Article

Transmission effect of entomopathogenic fungi on population of *Tetranychus kanzawai* (Kishida) (Tetranychidae: Acarina)

Yayan Sanjaya[1], Virginia R. Ocampo[2] , Barbara L. Caoili[2]
[1]Biology Program, Indonesia University of Education, Indonesia
[2]Crop Protection Cluster, College of Agriculture, University Philippines Los Banos, Philippines
E-mail: yayan229@yahoo.com

Received 18 November 2012; Accepted 20 December 2012; Published online 1 March 2013
IAEES

Abstract
Transmission Effect of *Beuaveria bassiana* and *Metarhizium anisopliae* on population of *Tetranychus kanzawai* was conducted .There were two activities, first by leaf contamination and secondly transmission from infected mite to a group population and were analyzed by probit wi resul LT50. The results showed that Fungal transmission occurs when there is contact with an infected cadaver or any residue left behind on the substrate. All healthy mites placed in contact with infected mites were infected at the end of the trial and covered with the fungus. The LT_{50} values for *T. kanzawai* mites, ranged from 2.865 to 4.150 days. All healthy mites placed onto leaf surface contaminated with entomopathogenic fungi through spraying also exhibited fungal infection at the end of the trial. LT_{50} values of the fungus isolates are summarized in Table 8. Three fungal species were found to have potentials within the value range of 2.223 - 4.206 days. The lowest LT_{50} value was found in *M. anisopliae* 6 (2.223 days) while others were not significantly different.

Keywords transmission; *Beuaveria bassiana*; *Metarhizium anisopliae*; population; *Tetranychus kanzawai*; LT50.

1 Introduction

Among insect pathogens, transmission is usually direct and follows a passage from host to host or host to environment to host pathway. Transmission can be horizontal or vertical. Horizontal transmission is the transfer of the pathogen from individual but not directly from parent to offspring. Vertical transmission is the transfer of pathogen from parent to the progeny. Vertical transmission transfers the pathogen from one host generation to the next and has also been referred to as congenital, parental or hereditary (Tanada and Kaya, 1993). Direct transmission is when the pathogen is transferred from an infected to a susceptible host without the intervention of any living agent; and indirect transmission involves one or more species of intermediate hosts or vectors (Tanada, 1963). In most insects, vertical transmission is through the mother and is referred to as matroclinal or maternal mediated (Tanada and Kaya, 1993). The pathogens can be transmitted to the surface of the egg (transovum transmission) or within the egg via the ovary (transovarial transmission).

Boucias and Pendland (1998) noted that insect pathogens have a variety of mechanisms and adaptations to perpetuate themselves. A fundamental aspect of epizootiology is the transmission pathways adapted by these pathogens to ensure their survival. The continual occurrence of a pathogen in a host population demonstrates its ability to be transmitted and the mechanism of transmission determines the changes in the host population and the spread of disease.

In horizontal transmission, the normal route into the host is through the integument in fungi and nematodes. Oral transmission is by ingestion of food contaminated with the infective stage of pathogen or by predation or cannibalism of infected insects. In this research was also to examine transmission effect from horizontal and leaf surface contamination

2 Materials and Methods

2.1 Horizontal transmission

Ten female mites were sprayed with 0.5 ml volume with 10^8 conidia ml^{-1} on 5 cm diameter petri dishes of each of the seven selected entomopathogenic fungi and kept until 90% mortality was achieved, approximately by sixth day after spraying. Ten dead female mites were transferred onto a 1.5 cm papaya leaf disc placed in the middle of a 2.5 cm diameter petri dish. A set of 10 live female mites was transferred onto another papaya disc measuring 1.5 cm in diameter and were placed in the same petri dish which contained the ten dead female mites. Controls were contained in identical dishes in which the cadavers of mites killed by starvation were sprayed with PBS and were deposited as previously described. The dishes were examined daily for dead mites under a dissecting microscope. The experiment was replicated three times.

2.2 Transmission of fungal infection between mites

Three hundred µl of 10^8 conidia mL^{-1} suspension from the 7 selected entomopathogenic fungi were applied into 1.5 cm diameter papaya leaf disc in a 2.5 cm petri dish. Control leaf disc was treated the same way with PBS. After washing for 5 minutes in the same buffer, 10 female mites were transferred into 2.5 cm diameter petri dishes. This experiment was replicated three times, for the three trials conducted. The dishes were checked every day under a dissecting microscope to observe for dead mites and LT_{50} was determined by probit analysis.

