Ethiop. J. Agric. Sci. 24:51-58 (2014)

Pathogenecity of *Beauveria bassiana* and *Metarhizium anisopliae*, to the Two Spotted Spider Mites, *Tetranychus urticae, (Acari: Tetranychidae*) at Different Temperatures and in Greenhouse Condition

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Abstract

Pathogenecity of entomopathogenic fungi, **Beauveria bassiana** *and* **Metarhizium anisopliae** *isolates of Ethiopian origin on eggs and adults of the two spotted spider mites,* **Tetranychus urticae,** *effect of temperature on virulence, as well as potentials of isolates in the greenhouse condition was assessed during October 2008 to May 2009 at Plant protection Research center in Ambo. All the isolates tested (B. bassiana 9614 and 9609, and* **M. anisopliae** *MM and PPRC 6) caused high mortality to the eggs of the two spotted spider mites compared to the control groups. Significant differences of pathogenicity were observed among isolates kept at different temperature regimes. Temperature regimes of 25°C and 30°C were suitable for the isolates* **M. anisopliae** ***MM*** *and* **B. bassiana** *9614 compared to 20°C and 35°C. All the four fungal isolates used caused significantly higher mortality compared with the untreated control groups under the greenhouse condition and the highest mortality was 89.4%. As a consequence leaves treated with the fungal isolates had less damage that may be attributed to fewer number of surviving mites. The study showed that* **M. anisopliae** *and* **B. bassiana** *are able to kill the two spotted spider mites eggs and adults, and can be used as substitute or complement to synthetic chemicals most of which lack ovicidal activity.*

Key words: Entomopathogens; Isolates; Mortality; Temperature; Two Spotted Spider Mites.

Introduction

The two-spotted Spider mite (Family *Tetranychidae*, Order Acari) is a common pest of many plants. It has been recorded on more than 150 hosts of economic value throughout the world and is a major pest in greenhouse cut roses (Blümel et al., 2003). It has become a big challenge to maintain quality of products and compete in international market because of the two-spotted Spider mite.

Spider mites are among pests with significant portion of all pesticides used on ornamentals (Hort Report, 2001). In addition, they are known to rapidly develop resistance to pesticides. The two spotted spider mites have evolved resistance to more than 91 pesticides and out of the total 491 reported cases of resistance for spider mites 321 of them are by the two spotted spider mite (APRD, 2012). The need to develop alternatives to conventional pesticides has become apparent in recent years because many pest species developed resistance to pesticides. Avoiding the use of chemicals has many advantages not only for the environment and the workers'health but also for the crop quality and yield because of phytotoxicity of pesticides sprayed directly on the crop (Belder and Elings, 2007; Belder and Akkerhuis, 2007).

Discoveries in Entomopathogenic fungi growth characteristics and methods of formulation have stimulated interest in their development (Bateman et al., 1993). Although pathogenecity of *Beauveria bassiana* and *Metarhizium anisopliae* to many pest species have been tested and reported, there are few studies on the two spotted spider mites. The present study investigated the virulence of *B. bassiana* and *M. anisopliae* isolates on the two spotted spider mite eggs, effect of temperature on virulence of the entomopathogenic fungi to adult spider mites, and evaluated the isolates for controlling the two spotted spider mites in the greenhouse condition.

Materials and Methods

This study was conducted during October 2008 to May 2009 at Plant protection Research Center at Ambo. Isolates of *Beauveria bassiana* and *Metarhizium anisopliae* were supplied by the Plant Protection Research Center (PPRC), Ambo, Ethiopia, previously collected and isolated from different arthropods in different agro-ecological zones of Ethiopia and stored as conidiaat 4°C. The conidia were sub-cultured and their virulence was maintained by passing them through the larvae of the wax moth, *Galleria mellonella* (L.). Conidial suspension of the isolates was prepared using 0.01% Tween 80 after culturing the isolates on Sabouraud's Dextrose Agar with Yeast extract (SDAY) media. The suspension was then adjusted to desired concentration based on the counts of the conidiain 1 ml of suspension. For each isolates tested, viability was determined by germinating conidia on SDAY media.

