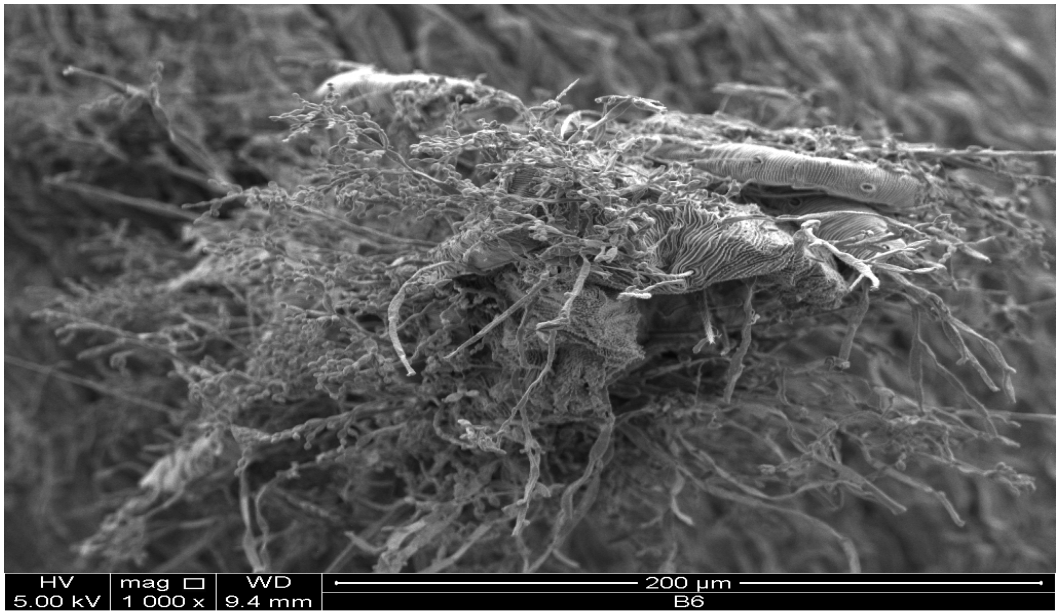

Fig. 7 Scanning electron micrograph of *Beauveria bassiana* Bb6 isolate infecting *Tetranychus kanzawai* at 4 days infection (1000 x).

Pathogenic fungi need to penetrate through the cuticle into the insect body to obtain nutrients for their growth and reproduction. Entry into the host involves both enzymic degradation and mechanical pressure as evidenced by the physical separation of lamellae by penetrated hyphae. A range of extracellular enzymes that can degrade the major components of insect cuticle, including chitinases, lipases, esterases and at least four different classes of proteases, have been suggested to function during the fungi pathogenesis. Although the complex structure of the insect cuticle suggests that penetration would require the synergistic action of several different enzymes, much of the attention has focused on the cuticle-active endoprotease as a key factor in the process (St. Leger, 1993).

The three most pathogenic isolates of *Beauveria bassiana* (Bb4, Bb5, Bb6) showed attachment, germination and penetration, extrusion and conidiogenesis fungal form. Bb6 was the most potential since had colonization on 24 hours.

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