3 Results

3.1 Horizontal transmission

Healthy mites were able to acquire fatal infections from the cadavers (Fig. 1). Fungal transmission occurs when there is contact with an infected cadaver or any residue left behind on the substrate. All healthy mites placed in contact with infected mites were infected at the end of the trial and covered with the fungus. The LT_{50} values for *T. kanzawai* mites, ranged from 2.865 to 4.150 days (Table 1). The LT_{50} values were lower for Ma isolates than Bb isolates and the highest LT_{50} value was for *P. lilacinus.*

Table 1 LT_{50} value (days) of seven entomopathogenic fungi to *T. kanzawai* female adults through contact through diseased mites under laboratory condition.

FUNGAL ISOLATES	LT_{50} (DAYS)	95% FIDUCIAL LIMIT
Bb4	3.42	3.128 to 3.723
Bb5	3.32	3.044 to 3.618
Bb6	3.21	2.921 to 3.518
Ma4	3.02	2.737 to 3.324
Ma5	2.88	2.596 to 3.140
Ma6	2.91	2.631 to 3.199
Pl	4.15	3.754 to 4.653

In this study, adult mites exposed to10^8 conidia ml^{-1} concentrations of conidia were found to be very susceptible to the three fungal species isolates. Indeed the fastest isolates were on Ma5 and Ma6 which caused 100% mortality on mites in 4 days after exposure while *P. lilacinus* was the slowest which caused 100% mortality at 7 days after exposure (data not shown). All infected mites exposed to conidia suspensions were covered with the fungus at the end of the trial.

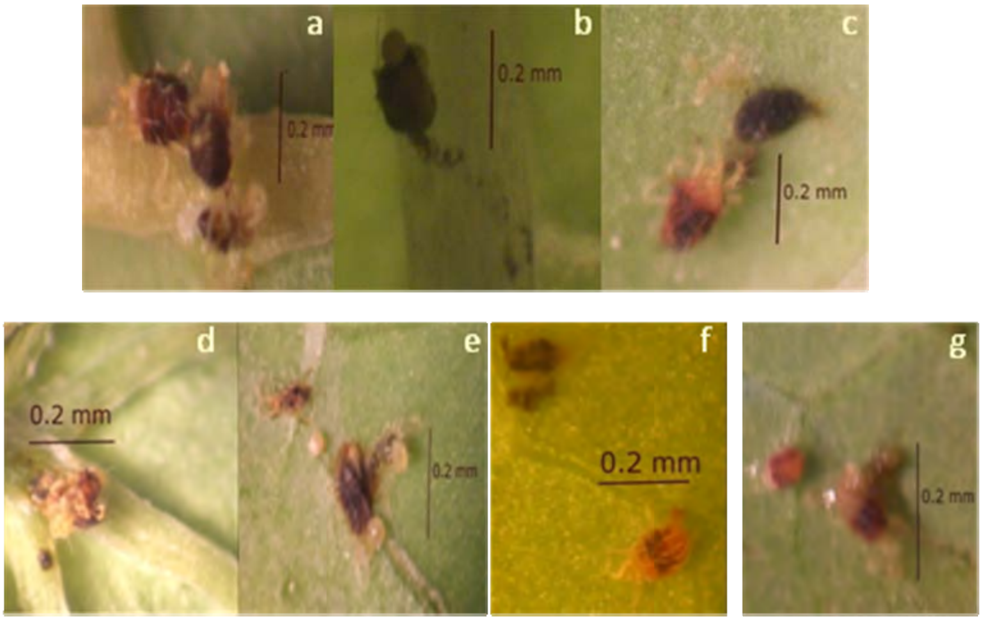

Fig. 1 *Tetranychus kanzawai* mites infected by contact with infected dead mites with conidia of *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces lilacinus* (400 x). (a) *B. bassiana* Bb4; (b) *B. bassiana* Bb5; (c) *B. bassiana* Bb6; (d) *M. anisopliae* Ma4; (e) *M. anisopliae* Ma5; (f) *M. anisopliae* Ma6; (g) *Paecilomyces lilacinus* Pl.