Effect of fungal isolates on eggs of the two spotted spider mites

The two spotted spider mites were reared on beans *(Phaseolus vulgaris*) planted in pots. Twenty-five large uniform sized adults were placed on leaf disks in a petri dish and allowed to freely lay eggs for 18 h as described by Shi and Feng (2004). Subsequently, all the adults were removed from the leaf, leaving eggs to receive fungal inoculation. Conidial suspensions of *B. bassiana* and *M. anisopliae* isolates (9614, 9609, MM and PPRC-6) were prepared using 0.01% Tween 80. Conidia dose of 1×10^8 ml^{-1} was used for inoculation and 0.01 % Tween 80 was used as blank control. Fifty eggs were counted and left in each leaf discexposed to a spray of half ml of conidial

suspension. The experiment was laid out in Completely Randomized Design with four replications. Data were collected on the number of hatched and un-hatched eggs starting from 18 hours after inoculation and continued for one week. Egg mortalities due to fungal infection was based upon the distortion or shrinkage in egg shape and non- emergence (Kongchuensin and Takafuji, 2005).

Effect of temperature on virulence of entomopathogenic fungi to adult spider mites

Batches of 25 adult spider mites were sprayed with suspension of two fungal isolates, 9614 (*B. bassiana*) and MM (*M. anisopliae*), at concentration of 1 $\times 10^8$ conidia per ml. Petridishes were sealed with parafilm and small holes were created for aeration. The petridishes were incubated at temperatures of 20, 25, 30 and 35°C. The design of the experiment was Completely Randomized Design with four replications and the control treatments were treated with 0.01% tween 80 for each temperature treatment and replicated four times. New fresh leaves were provided when the original leaves dried and dead and live spider mites were counted daily.

The effect of entomopathogenic fungi to adult spider mites and damage levels in a greenhouse experiment

The experiment was conducted on potted green bean plants *(Phaseolus vulgaris*) in a greenhouse. Five random samples of infested bean leaves from each pot were tagged and the number of adult *T. urticae* on each side of the bean leaves was counted on the day of treatment application. Four fungal isolates (MM, PPRC 6, 9614 and 9609) using dose of 1×10^8 conidia ml^{-1} were sprayed on leaves and a treatment with 0.01 % Tween 80 was used for the control groups. Each pot with the bean plant was placed in a cage covered with black plastics to avoid drifting of the fungal conidia during spraying. Application was using a small hand held sprayer until run off as described by Cote (2001). After treatment application the pots were placed in the greenhouse by leaving some space between them to prevent migration of mites with in the pots. Each treatment was replicated four times and laid in a completely randomized design. The number of spider mites on both sides of each bean leaf was counted daily for 20 consecutive days after application. Leaf damagewas assessed based on Kondo (2004) using 0 to 5 scales whereby 0= no damage; 1= 20% of the lower leaf surface is spotted; 2= 40%; 3= 60%; 4= 80% and 5= 100% and data on the leaf damage was taken at 10, 15 ad 20 days after treatment.

Statistical analysis

For all experiments mortality data were adjusted for control mortality (Abbott, 1925) and subjected to analysis of variance. Percentage mortality was arcsine transformed before analysis of variance (ANOVA). Significant differences between treatment means were compared at 0.1% significance level using least significance difference (LSD).

Results and Discussion

Susceptibility of the two spotted spider mite eggs to fungal isolates

Untreated eggs started to hatch within two days and hatching completed within 7 days. The fungal isolates significantly differed in the levels of mortality they caused on spotted spider the eggs ($F= 238.70$, $DF=4$, $P<0.001$) (Table 1).The highest egg mortality (82%) was recorded for isolate *B. bassiana* 9614 followed by isolate *M. anisopliae* MM (77%) seven days after treatment and no mortality was observed in the control group treated with only 0.01 % Tween 80 (Table 1). The remaining two isolates, *M. anisopliae* PPRC 6 and *B. bassiana* 9609 caused 61% and 65% egg mortality, respectively. About one week after exposure to fungal treatments, un-hatched eggs looked distorted and shrunken. Fungal outgrowths appeared on the un-hatched eggs after maintenance in moist chambers at 25 ±2°C for about 4-5 days but no fungal growth was observedon the few un-hatched eggs in the control group though they might turn gray or less glossy. The fungal outgrowths were identified as *B. bassiana* or *M. anisopliae* based on their characteristic color when the wizened eggs were individually placed in SDAY media.

