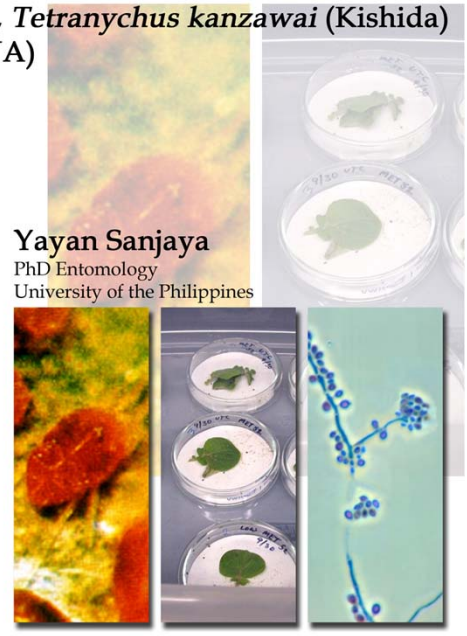
INFECTIVITY OF ENTOMOPATHOGENIC FUNGI AGAINST THE SPIDER MITE, *Tetranychus kanzawai* (Kishida) (TETRANYCHIDAE: ACARINA)



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OUTLINE

Study 2. Characterization of the Selected Entomopathogenic Fungi

ITS Amplification

RESULT AND DISCUSSION

Study 1. Selection of the Most Pathogenic Isolates

Koch's Postulates

Transmission of Fungal Infection between Mites

Transmission through a Contaminated Surface

Efficacy of Selected Entomopathogenic Fungi in the Greenhouse

Study 2. Characterization of the Entomopathogenic Fungi

Infection Process

Characterization and Identification of Entomopathogenic Fungi using ITS Sequence

Phylogram

SUMMARY AND CONCLUSION

INTRODUCTION

(back)

Spider mite (Tetranychidae) is widely distributed as well in the Philippines and Indonesia. *T. kanzawai* is one important throughout East and South Asia and is a polyphagous mite.

In the Philippine it commonly infests cassava and papaya plants The mites attack and severely damage the older leaves of papaya and sometimes, its seedlings.

Application of entomopathogenic fungi is an important factor in Integrated Pest Management (IPM) concerning safety of environment. There are some advantages in using these

next

INTRODUCTION

back

Some researches were also carried out using entomopathogenic fungi to mite for bioasssay and examine morphologically and genetically

Alves et. al (2002) → *B. bassiana* → *T. urticae*

Bareto et. al (2002) → *B. Bassiana & M.anisopliae* → *Mononychellus tanajoa*

Ihsan and Ibrahim (2004) → *B. bassiana, M. anisopliae* and *P. fumosoroseus*

↓

Polyphagotarsonemus latus

OBJECTIVES

(back)

Specifically, this study

- Screened the entomopathogenic fungi *M. anisopliae*, *B. bassiana* and *P. lilicanus* to *T. kanzawai*;
- Investigated the transmission process through surface contamination and through infected *T. kanzawai*;
- Investigated the efficacy of selected entomopathogenic fungi against *T. kanzawai* in the green house; and
- Characterized the most virulent entomopathogenic fungi against *T. kanzawai* using morphological and molecular techniques

Materials and Methods

(back)

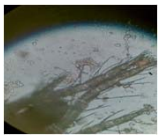
Time and Place of Study →

Insect Pathology Laboratory and Entomology Green House of the Crop Protection Cluster, University of the Philippines Los Baños and also Plant Breeding Genetics and Biotechnology Laboratory, IRRI

Mass Rearing of the Red Spider Mites, *Tetranychus kanzawai*

Maintenance of Host Plants

Male



Female

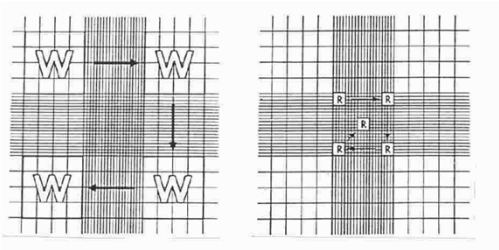
Test Fungi

Fourteen isolates of Entomopathogenic Fungi

Maintenance Fungi

Study 1. Screening of the Entomopathogenic Fungi against *T. kanzawai*

BACK



Cell concentration per milliliter = Total cell count in 4 squares x 2500 x dilution factor

Cell concentration per milliliter = Total cell count in 5 squares x 50,000 x dilution factor

Bioassay Tests for Virulence Laboratory Screening

back

Preliminary phase.

10 adult female mite with 3 replication

spray →

Five series dilution 10^4 - 10^8 conidia/mL

Final phase.

10 adult female mite with 3 replication →

Eight series dilution 10^1 - 10^7 conidia/mL →

Koch‘s Postulates

back

Reinoculation & reisolation

Observe fungi on microscope → Spray the *T. kanzawai* → Death mite

Dead mite → Isolate → Compare with initial isolate

Transmission of Fungal Infection between Mites

back

10^8 conidia/mL

Spray →

← 10 adult female mite with 3 replication

Horizontal Transmission

Spray

10^8 conidia/mL →

10 mites

cadaver →

Green House Bio-assay

back

Papaya seedlings → Surface contact of 10 mite per treatment → 3 replication with 10 days observation → LC50 and LC90



Study 2. Characterization of the Selected Entomopathogenic Fungi

Scanning electro microscope (sem) → Send to biotech

Morphological characterization of fungi.

microcamera and binocular microscope 1000 X → The observations covered color, mycelia structure, colony shape, growth rate, number of sporulation

next

Characterization and Identification of Entomopathogenic Fungi using ITS Technique

1 ml of PDB culture

1.5 ml microtube

15000 g
1 min

pelet

resuspend

300 µl CRS

Cell lysis for fungi

+ 1.5 µl proteinase k

inverting

incubated at 55°C for 60 min

15000 g
3 min

Protein precipitation

Resususpend the pellet with 300 µl Cell Lysis Solution

+ pps

20 sec

Sentrifuge 15000 g 3 min

Transfer Supernatant

300 µl isopropanol >99%

Mix the sample

Inverting gently three times

15000 g
1 min

DNA will be visible as pellet

White to light green

Discard supernatant

Drain tube briely

300 µl of 80% ethanol

Inverting several times

Sentrifuge 15.000 g

1 min

Discard ethanol

Air dry

At room temparature

+ 75 µl dna hydratiom

dna precipitation

Dry dna pellet

Mix the sample

hydrate dna at 65°C for 60 min

Store at 4°C.

