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Efficacy of Ethiopian *Beauveria bassiana* and *Metarhiziumanisopliae* Isolates on Spotted Spider Mites, *Tetranychusurticae* (Acari: Tetranychidae) under Laboratory Conditions

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ከኢትዮጵያ የተገኙ ስድስት የቢቬሪያ ባሲያና (Beauveria bassiana) እና ዘጠኝ የሜታሪሂዚየም አኒሶፓሊ (Metarhizium anisopliae) የፈንገስ ዓይነቶች በቤተ ሙከራ ዉስጥ አራት ጊዜ በመደጋገም ትልልቆቹን (አዴልት) Tetranychus urticae የመግደል ሁኔታዉን ለመገምገም ተሞክሯል። ይህም የተደረገዉ የቅንቅን ዓይነቶችን በተፈጥሯዊ መንገድ ለመቆጣጠርና IPM ዘዴ ዉስጥ ማካተት ይቻል እንደሆነ ለማየት በሚል ዓላማ ነዉ። በዚሁም መሰረት ሁሉም የተሞከሩ የፈንገስ ኣይነቶች በ $1x10^8$ conidia ml-መጠን ባለሁለት ነጠብጣብ ቅንቅኖችን መግደል ችለዋል። ነገር ግን የመግደል አቅማቸዉ ከ45.4% እስከ 90.0%፤ 50% መግደል የተቻለበት ሰዓት (LT50) ደግሞ 3.19 እስከ 11.81 ቀናቶች ዉስጥ ነበረ። ከመግደል አቅማቸዉ በመነሳት PPRC 29, PPRC 27, PPRC 19, PPRC 66, እና EE የተባሉት የሜታሪሂዚየም ዓይነቶች እና GG እና HH የተባሉት የቢቬሪያ ዓይነቶች መካከለኛ (60-80%) የመግደል አቅም ሲኖራቸዉ የሜታሪሂዚየም PPRC 2 እና PPRC 61 እና የቢቬሪያ 9615 እና 9604 ደካማ የሚባል የመግደል አቅም ነበራቸዉ (<60%). አራት የፈንገስ ኣይነቶች (የሜታሪሂዚየም MM እና PPRC 6፤ እና የቢቬሪያ 9614 እና 9609 የመግደል አቅማቸዉ 86% እስከ 90% በመሆኑ ከፍተኛ ገዳይ በመባል ሲፈረጁ ይህን አቅማቸዉን በበለጠ ለመገምገም በተለያዩ 4 መጠኖች ($1x10^5$, $1x10^6$, $1x10^7$ እና $1x10^8$ ml-1) ታይተዋል። በተለያየ መጠን የተጨመሩ የፈንገስ ኣይቶች በመግደል አቅማቸዉ በጣም የሚለያዩ ሲሆኑ ከፍ ባለ መጠን ($1x10^8$ conidia ml-1) የተሞከሩት የመግደል አቅማቸዉ በትንሽ መጠን ($1x10^5$ conidia ml-1) ከተሞከሩት በልጦ ታይቷል። 9614 የተባለዉ የፈንገስ ኣይነት ከሁሉም አነስተኛ LC50 ማለትም 50% መግደል የቻለበት መጠኑ አነስተኛ ሲሆን፤ በ $1.9x10^5$ LC50 MM የሚባለዉ የፈንገስ ኣይነት በሁለተኝነት ተፈርጇል። በዚሁም መሰረት ቢቬሪያ ባሲያና 9614 እና የሜታሪሂዚየም ኣይነት MM በአጭር ጊዜ ዉስጥ ባለሁለት ነጠብጣብ ሸረሪት መሳይ ቅንቅኖችን በተፈጥሯዊ መንገድ ለመቆጣጠር ተሰፋ ሰጪ ሆነዉ ተገኝተዋል።

