LABORATORY BIOASSAY OF *METARHIZIUM ANISOPLIAE* (METSCH.) AGAINST THE TWO SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE)

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Abstract

Laboratory bioassay of an entomopathogenic fungi, *Metarhizium anisopliæ* (Metsch.) Sorokin, which maintained on potato dextrose agar (PDA) Difco, and liquid medium was evaluated against the two spotted spider mite, *Tetranychus urticae* Kock adult females. This bioassay revealed that liquid medium supernatants (LMS) caused mortality up to 90% while *M. anisopliae* spores resulted, from PDA (solid medium) caused 76.67% *mortality* at 8×10^5 spores/ml.

EC_{50} and EC_{90} values of *M. anisopliae* spores were 3.38×10^5 and 16.62×10^5 spores/ml , respectively.

Key words: Bioassay, *Metarhizium anisopliae*, *Tetranychus urticae*

INTRODUCTION

Avilable knowledge of mite diseases is increasing in recent years because of the economic importance of many mite species especially eriophyoids and spider mites.Fungal pathogens often an important role in the regulation of natural mite populations (Van Der Geest et al., 2000).

Tetranychid mite, *Tetranychus urticae* can be infested by entomophthorales fungi, such as Neozygites, which posses a restricted host spectrum and are obligatory pathogens. Also, deuteromycetes are able to kill *T.urticae* even by true spores or mycotoxins which often cause host death prior to complete colorization of the body (Hajek, 1997).

Metarhizium is a clear example of Deuteromycetes fungi. The first species of the genus metarhizium (Subdivision. Deuteromycotina; Class Hyphomycetes; Order Moniliales), *Metarhizium anisopliae*, was isolated from the coleopteran species, *Anisopliae austriacae* by Metachinkoff in 1878. *Metarhizium anisopliae* can produce three spore types in vitro. Aerial conidia in solid culture and both of blastospores and submerged conidia in liquid culture (Jenkins and Prior, 1993). This fungi improved its efficacy against many insects such as grasshoppers and locusts (Lomer et al., 2001)

and mites. *M. anisopliae* provided an excellent control to varroa mites infested honeybees (Flores,2004). Most infested mites died within 3-5 days without any bad hazards to bees.

M. anisopliae hyphae could easily pentrate the soft unsclerotised exoskeleton of astigmatid sheep scab mite, *Psorptes ovis* and casued 77% mortality of mites which were exposed to $1x10^8$ conidia/ml at 25°C (Brooks et al., 2003).

Moreover, *M. anisopliae* produce many toxins, the most important group named destruxins which can be used as pesticides (Glover, 2005). Destruxins has a lack of mammalian toxicity at high exposure levels. Beside that the quantities of toxic secondary metabolites produced by fungi in vivo were much less than those in nutrient rich liquid media. Therefore, use of myocoinsecticides was not expected to result in harmful levels to the environment (EPA, 2005). So, *M. anisopliae* is generally safe to natural enemies.

This research aimed to evaluate the lab. efficacy of *M. anisopliae* spores and *M.anisopliae* liquid medium supernatant (LMS) against *Tetranychus urticae* adult females.

MATERIALS AND METHODS

A)Fungal medium

a) **Solid media:** *Metarhizium* anisopliae spores were isolated from naturally infested *Tetranychus urticae* adult females. Spores were maintained on PDA (Potato Dextrose Agar) supplemented with 5% yeast extract (Difco) which had been sterilized, for 20 min at 121°C. To prepare fungal inocula conidia from 2-3 weeks old cultures (20 + 2.70°C and 70%RH) were scarped from the surface of the plates with a sterile scalpe and suspended in 0.05% aqueous Tween 80. Haemocytometer was used to estimate the conidial concentration and subsequent appropriate dilutions were made.

A)Fungal medium

b) Solid media: *Metarhizium* anisopliae spores were isolated from naturally infested *Tetranychus urticae* adult females. Spores were maintained on PDA (Potato Dextrose Agar) supplemented with 5% yeast extract (Difco) which had been sterilized, for 20 min at 121°C. To prepare fungal inocula conidia from 2-3 weeks old cultures (20 + 2.70°C and 70%RH) were scarped from the surface of the plates with a sterile scalpe and suspended in 0.05% aqueous Tween 80. Haemocytometer was used to estimate the conidial concentration and subsequent appropriate dilutions were made.

summer and on castor oil leaves at winter. This colony was left for one year under the previous conditions in order to get a homogenous and sensitive colony.

C)Laboratory bioassay: *Metarhizium anisopliae* was bioassayed using an adulticidal technique (Edge and James ,1982). Briefly, 20 young adult female mites were transferred to castor oil leaf discs (2cm diameter). Mites on the leaf discs were then sprayed with aqueous suspensions of *M.anisopliae* spores and with 10 ml (LMS) of each spores concentration. Controls of *M.anisopliae* spores treatment were sprayed with water + 0.05% Tween 80 but in the case of (LMS) treatments, controls sprayed with water only. Sprayed leaf discs (three per concentration) were maintained under constant light on moistened cotton wool for 72h (25+2°C,60+5%RH). Mortality percentages were determined and corrected by using Abott's formula (1925). Data were analysed using a probit program written in GENSTAT5 statistical software (Barchia ,2001). LC_{50} and LC_{90} values plus their 95% fiducial limits, were calculated using the probit method outlined in (finney, 1971).