DNA hydration

back

ITS Amplification

Amplification reaction
20 µl

10 µl master mix

1 µl primer a

1 µl primer b

6 µl ultra fresh sterile water

2 µl DNA template

DNA quantification

Nano drops

The temperature was set using thermal cycling

initial denaturation at 95°C for 5 min

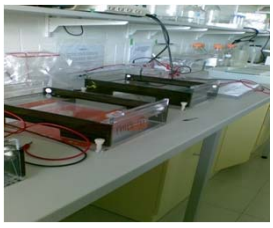
35 cycles with denaturation at 94°C set for 1 min and 30 sec

annealing at 55°C for 2 min

extended at 72°C for 3 min

a final extension at 72°C for 5 min.

Electrophoresis



electrophoresis in 1.2% agarose gel mixed with cyber safe

DNA 10 µl and 2µl loading dye

450 mV, then at 400 mA for 45 min.

geldoc

next

RESULT AND DISCUSSION

Study 1. Selection of the Most Pathogenic Isolates

. Mean of mortality of *T. kanzawai* on 5th day after treatment with 14 isolates of entomopathogenic fungi

	Concentration						
FUNGAL ISOLATES	0	1.0×10^4	1.0×10^5	1.0×10^6	1.0×10^7	1.0×10^8	Mean
Ma1	0.00	18.00	20.50	24.33	26.00	28.00	19.47^{cde}
Ma2	0.50	18.17	20.67	24.00	26.67	28.50	19.75^{cd}
Ma3	0.33	15.83	19.00	23.33	26.17	28.17	18.81^{e}
Ma4	0.67	19.83	22.33	26.17	28.50	29.17	21.11^{b}
Ma5	0.67	21.00	24.67	27.33	28.67	29.50	21.97^{a}
Ma6	0.50	20.67	23.00	26.50	28.00	29.33	21.33^{ab}
Ma7	0.20	15.17	17.33	22.00	24.67	27.00	17.73^{f}
Bb1	0.33	14.67	17.17	17.17	22.00	25.50	16.14^{f}
Bb2	0.33	15.00	17.83	20.67	24.17	26.33	17.39^{f}
Bb3	0.45	17.72	20.39	23.31	26.00	27.81	19.28^{f}
Bb4	0.50	16.83	19.17	24.00	27.33	28.67	19.42^{e}
Bb5	0.17	17.83	19.83	23.67	27.00	28.50	19.50^{c}
Bb6	0.33	17.50	19.17	23.17	27.00	28.83	19.33^{de}
Pl	0.50	14.33	16.67	20.17	24.67	27.00	17.22^{f}
	0.39A	17.33 B	19.84 C	23.27 D	26.20 E	28.02 F	

Means with the same letter are not significantly at Duncan 0.05

Refined LC_{50} Values for the 7 Selected Isolates

back

Table . LC_{50} values (conidia/ml) of 7 entomopathogenic fungi at 5 days after application on *T. kanzawai* adult females

SPECIES	LC_{50}	FIDUCIAL LIMITS	SLOPE
M. anisopliae 4	1441.13	664.70 – 2905.71	.338+-.028
M. anisopliae 5	722.00	312.27 – 1502.98	.332+-.028
M. anisopliae 6	496.46	225.39 – 97.29	.363+-.028
P. lilacinus	17775.56	9241.89 – 34819.90	.358+-.028
B. bassiana 4	2348.58	1110.42 – 5046.76	.318 +-.027
B. bassiana 5	1959.63	902.69 – 3988.75	.330+-.028
B. bassiana 6	1161.92	590.23 – 2153.39	.392+-.029

Table . LC_{98} values (conidia/ml) of 7 entomopathogenic fungi at 5 days after application on *T. kanzawai* adult females

SPECIES	**LC_{98}**	**FIDUCIAL LIMIT**
M. anisopliae 4	9.7×10^8	1.6×10^8 to 1.1×10^9
M. anisopliae 5	6.0×10^8	1.0×10^8 to 7.1×10^9
M. anisopliae 6	1.3×10^8	$3\,0 \times 10^7$ to 9.4×10^8
P. lilacinus	55×10^8	8.8×10^7 to 12.2×10^{10}
B. bassiana 4	3.8×10^8	5.0×10^8 to 6.0×10^{10}
B. bassiana 5	1.9×10^8	2.9×10^8 to 25.4×10^9
B. bassiana 6	1.2×10^8	3.0×10^7 to 7.4×10^8

Refined LC_{50} Values for the 7 Selected Isolates

Table 4. Mean of mortality of *T. kanzawai* on 5th day after treatment with 7 isolates of entomopathogenic fungi