Abstract

The efficacy of six Beauveriabassiana and nine Metarhiziumanisopliae isolates of Ethiopian origin were bioassayed in the laboratory replicated 4 times for their lethal effects against the adult of Tetranychusurticae with the aim to incorporate as bio- agent component for IPM of the two spider mites. All the tested isolates were pathogenic tothe two spotted spider mite at $1x10^8$ conidia ml- concentration, but virulence ranged from 45.4% to 90.0% with LT_{50} from 3.19 to 11.81 days. Based on the extent of mortality, isolates PPRC 29, PPRC 27, PPRC 19, PPRC 66, and EE of Metarhizium; and GG and HH of Beauveria were moderately virulent (60-80%) and PPRC 2 &PPRC 61 of Metarhizium; and9615 & 9604 of Beauveria are weak (<60%). Four isolates (MM &PPRC 6 of Metarhizium; and 9614&9609 of Beauveria) which caused mortality ranges of 86% to 90%,were categorized as highly virulent and further evaluated at four different doses (1×10^5, 1×10^6, 1×10^7 and 1×10^8 ml^{-1}). The isolates inoculated at different concentrations significantly differed in their efficacy and the higher concentration (1×10^8 conidia ml^{-1}) caused higher mortality than the lower (1×10^5 conidia ml^{-1}). Isolate 9614 showed the least LC_{50} (1.37×10^5) followed by MM (1.9×10^5). Therefore the two isolates, B.

bassiana 9614 and M.anisopliae MM, which caused higher mortalities within shorter periods, were promising bioagents for the management of the two spotted spider mites.

Introduction

Spider mites belong to the family Tetranychidae of the order Acari. They are so named because many members of this family produce silk webbing on host plants. Most spider mite species are polyphagous (Miresmailli, 2005; Martin, 2000a). The Tetranychidae is a large family of worldwide distribution, which includes other plant-feeding mites and contains about 900 species worldwide (Martin, 2000a). The two-spotted spider mite *Tetranychusurticae* Koch is the most important species in this subfamily. They remove chlorophyll from plant cells that reduces photosynthesis and results in loss of plant vigor and quality (Gilrein, 2004; Kerns and Trostle, 2007).

Spider mites are among the most difficult pests to manage, and responsible for a significant portion of all pesticides used on ornamentals (Hort Report, 2001). Current management of *T. urticae* in Ethiopia heavily relies on the use of synthetic acaricides which is associated with ecological problems, effects on non-target organisms, development of acaricide resistance, and health risks to farmers and consumers. Red spider mites are known to exhibit resistance even after few applications of insecticides (Irigaray *et al*. 2002). The widely– occurring problem of pesticide resistance in mites justifies the need for alternative strategies for mite control (Cote, 2001). These factors, combined with concerns over environmental and human safety, have provided major techniques to minimize negative ecological impacts and other significant problems that can arise from extensive pesticide use. An integrated pest management (IPM) approach, where biological control agents play a major role in pest regulation, is increasingly advocated (Gouli et al., 2005). In response to the resistance, growers have increased their use of biological control by applying predatory phytoseiid mitessuch as*Phytoseiulus persimilis,*ladybird beetles (*Stethorus*species) and predatory midges (*Feltiella (= Theridoplosis) acarisuga*(Michaud et al., 2008).

The entomopathogenic fungus,*Beauveriabassiana* (Balsamo) Vuillemin, is widely distributed in nature and has the potential to control many insect pests. *Metarhiziumanisopliae*(Metschnikoff) Sorokinis another very important fungus known to control many species of plant pests (Bhattacharyya, 2004; Magalhães*et al*., 2005).However, studies of pathogenicity of entomopathogenic fungi on mites have received much less attention, despite their potential for use against large number of pest species (Irigarayet al. 2002).

Little is known about entomopathogenic fungi associated with the two spotted spider mites. The present study evaluated the virulence of different strains of *B.bassiana* and *M. anisopliae*which originated from Ethiopia against *T. urticae* and establish the dose response relationship between the concentration of potent fungal strains and the extent of mortality.

Materials and Methods

Fungal isolates

Fifteen different isolates of *B. ba*ssiana and *M. anisopliae* were supplied bythe Ethiopian Institute of Agricultural Research (EIAR), Plant Protection Research Center (PPRC),Ambo, Ethiopia. The isolates originated from different arthropods in different agro-ecological zones of Ethiopia, which were isolated and stored in PPRC laboratory as conidia in powder form at 4°C.