Table 1. Mortality of two spotted spider mites eggs treated with isolates of *M. anisopliae* (MM, PPRC 6) and *B. bassiana* (9614, 9609) seven days after treatment

Entomopathogenic Fungi Isolates	Mortality ± SE*
MM	76.63±1.07[a]
PPRC 6	61.19±1.86[b]
9614	82.38±3.44[a]
9609	65.39±0.77[b]
Control	0±0[c]

*Values followed by the same letter in the same column do not differ significantly ($P>0.05$) according to least significance difference (LSD) test.

Gouli et al. (2005) also reported that entomopathogenic fungi, *B. bassiana* and *M. anisopliae* are often able to infect several developmental stages, including eggs. Shi et al. (2008) confirmed the ovicidal activities of the aerial conidia of *B. bassiana* in an emulsifiable formulation on two-spotted spider mite, *T. urticae*. Weibin and Mingguang (2004) found that both *B. bassiana* and *Paecilomyces fumosoroseus* infections decreased the hatch rates of *Tetranychus cinnanarinus* eggs and the higher the conidial concentrations resulted in greater reduction in the hatch rates. The current study showed that *M. anisopliae* and *B. bassiana* are virulent to the adult and also eggs of the two spotted spider mite.

Effect of temperature on efficacy of fungal isolates

Temperature significantly affected the virulence of the fungal isolates four days after treatment (F=160.61, DF=8, P=0.001), six days after treatment (F=46.52, DF=8, P< 0.001) and eight days after treatment (F=460.81, DF=8, P<0.001) (Table 2).

Table 2: Percent mortality of two spotted spider mites treated with MM and 9614 and kept under different temperature regimes.

Temperature (°C)	Mortality ± SE* 4 days after treatment		Mortality ± SE* 6 days after treatment		Mortality ± SE* 8 days after treatment	
	MM	9614	MM	9614	MM	9614
20°C	32.6±4.89^{cd}	36.7±2.87^{c}	41.9±2.87^{de}	50.0±1.7^{d}	47.5±5.23^{d}	65.3±2.98^{c}
25°C	54.8±1.07^{ab}	60.1±3.44^{a}	79.7±6.48^{b}	90.01±0.0^{a}	90.0±0.0^{a}	90.0±0.0^{a}
30°C	52.8±2.34^{b}	51.3±0.97^{b}	70.3±3.40^{c}	65.4±1.92^{cd}	90.0±0.0^{a}	72.7±5.07^{b}
35°C	36.91±5.32^{c}	28.6±3.37^{d}	35.5±4.76^{e}	30.0±2.36^{f}	39.2±4.20^{e}	30.3±3.69^{f}
Control	0±0^{e}		0±0^{g}		0±0^{g}	

*Values followed by the same letter in the same day do not differ significantly (P>0.05) according to least significance difference (LSD) test.

MM isolate at 1×10^{8} conidia ml^{-1} caused 90.0% mortality at temperature of 25°C eight days after treatment (Table 2) while the same isolate caused reduced mortality of 57.5% at 20°C and 39.2% at 35°C. Similarly, Beauveria isolate, 9614, caused higher mortality at 25°C (90.0%) while mortalities of 65.3% and 30.3% were recorded for 20°C and 35°C temperatures, respectively. Temperatures 25°C and 30°C were found to be more suitable for both isolates compared to 20°C and 35°C.

There were differences in the extent of mortality caused by Metarhizium isolate MM and Beauveria isolate 9614 at different temperatures. Isolate MM caused higher mortality than isolate 9614 at higher temperatures (25°c and 30°c) in all of the three observation days (4, 6 and 8 days after treatment). On the other hand, isolate 9614 caused higher mortality at 20°c than Isolate MM. At 35°c, both isolates caused lower mortality, although isolate MM out performed isolate 9614. The study indicated that both higher and lower temperatures are not suitable for the two isolates tested.

Similar studies have shown that Temperature variations affect the performance of entomopathogens. Bugeme et al. (2009) observed better *B. bassiana* and *M. anisopliae* germination and virulence on *T. evansi* at 25 and 30°C. Tadele and Pringle (2003) also reported significant differences between Ethiopian isolates Bb-01, PPRC 4, PPRC 19, PPRC 61 and EE-01 in causing mortality to larvae of *Chilo partellus* at different

temperatures and all the tested isolates induced 100% mortality at 25°C and 30°C in six days after treatment.