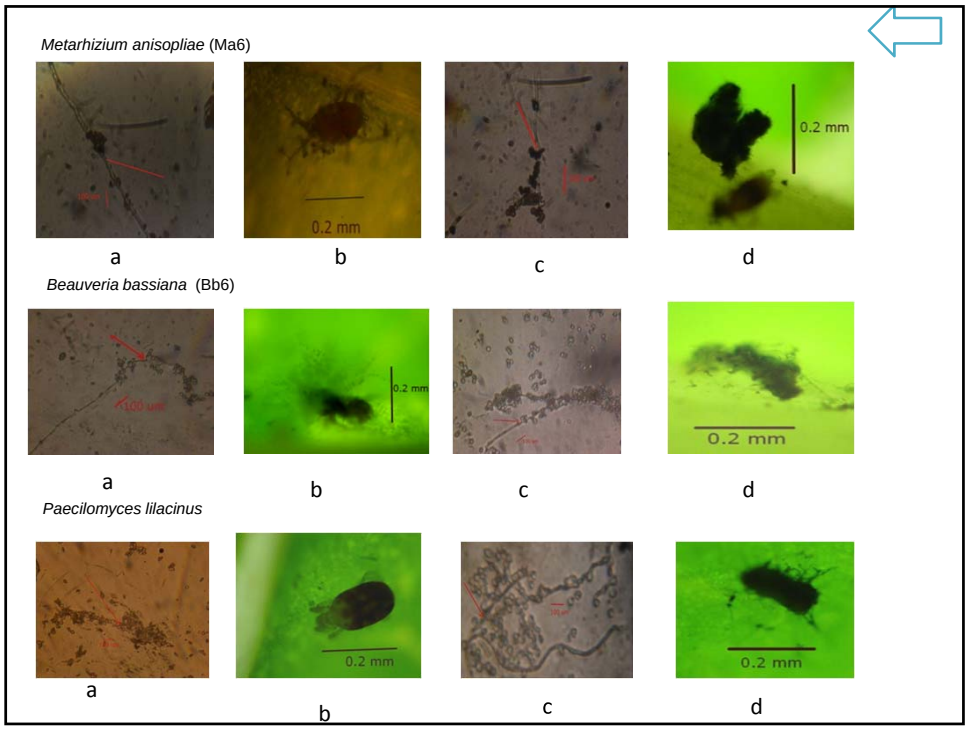
	CONCENTRATION								
FUNGI ISOLATE	0	1.0 X10^1	1.0 x 10^2	1.0 x 10^3	1.0 x 10^4	1.0 x 10^5	1.0 x 10^6	1.0 x 10^7	Mean
Ma4	0.33	8.00	10.83	14.17	16.67	20.33	25.67	28.17	15.52c
Ma5	0.50	9.67	11.50	15.50	18.00	20.50	25.67	29.33	16.33b
Ma6	0.67	9.00	12.67	16.17	18.50	22.50	27.17	29.50	17.02a
Bb4	0.33	7.83	10.00	13.67	15.67	19.17	24.00	27.83	14.81d
Bb5	0.50	8.00	10.33	14.00	16.00	18.83	25.00	28.33	15.13d
Bb6	0.50	6.83	11.33	14.50	17.33	21.83	26.33	29.67	16.04b
Pl	0.83	3.33	7.00	9.67	14.67	17.00	21.33	26.00	12.48e
Means with the same letter are not significantly at Duncan 0.05									
Mean	0.52A	7.52B	10.52C	13.95D	16.69E	20.02F	25.02G	28.40H	

Koch's Postulates

*Conidiophores of three *Metarhizium* isolates aggregated in dense tuft with repeated, more or less, verticillate branching on Ma4 and Ma6, with Ma5 having the most tufts.

*Mycelia grew on Bb4, Bb5 and Bb6. Conidia hyaline had a barely yellowish color, smooth, globose to broadly ellipsoidal, sometimes with an apiculate base.

**P. lilacinus* showed a cottony-textured colony on PDA. Spores which were pinkish in color could be seen covering the whole body of the cadaver.



Transmission of Fungal Infection between Mites

Horizontal Transmission

Table . LT_{50} value (days) of 7 entomopathogenic fungi to *T. kanzawai* female adults through contact through diseased mites under laboratory condition

NO.	NAME	CODE	LT 50 (CADAVER)	FIDUCIAL LIMIT 50 %	SLOPE
1.	*M. anisopliae*	Ma4	3.02	2.737 to 3.324	3.641+-.355
2.	*M. anisopliae*	Ma5	2.88	2.596 to 3.140	3.811+-.362
3.	*M. anisopliae*	Ma6	2.91	2.631 to 3.199	3.654+-.354
4.	*P. lilacinus*	Pl	4.15	3.754 to 4.653	3.399+-.385
5.	*B. bassiana*	Bb4	3.42	3.128 to 3.723	4.125+-.405
6.	*B. bassiana*	Bb5	3.32	3.044 to 3.618	4.197+-.406
7.	*B. bassiana*	Bb6	3.21	2.921 to 3.518	3.817+-.374

The LT_{50} values for *T. kanzawai* mites as shown in Table 6, ranged from 2.88 to 4.150 days. The LT_{50} values were lower for Ma isolates than Bb isolates and the highest LT_{50} value was for *P. lilacinus.*

Transmission through a Contaminated Surface

Table 8. .LT_{50} value (days) of 7 entomopathogenic fungi to *T. kanzawai* female adults through contact with contaminated surface under laboratory condition

NAME	CODE	LT50 (SURFACE)	FIDUCIAL LIMIT	SLOPE
M. anisopliae 4	Ma4	2.87	2.613 to 3.136	4.065+-.374
M. anisopliae 5	Ma5	2.55	2.286 to 2.807	3.613+-.339
M. anisopliae 6	Ma6	2.22	1.977 to 2.462	3.591+-.335
P. lilacinus	Pl	4.21	3.847 to 4.645	3.941+-.435
B. bassiana 4	Bb4	3.42	3.127 to 3.731	4.050+-.400
B. bassiana 5	Bb5	3.25	2.965 to 3.553	3.952+-.382
B. bassiana 6	Bb6	2.98	2.717 to 3.240	4.228+-.374

Three fungal species were found to have potentials within the value range of 2.223 - 4.206. The highest LT_{50} value was found in *P.lilacinus* (4.206) which was statistically different to others. The lowest LT_{50} value was found in *M. anisopliae* 6 (2.223) while others were not significantly different.