RESULTS AND DISCUSSION

Data showed that adult females of *T.urticae* were affected by serial concentrations of *M.anisopliae* spores and its liquid media supernatants (LMS).

Mortality percentage caused by fungi spores of mite females at high concentration 8 $x10^5$ spores/ml was 76.67%. Table (1), but It was increased to 93.33% in the case of (LMS). Table (2).

Estimates of the EC_{50} and EC_{90} with 95% fiducial limits computed for *M. anisopliae* spores against *T.urticae* were 3.38 X 10^5 (5.61 x 10^5- 2.05 x 10^5) spores/ml and 16.2 x 10^5 (27.59X10^5-10.0x10^5) spores/ml, respectively. Table (1).

Materihizium anisopliae as an entomopathogenic fungi improved its pathogenicity against *T.urticae* adult females in this research as shown previously.

The basic infective unit of fungi was spore which had to adhere to the cuticle of host and germinate. Attachment, the first step of infection process, was mediated by chemical components in the outer layers of the spore and the metal cuticle (parker et al., 2000). *M.anisopliae* hydrophobic spores were covered by proteins, lipoproteins and polysaccharides which helped spores to adhere themselves to the host beside that fungi secreted N-acetylglucosaminidase which concentrated on the spores surfaces when they touched hosts body, immediately (St Leger et al., 1991).

After that, spores germination took place, fastly within few hours. In the case of *T.urticae* adult females fungi infected them at moisturized ares (Charnley et al., 1997). Such as mouth parts, secretion pores (excretory proe, etc), and natural pores in tetranychid mite's such as the couple spiracles which located on ganthosoma, joints of legs, chelicerae, and pedipalps and where the cuticle is less sclerotised plus humidated conditions.

The next step of infection process was penetration. *M.anisopliae* produced appressoria to help it to stuck in vitro on hard hydrophobic surfaces like cuticle. (St Leger et al., 1989) and cuticle degrading enzymes such as lipases, proteinases, and chitinases.

Once fungi reached hemoceol, it encountered the host's immune system, by secreted toxins. Then host died and it was good resource of infection of fungi.

(Nugroho and Ibrahim, 2004) investigated the effect of *M.anisopliae* against broad mite (Polyphagotarsenomus *latus* Bank) and they found that EC_{50} was 2.77 x 10^7 conidia/ml.

Beside that several scientists prefered using entomophthorales fungi as control agents of *T.urtica* (Leit et al., 2000) more than deuteromycetes fungi (Tamai et al., 1998). On the other hand, data concerning this research not agree with them generally, but if there was a comparison between the ability of pathogenicity of *M.anisopliae* spores and its (LMS), the last one could be the superior against *T.urticae*.

Concerning the highly good response of treatment with *M.anisopliae* (LMS), it could be explained upon two points.

The most important one depend on mycotoxins which secreted by fungi as a secondary metabolite products caused mite death prior to complete colonization of the body (Van Der Geest et al., 2000). These included cyclic depsipeptides; Cytochalsins and Destruxins (DTXs) (Clarkson and Charnely, 1996). DTXs showed highly effect as an insecticidal when ingested by mouth.

DTXs had diverse modes of action including depolarization of muscle membrane Ca^{+2} channels, inhibition hemocyte (Blood cells) function and inhibition of vascular – type ATP ase. This can be an answer of the main question: Why (LMS) of *M.anisopliae* had a fast reponse against *T.urticae* comparable with treatments by M. anisopliae spores only.

Secondly, blastospores which were only produced in liquid medium, thought to have faster germination rates than conidia. So it contributed in fast infection and

mortality. (Thomas et al., 1987). Spores with faster germination rates might have a greater potential for infection by reducing potential for desiccation, effects of other organisms, or less during molting while on the insect cuticle, (Dillon and Charnely 1985). Beside blastospores in liquid media there were submerged condia, which were hydrophilic and had similar characteristics of aerial conidia(Jenkins and Prior 1993).

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Table1. Results of probit analysis of *Metarhizium anisopliae* spores against *Tetranychus urticae* Koch adult females

Conc. Spores/ml	% mortality	EC_{50}		EC_{90}		Slope
		Value	95% fiducial limits	Value	95% fiducial limits	
$1x10^5$	16.67	3.38	5.61 – 2.05	16.2	27.9-10.01	1.85
$2x10^5$	33.33					
$4x10^5$	53.33					
$8x10^5$	76.67					

Table 2. Effect of *Metarhizium anisopliae* liquid media supernatants against *Tetranychus urticae koch* adult females.

Spores/ml water/100ml liquid media	% Mortality
$1x10^5$	30
$2x10^5$	50
$4x10^5$	66.67
$8x10^5$	93.33