Efficacy of isolates in the greenhouse condition

All the fungal isolates caused significantly higher levels of mortality compared to the control treatment (Table 3). However, there were significant differences between treatments ($F= 100.42$, $DF=4$, $P<0.001$)10 days ($F= 181.02$, $DF= 4$, $P<0.001$), 15 days and ($F= 450.11$, $DF= 4$, $P< 0.001$) 20 days after treatment.

Table 3: Effect of four entomopathogenic fungi isolates on the two spotted spider mites at the rate of 1×10^8 conidia ml^{-1} in the greenhouse

Fungal isolates	Mortality ±SE* 10 days after treatment	Mortality ±SE* 15 days after treatment	Mortality ±SE* 20 days after treatment
MM	81.7±3.29[a]	85.9± 1.92[a]	89.4±5.23[a]
PPRC 6	69.2±5.45[b]	76.6± 5.98[b]	83.0±7.07[b]
9614	80.3±3.07[a]	85.9± 4.07[a]	88.7±6.67[a]
9609	68.6±1.89[b]	70.2±4.76 [b]	79.8± 4.20[b]
Control	0±0.0[c]	0±0.0[c]	0±0.0[c]

*Values followed by the same letter in the same column do not differ significantly ($P>0.05$) according to least significance difference (LSD) test.

Mortalities recorded in the green house experiment were lower than the laboratory. For example, mortality by MM, and 9614 were about 80% 10 days after treatment in the greenhouse while it was about 90% in the laboratory experiment in a relatively shorter duration (6 days after treatment) (Table 3). This variation could be due to high probability of inoculation of eggs in case of the laboratory study which was conducted in petridishes.

Metarhizium isolate MM and PPRC 6 were more virulent in the greenhouse study compared to Beauveria isolates 9614 and 9609. This may be due to their ability to withstand the greenhouse conditions better than others. The result agree with temperature dependent virulence results, as MM and PPRC 6 caused higher levels of mortality compared to *Beauveria* spp. at higher temperatures.

There were significant differences of leaf damage levels between the leaves treated with the four entomopathogenic isolates and the untreated control (0.01% Tween 80) in all the three observation days; ($F=3.71$, $DF=4$ $P=0.03$) 10 days after; ($F=18.15$, $DF=4$, $P<0.001$) 15 days after and ($F=68.09$, $DF=4$, $P<0.001$) 20 days after treatment (Table

4).There were variations in leaf damage levels among the entomopathogens treatments and the lowest was recorded on MM treated plants.

Table 4: Effect of *B. bassiana* and *M. anisopliae* isolates on leaf damage level (Scales±SE).

Fungal isolates	damage ± SE* 10 days after treatment	damage ± SE* 10 days after treatment	damage ± SE* 10 days after treatment
MM	2.7±1.24^{b}	2.0±0.68^{b}	1.3±0.86^{d}
PPRC 6	3.2±0.62^{b}	2.0±0.42^{b}	1.6 ± 0.20^{cd}
9614	3.3±1.24^{b}	2.1± 1.04^{b}	1.9±0.67^{bc}
9609	3.4±1.18^{ab}	2.4± 0.48^{b}	2.4±1.27^{b}
Control	4.2±2.40^{a}	4.5± 1.24^{a}	4.9±0.13^{a}

*Values followed by the same letter in the same column do not differ significantly (P>0.05) according to least significance difference (LSD) test

The study showed that *M. anisopliae* and *B. bassiana* are able to kill eggs of the two spotted spider mites and entomophatogenic fungi can be used as substitute or complement to synthetic chemicals most of which lack ovicidal activity. Integrating the use of entomopathogenic fungi in the management of the two spotted spider mites may help to reduce resistance development among mite populations and create a competitive market advantage by reducing the associated miticides costs. Moreover, entmopathogens are safe to the environment and the workers. Future research should also focus on collection and testing of entompathogenic fungi from various areas of the country including different agro ecologies, farming systems, crops, pests, etc. with the main aim of developing highly virulent isolates.

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