Efficacy of Selected Entomopathogenic Fungi in the Greenhouse

Table 9. LT_{50} values (days) of spray suspension of 7 entomopathogenic fungi to *Tetranychus kanzawai* through papaya seedlings (60 days) under green house conditions

NAME	LT50	FIDUCIAL LIMIT	SLOPE
M. anisopliae 4	3.97	3.674 to 4.258	3.423 +-. 0.229
M. anisopliae 5	3.63	3.341 to 3.917	3.275 +-. 0.219
M. anisopliae 6	3.54	3.260 to 3.805	3.495 +-.0.229
P. lilacinus	6.14	5.783 to 6.528	3.979+-.0.292
B. bassiana 4	4.93	4.552 to 5.331	3.863+-.0.260
B. bassiana 5	4.53	4.261 to 4.806	4.265+-.0.280
B. bassiana 6	4.18	3.858 to 4.492	3.729+-.0.492

Table 10. LT_{90} values (days) of spray suspension of 7 entomopathogenic fungi to *Tetranychus kanzawai* through papaya seedlings (60 days) under green house conditions

NO.	NAME	CODE	LT90	FIDUCIAL LIMIT
1.	*M. anisopliae*	Ma4	9.38	8.470 to 10.627
2.	*M. anisopliae*	Ma5	8.95	8.068 to 10.150
3.	*M. anisopliae*	Ma6	8.23	7.485 to 10.150
4.	*P. lilacinus*	Pl	12.89	11.508 to 14.918
5.	*B. bassiana*	Bb4	10.60	9.381 to 12.444
6.	*B. bassiana*	Bb5	9.05	8.330 to 10.018
7.	*B. bassiana*	Bb6	9.22	8.300 to 10.507

Table 9 and 10 . LT_{90} values (days) of spray suspension of 7 entomopathogenic fungi to *Tetranychus kanzawai* through papaya seedlings (60 days) under green house conditions . Green house experiment results indicate that pathogenic fungi have great potential for control of *T. kanzawai*. The LC_{90} of *T. kanzawai* infected with all isolates ranged from 8.23 to 12.89 among experimental units. These data show that the LC90 was more or less the same prior to the trial except on *P lilacinus*. The lowest LC50 (12.89) was observed at the plots treated with *P. lilacinus* while the highest was found on Ma6 (8.23).

Mean of mortality of *T. kanzawai* on 5th day after treatment with 7 isolates of entomopathogenic fungi

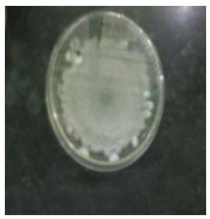
FUNGI ISOLATE	CONCENTRATION 0.00	1x 10 6	1 X 10 7	1 X 10 8	MEAN
Ma4	0.00	6.33	7.89	9.00	5.81b
Ma5	0.00	7.11	8.56	9.22	6.22a
Ma6	0.00	7.67	9.11	9.89	6.67a
B4	0.00	5.89	7.22	8.11	5.31c
B5	0.00	6.56	8.00	8.78	5.83b
B6	0.00	7.33	8.44	9.56	6.33a
Pl	0.00	5.33	7.00	7.67	5.00d
MEAN	0.00 A	6.60 B	8.03 C	8.89 D	

Means with the same letter are not significantly at Duncan 0.05

Study 2. Characterization of *M. anisopliae, B. Bassiana* and *P. lilacinus*

Table . Cultural characteristic of Entomopathogenic fungi

ISOLATE	COLOR Top	Bottom	MYCELIA STRUCTURE	COLONY SHAPE MEAN	GROWTH RATE MM	SIZE (MM)	NO. OF DAYS TO SPORULATION	SHAPE OF CONIDIA	SIZE OF CONIDIA (µM)
Ma4	white micelia with circular green conidia	Brown with circular ring	Thin and adpressed	Round	0,20	21	3	cylindrical	3 X 1.5
Ma5	Green conidia covering whole colony with white outside rings	Brown with circular ring	Thick and adpressed	Round	0,21	22	3	cylindrical	3.5 x 1.5
Ma6	Yellow dark with circular green conidia	brown with circular ring	Thick and adpressed	Round	0,21	22	3	cylindrical	5.5 x 1.5
pl	Pinkish	White	Thick and adpressed	Round	0,19	20	4	ovoid	4 x1.5
Bb4	White	Brownish white	Thick and adpressed	Round	0,15	16	4	globose	2 x 2.5
Bb5	White	Yellowish White	Thick and adpressed	Round	0,16	17	4	Sub-globose	2 x3
Bb6	White	White	Thick and adpressed	Round	0,21	22	3	globose	2 x 1.5



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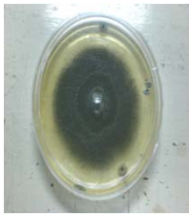

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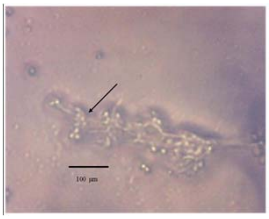

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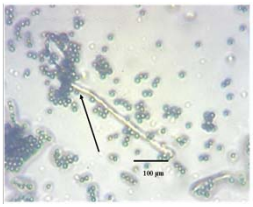

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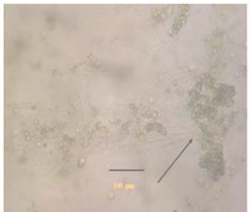

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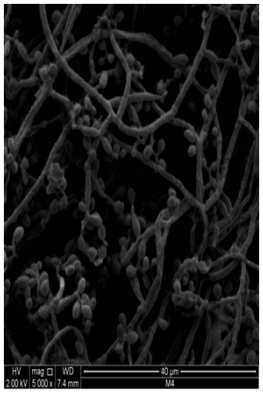
Colony and conidiophore of *Metarhizium anisopliae*



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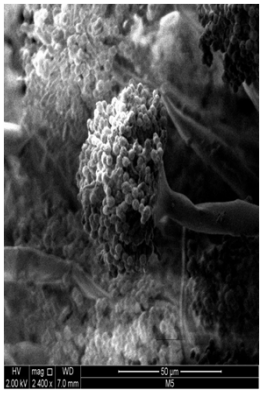