Table 1: Fungal isolates tested against the two spotted spider mites

Species	Isolate code	Location Collected	Origin/Host
	PPRC 29	Gobenayetu (N.Shoa)	*Pachnodainterrupta*
	PPRC 2	Ashan (N. Shoa)	*Pachnodainterrupta*
	PPRC 61	Awaketu (N. Shoa)	*Pachnodainterrupta*
	PPRC 66	Awaketu (N. Shoa)	*Pachnodainterrupta*
Metarhiziumanisopliae	PPRC 19	Rufe Kure (N. Shoa)	*Pachnodainterrupta*
	PPRC 6	Kewot (N. Shoa)	*Pachnodainterrupta*
	PPRC 27	Dedeaa (N. Shoa)	*Pachnodainterrupta*
	EE	Alamata (Tigray)	Crustacean (sow pill)
	MM	Arbaminch	From soil
	9615	Awassa	Spider (Arachnida)
	9614	Awassa	Ground beetle
Beauveriabassiana	9604	Bugae(Arbaminch road)	*Aceraeaacerata*
	9609	Mugundo (Dila road)	*Blosyrusrugulosus*
	HH	Ashengae (Tigray)	Coleoptera (Adult)
	GG	Ashengae (Tigray)	Coleoptera (Adult)

Source: PPRC/ EIAR, Ethiopia

Preparation of entomopathogenic fungal isolates

Conidia of the isolates for the experiment were from two weeks sporulating cultures on 0.25% weight by volume (w/v) Sabouraud's Dextrose Agar with Yeast extract (SDAY) media. The virulence of the isolates was maintained by passing them through the larvae of the wax moth, *Galleria mallonella* (L.). Conidial suspensions of the isolates were prepared using 0.01% Tween 80 after culturing the isolates on SDAY media. The suspensions were then adjusted to desired concentrations(1×10^5, 1×10^6, 1×10^7 or 1×10^8) ml^{-1} based on the counts of the conidia, in 1 ml of suspensions, made with an improved Neubauerhaemo-cytometer under compound microscope (40x magnifications).

The viability of each isolate was tested by germinating conidia on SDAY media. Suspension of the isolates prepared using 0.01 % Tween 80, at a concentration of 10^6 conidia in 100 µl was used as described in Goettel and Inglis (1997). A conidium was considered to have germinated if the germ tube was at least as long as the width of the conidium (Tadele and Pringle, 2004).

The two spotted spider mites were reared on beans (*Phaseolus vulgaris*) planted in pots.The pots with bean plants were placed in wire house table at photoperiod of 12: 12h light and dark and mean daily temperature measured using thermohygrograph was 26 °C.As indicated in many literatures, beans are the main host of the two spotted spider mites. Twenty-five uniform larger sized adults from the stock culture were transferred and placed on bean leaf discs in the petridishes. Each of the 16 treatments including the

control were replicated 4 times and laid in completely RandomizedDesign. Petridishes were sealed with parafilm and small holes were created on the lid with a hot needle for aeration. The petridishes were placed in growth chamber at 16: 8 (L: D) photoperiod and 25 ± 1°C temperature. Dead and live mites were separated under a dissecting microscope and counted starting 24 hours after treatment application for 10 consecutive days. The mortality data were corrected for the corresponding control mortality by the formula:

$$\%CM = \frac{(\%T - \%C)}{(100 - \%C)} * 100;$$

Where CM is corrected mortality, T is mortality in treated insects and C is mortality in untreated insects (Abbott, 1925). The LT_{50} (Time required to kill 50% of the treated mite population) was determined using probit analysis.

Screening of fungal isolates against two spotted spider mites

Nine isolates of *M. anisopliae* (EE, PPRC-2, PPRC-6, PPRC-66, PPRC-27, PPRC-29, PPRC-19, PPRC-61, MM), and six isolates of *B. bassiana* (9604, 9609, 9614, 9615, GG, HH)were evaluated for their efficacy in the laboratoryin November 2008. Suspensions of the isolates were adjusted to final concentration of 1×10^8 ml^{-1}. Half ml of each isolate suspension was used to spray on both sides of 5cm diameter bean leaf disc and allowed to dry for about 30 minutes at room temperature. The control group leaf discs were treated with 0.01 % Tween 80. The treated leaf discs were placed in sterile petridishesof 9 cm diameter.

Dose response bioassay

The four potent isolates (Metarhizium isolates MM and PPRC-6; and Beauveria isolates 9614 and 9609) from the screening experiment were further evaluated at four different doses (1×10^5, 1×10^6, 1×10^7 and 1×10^8 ml^{-1})with the same procedures described above. Lethal concentration of conidia required to kill 50% of the treated the mite population (LC_{50}) was determined byprobit analysis using SAS software.