Exposure of *Psoroptes ovis* to *Metarhizium anisopliae* infected cadavers led to 65% mortality in just one day (Brooks and Wall, 2001a). Likewise, exposure of *P. ovis* to *B. bassiana* infected cadaver resulted to 50% mortality in 1.95 days (Lekime et al., 2006).

3.2 Transmission through a contaminated leaf surface

All healthy mites placed onto leaf surface contaminated with entomopathogenic fungi through spraying also exhibited fungal infection at the end of the trial (Fig. 2). LT_{50} values of the fungus isolates are summarized in Table 2. Three fungal species were found to have potentials within the value range of 2.223 - 4.206 days. The lowest LT_{50} value was found in *M. anisopliae* 6 (2.223 days) while others were not significantly different. The highest LT_{50} value was found in *P. lilacinus* (4.206 days) which was statistically different from the others (Table 2).

Contaminated surface of papaya leaf with *B. bassiana*, *M. anisopliae* and *P. lilacinus* were very infectious to *T. kanzawai* (Table 2). These results are similar to the results of the study conducted by Brooks and Wall (2001b) which exposed *Psoroptes ovis* mites to a 10^8 conidia ml^{-1} suspension of resulting to 70% mortality 1.0 day after exposure. In a study by Lekime et al. (2006), 50% mortality was achieved 1.73 days after exposure of *P. ovis* to *B. bassiana*. In this study, however, it took 2.22 days after exposure to achieve 50% mortality for *M. anisopliae* and 2.79 days for *B. bassiana* 6.

Table 2 LT$_{50}$ value (days) of 7 entomopathogenic fungi to *T. kanzawai* female adults through contact with contaminated surface under laboratory condition

FUNGAL SPECIES	LT$_{50}$ (DAYS)	95% FIDUCIAL LIMIT
Bb4	3.42	3.127 to 3.731
Bb5	3.25	2.965 to 3.553
Bb6	2.98	2.717 to 3.240
Ma4	2.87	2.613 to 3.136
Ma5	2.55	2.286 to 2.807
Ma6	2.22	1.977 to 2.462
Pl	4.21	3.847 to 4.645

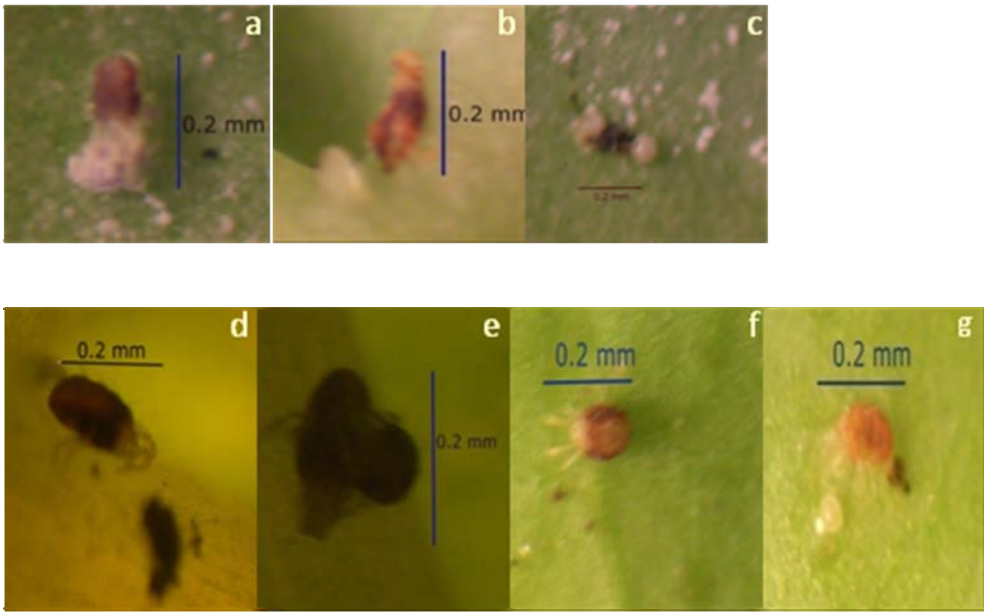

Fig. 2 *Tetranychus kanzawai* mites infected by surface contact with conidia of *Beauveria bassiana, Metarhizium anisopliae* and *Paecilomyces lilacinus* and (400 x). (a) *B. bassiana* Bb4; (b) *B. bassiana* Bb5; (c) *B. bassiana* Bb6; (d) *M. anis0pliae* Ma4; (e) *M. anisspliae* Ma5; (f) *M. anisopliae* Ma6; (g) *Paecilomyces lilacinus* Pl.