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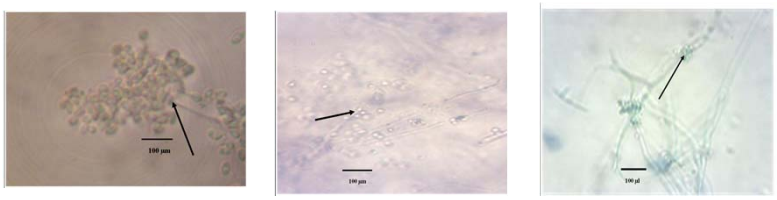
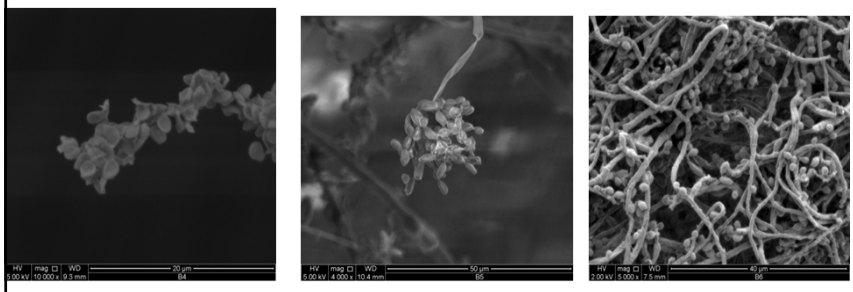
Mycelia and conidiophore of *M. anisopliae*

a b c



d e f

Colony and conidiophore of *B. bassiana*



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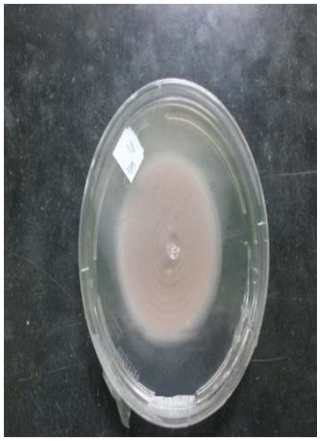
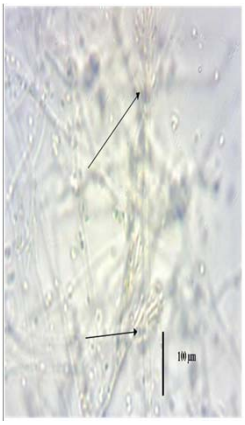
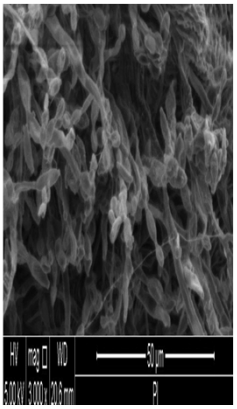
Mycelia and conidiophore of *B. basianna*







Colony and conidiophore of *P. lilacinus*

Infection Process to *M. anisopliae*, *B. bassiana* and *P. lilacinus*

Ma4

Diseasephases	0-24	24-48	48-72	72-96
Attachment & Adhesion				
Germination				
Penetration				
Colonization				
Extrusion				
Conidiogene-sis				

Ma5

Disease	0-24	24-48	48-72	72-96
Adhesion				
Germination				
Penetration				
Colonization				
Extrusion				
Conidiogene-sis				

Ma6

Disease phases	0-24	24-48	48-72	72-96
Attachment				
Germination				
Penetration				
Colonization				
Extrusion				
Conidiogene-sis				

B4

Disease phases	0-24	24-48	48-72	72-96
Adhesion				
Germination				
Penetration				
Colonization				
Extrusion				
Conidiogene-sis				

Bb6

Disease	0-24	24-48	48-72	96
Adhesion				
Germination				
Penetration				
Colonization				
Extrusion				
Conidiogene-sis				

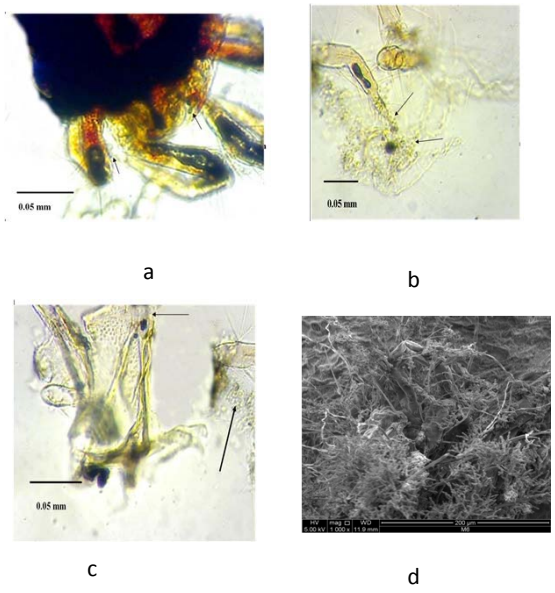
Bb5

Disease phases	0-24	24-	48-72	72-
Adhesion				
Germination				
Penetration				
Colonization				
Extrusion				
Conidiogenesis				

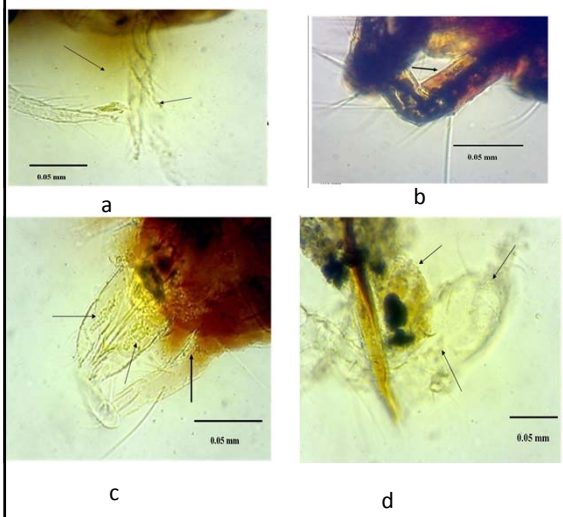
PI

Disease phases	0-24	24-48	48-72	72-96
Adhesion				
Germination				
Penetration				
Colonization				
Extrusion				
Conidiogenesis				

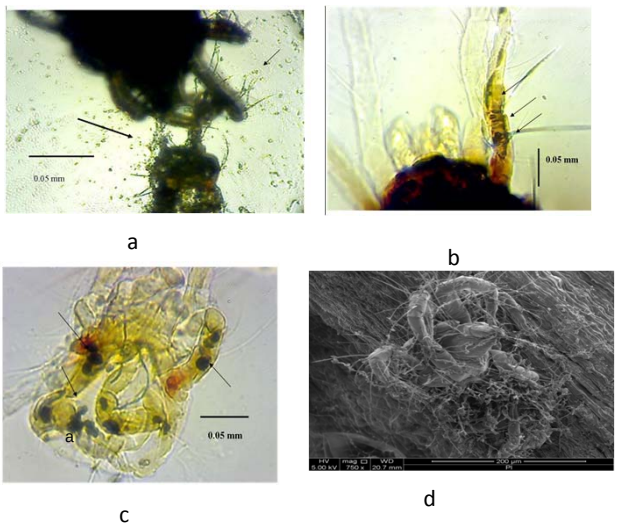
Figure . Duration, in hours, of the different developmental phases of *M. anisopliae* , *B. bassiana and P lilacinus* on the red mite *T. kanzawai*.



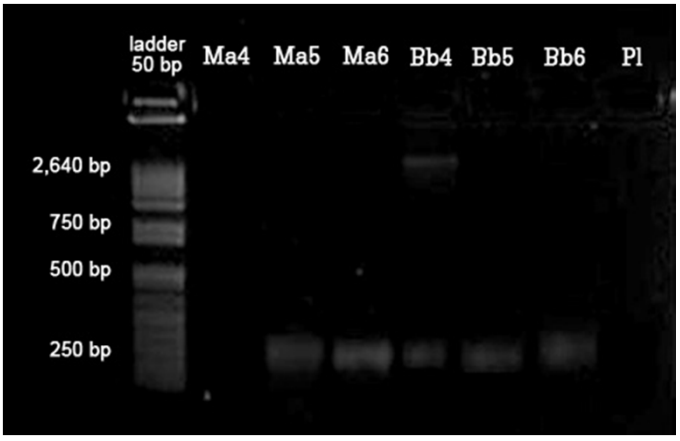
Infection process of *M.anisopliae 6* to *T. kanzawai* with 1000 x magnification a) attachment and adhesion 24 h; b) germination and colonization 48 h; and c) colonization and extrusion 72 h on ophistosome



Infection process of *B. bassiana 6* to *T. kanzawai* with 1000 x magnification a) Adhesion 24 hours; b) germination 24 hours; c) penetration, colonization and extrusion 24-48 hours; and d) Extrusion and conidiogenesis 48-72 hours in hypostome



Infection process of *P.lilacinus* to *T. kanzawai* with 1000 x magnification a) attachment and adhesion 24 h; b) germination 48 h;c) colonization 48-72 h on ophistosome; and d) colonization 48-72 on intersegmental



PCR amplification results with ITS1-ITS 2 primers on 7 isolates

Product of ITS1-ITS2 pair of approximately 200 bp was obtained from all the PCR amplifications for five isolates except with Ma4 and Pl which had no product result of three *Beauveria* isolates that can be detected with ITS1 and 2 primers.

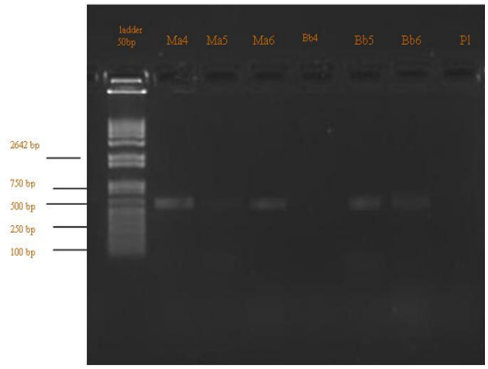


Figure 33. PCR amplification results with ITS1-ITS4 primers on seven isolates

Product of ITS1-ITS4 pair of approximately 560 bp was obtained from the PCR amplifications for five isolates except with Bb4 and Pl where there was no result. Three *Metarhizium* isolates can be detected with ITS1 and 4 primers.

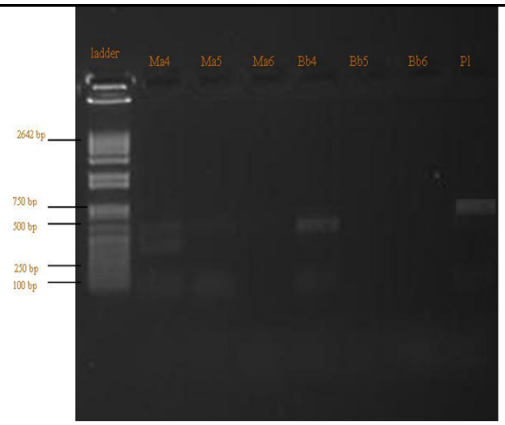


Figure 34. PCR amplification results with ITS4-ITS 5 primers on seven isolates

Product of ITS4-ITS5 pair of approximately 750 bp was obtained from the PCR amplifications for four isolates Ma4, Ma5, Bb4 and Pl.

Phylogram

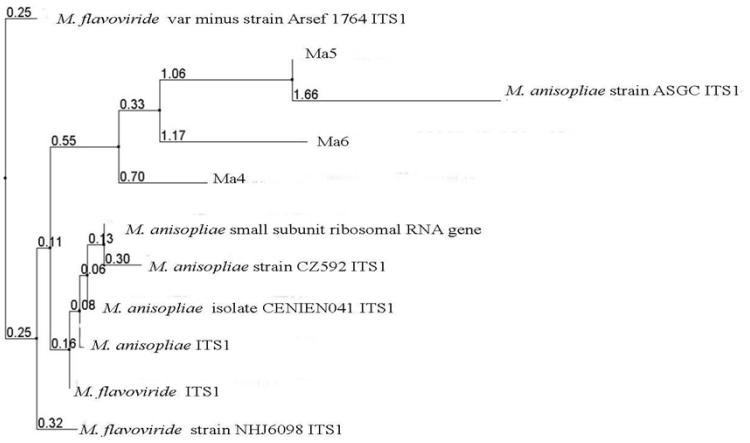


Figure 35. Rooted phylogenetic tree of rDNA ITS region of Ma4, Ma5 and Ma6 compared to *M. flavoviride* and *M. anisopliae*