4 Discussion

Transmission is the process by which a pathogen or parasite is passed from a source of infection to a new host. Direct transmission is when the pathogen is transferred from an infected to a susceptible host without the intervention of any living agent; and indirect transmission involves one or more species of intermediate hosts or vectors (Tanada, 1963). Among insect pathogens, transmission is usually direct and follows a passage from host to host or host to environment to host pathway. Transmission can be horizontal or vertical. Horizontal transmission is the transfer of the pathogen from individual but not directly from parent to offspring. Vertical transmission is the transfer of pathogen from parent to the progeny. Vertical transmission transfers the pathogen from one host generation to the next and has also been referred to as congenital, parental or hereditary (Tanada and Kaya, 1993). In most insects, vertical transmission is through the mother and is referred to as matroclinal or maternal mediated (Tanada and Kaya, 1993). The pathogens can be transmitted to the surface of the egg (transovum transmission) or within the egg via the ovary (transovarial transmission).

Boucias and Pendland (1998) noted that insect pathogens have a variety of mechanisms and adaptations to perpetuate themselves. A fundamental aspect of epizootiology is the transmission pathways adapted by these pathogens to ensure their survival. The continual occurrence of a pathogen in a host population demonstrates its ability to be transmitted and the mechanism of transmission determines the changes in the host population and the spread of disease.

In horizontal transmission, the normal route into the host is through the integument in fungi and nematodes. Oral transmission is by ingestion of food contaminated with the infective stage of pathogen or by predation or cannibalism of infected insects. Some fungi (Steinhaus, 1963) and nematodes (Poinar, 1979) infect through the mouth, while spores of *B. bassiana* and *M. anisopliae* can infect through the spiracle (Pekru and Grula, 1979).

The most virulent insect pathogens, which include a range of species of entomopathogenic fungi, have a 'sit & wait' strategy (Chandler et al., 1997). These organisms are primarily transmitted horizontally, and produce infective stages which are released into the environment when the host dies. The infection cycle is continued when a new susceptible host acquires the infective stages from the environment. The efficiency of transmission in this system is affected very strongly by the density of susceptible hosts. This is a critical issue for insect pathogenic fungi as many insect species have patchy distributions, and hence the probability of contacting new hosts is often low. Brown and Hasibuan (1975) likewise state that under such circumstances, there is a strong selection pressure in favor of traits that increase the probability of success. Hence, the longer the transmission and the infective stages last in the environment, the more likely it is to contact a susceptible host.

Since the chances of successful transmission are positively correlated with the number of infective stages released into the environment, there is also selection pressure for producing large numbers of these stages on susceptible hosts. In fact, under the sit and wait strategy, the chances of transmission are maximized if the pathogen can convert as much host biomass as possible into new pathogen infective stages, which will result in the death of the host (Tanada, 1963). Hence sit and wait pathogens are usually highly virulent. Having said that, the fact that complete eradication of a host population by horizontally transmitted "sit & wait" pathogens will incur a severe fitness cost (disease 'burn out') should not be overlooked unless the pathogen can survive in the environment until new susceptible hosts arrive (Tanada and Kaya, 1993).

A basic characteristic of the fungi is the production of spores, which are resistant propagules released into the environment for dispersal. Perhaps, then, many species of entomopathogenic fungi are predisposed to 'sit and wait' transmission because of their ability to produce spores (Chandler et al., 2000).

The result indicate that tetranychus kanzawai can be affect by horizontal and leaf surface Contamination. That can be support the advantage of Beauveria and Metarhizium by self regulating of mite population. The further implication that it can compatible with other Integrated pest management tools to produce clean papaya product.

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