Beauveria sp

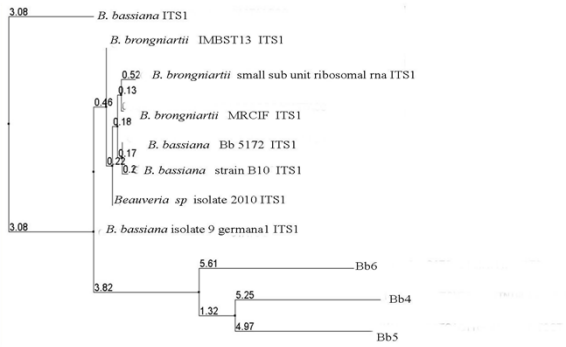


Figure 36. Rooted phylogenetic tree of rDNA ITS region of Bb4, Bb5, Bb6 compared to *Beauveria* sp, *B. brongniartii* and *B. bassiana*

Paecilomyces sp

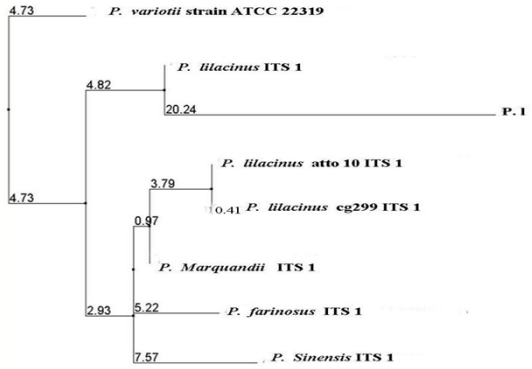


Figure 37. Rooted phylogenetic tree of rDNA ITS region of Pl compared to *Paecilomyces* sp, P. *marquandii, P. farinosus* and *P. sinensis, and P. lilacinus*

SUMMARY AND CONCLUSION

- The 14 most pathogenic isolates of *M. anisopliae* at 5 DAT in order of decreasing pathogenecity were Ma5>Ma6>Ma3>Ma2>Ma1>Ma3>Ma7. For *B. bassiana*, Bb5>Bb6>Bb4>Bb3>Bb1>Bb2.

- The refined LC_{50} values The most pathogenic isolates remained to be Ma5>Ma6>Ma4 and Bb6>Bb5>Bb4

  Reinoculation and reisolation from infected *T. kanzawai* with definite signs of infection were observed among insects treated with seven isolates demonstrating its entomopathogenic capacity

- The LT_{50} values ranged from 2.865 to 4.150 days. The LT_{50} values were lower for Ma isolates than Bb isolates and the highest LT_{50} value was for *P. lilacinus.*

- Greenhouse experiment results is The LC_{90} of *T. kanzawai* infected with all isolates ranged from 8.23 to 12.82 among experimental units.

- Infection process showed attachment, germination and fungal form *M. anisopliae 5, M. anisopliae* isolate 6 and *Beauveria bassiana* 6 were the fastest to infect mite

- All *M. anisoplia*e had cylindrical shapes with different characteristic. *B*. bassiana had a globose with variation of characteristic and *Paecilomyces* is characterized by having flasked phialides or phialides with swollen base structure; the phialides taper into a distinct neck and generate conidia that are dry and hyaline or slightly pigmented.

- Further analysis were confirmed molecularly by subjecting to polymerase chain reaction (PCR) amplification with set of ITS primers (ITS1, ITS2, ITS4, and ITS5) which are commonly used among fungal species. Lengths of amplified fragments of the two isolates were approximately 200bp, 560bp and 750bp, respectively.

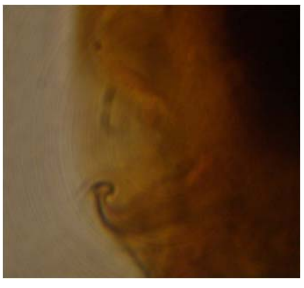
- Alignment sequence showed that isolate Ma4, Ma5, Ma6 had close similarity with Metarhizium anisploliae. The analyses indicated that Bb4, Bb5, Bb6 had close related to *B. bassiana* isolates. A dendrogram showed that *Pl* is close similar with *P. lilacinus*

THANK YOU VERY MUCH

SALAMAT PO

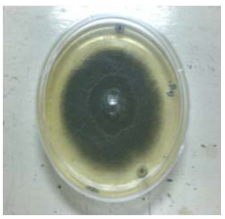
(Back)

T. kanzawai



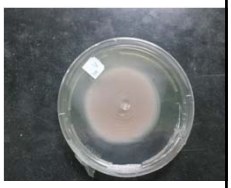
male

Female


next



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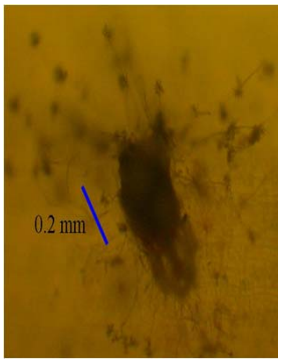
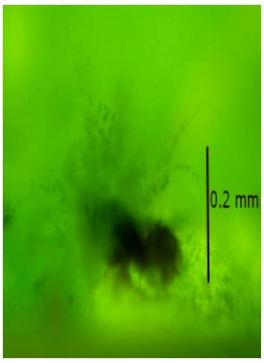
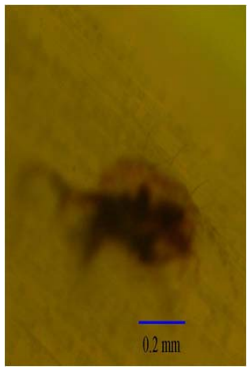


M. anisopliae



B. Bassiana



P. lilacinus

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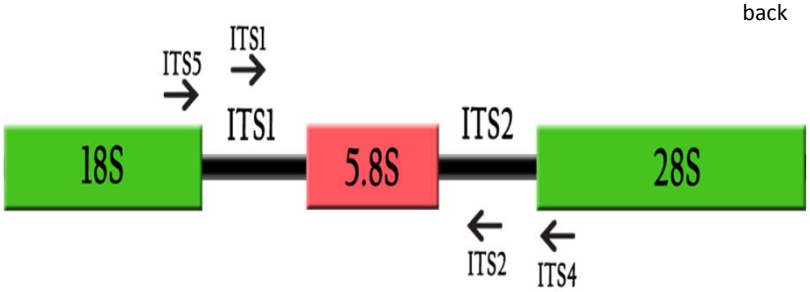
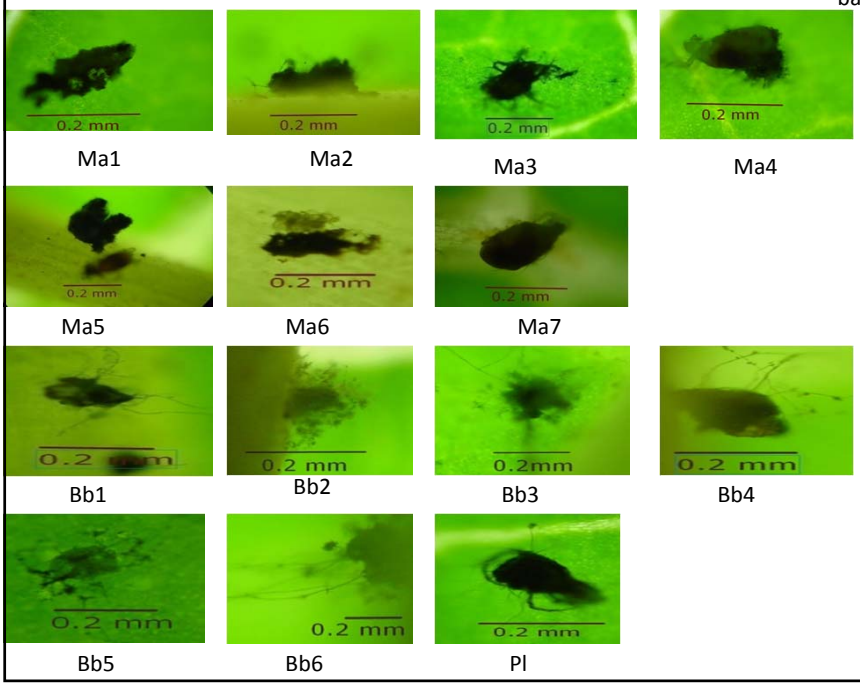
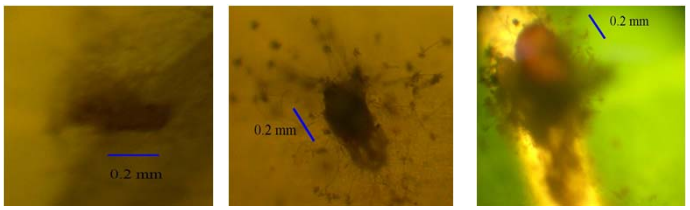


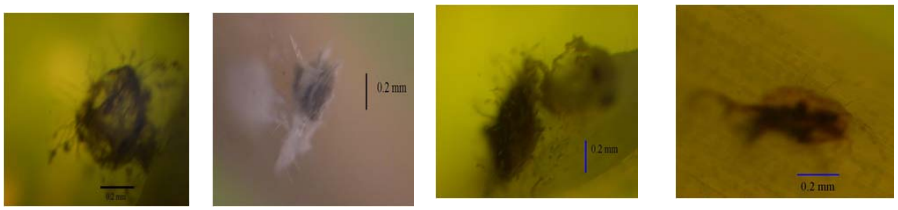
Genetic map of the rDNA region of fungi showing the ITS regions (ITS1 and ITS2), nested between highly conserved ribosomal genes 18s, 5.8s, and 28s (White et al., 1990). Arrows represent positions of the ITS1, ITS2, ITS4, and ITS5 primers.

back





a b c



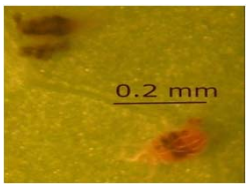
d e f

T. kanzawai mites infected by surface contact with conidia of *M. anisopliae, P. lilacinus* and *B. bassiana by* 400 X

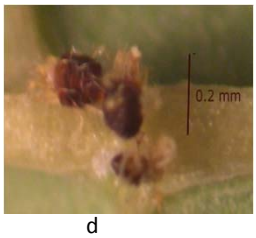
Horizontal Transmision

a

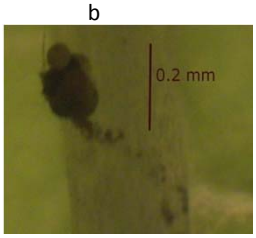
b



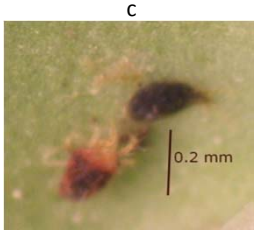
c



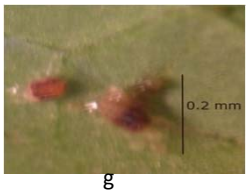
d



e



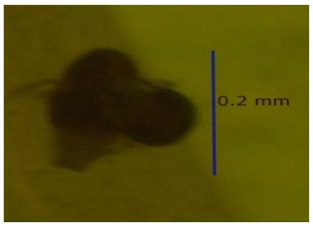
f



g

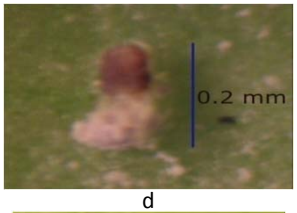
Surface Contamination

a



b

c



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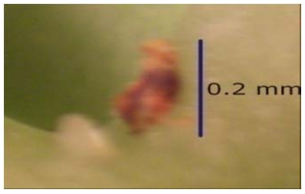

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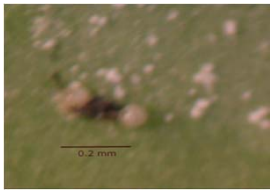

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