Statistical analysis

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

Results and Discussion

Screening of fungal isolates against the two spotted spider mites

All the fifteen isolates tested were able to infect and kill the two spotted spider mites as confirmed by fungal growth (mycosis) on surface of cadavers. However, the effectiveness of isolates varied in the extent of mortality and time taken to kill the spider mites. There were significant differences in mortality at 4 days after treatment (F= 17.49, DF= 15,

P<0.001),6 days after treatment (F= 92.97, DF=15, P< 0.001), and 8 days after treatment (F=78.86, DF=15, p<0.001) (Table 2).

All fungal isolates reduced the population density of mites as compared to controls, which was treated only by 0.01% Tween 80.Based on the bioassay the isolates were placed in to three virulence categories; high, moderate and weak (Table 2). Isolates ofBeauveria9614, Beauveria9609, Metarhizium MM, and Metarhizium PPRC 6 caused the highest mortality with corrected mortalities of 90.01%, 90.01%, 86.52% and 86.11%, respectively, 8 days after treatment. Metarhizium isolates EE, PPRC-19, PPRC-27, PPRC-29, PPRC-66, and Beauveria isolates HH and GG were moderately virulent, causing mortality ranging from 60 to 72%. Isolates 9604, 9615, PPRC 2 and PPRC 61 were weakly virulent and caused mortality of 56.75%, 47.99%, 47.80% and 45.43%, respectively(Table 2).

Isolate 9614 and MM took3.19 and 3.68 days, respectively, to cause 50% mortality (Table 2) which is faster than the LT_{50} reported by Wekesa *et al.* (2005) of *Tetranychusevansi* by the most active isolates of *B bassiana* and *M. anisopliae* which *v*aried between 4.6 and 5.8 days. Similarly,Bugeme *et al*. (2008) reportedsignificant variations in lethal time to 50% mortality (LT_{50}) of adult females of *T. evansi*. In the current study the weakly virulent isolates PPRC 61, PPRC 2, 9615 and 9614 had longer LT_{50} of 11.81, 10.67, 8.56 and 8.24 days, respectively, compared to those categorized as highly virulent.

For all isolates cumulative mortality increased through time. Isolates of both *B. bassiana*and *M. anisopliae*(HH, GG, EE, PPRC-56, FF, PPRC-6 and MM) have been reported to have the potential as control agents in Ethiopia for different pests such as western flower thrips, sorghum chafer, leaf miners, desert locust, and root mealy bugs (Sinishaw, 2002; Mohammed, 2003;Brownbridge, 2003; Sisay,2008). The isolates tested in this study were not specific to their original host as none of them originated from spider mites, not even from order Acari except *B. bassiana* isolate 9615.

Table 2: Percentage mortality and LT_{50} of *T. urticae* 8 days after treatment with isolates of *M. anisopliae* and *B. bassiana* at the rate of $1x10^8$ conidia ml^{-1}

Fungal Species	Fungal isolates	Mortality ±S.E*	LT_{50} (days)	95% CI	Slope ±S.E	X^2	P-value	Virulence Category
B. bassiana	9614	90.01±0.0 ^{a}	3.19± 0.36	1.74-4.38	2.63±0.48	30.63	0.0001	High
	9609	86.52±4.3 ^{a}	5.23±0.33	4.25-5.95	4.04±0.62	32.26	0.0001	High
	HH	69.41±8.7^{bc}	7.67±0.31	6.90-8.29	5.58±0.87	35.83	0.0001	Moderate
	GG	64.08±5.2^{cde}	7.37±0.36	6.61-7.88	7.19±1.35	28.48	0.0001	Moderate
	9604	56.75±5.74^{e}	8.24±0.18	6.93-8.97	4.44±0.74	33.24	0.0001	Weak
	9615	47.80±4.35 ^{f}	8.56±0.28	7.85-8.93	11.06±2.9	13.26	0.0003	Weak
M. anispoliae	MM	90.01±0.0 ^{a}	3.68±0.43	1.78-5.20	2.62±0.56	21.78	0.0001	High
	PPRC-6	86.11±6.47^{a}	6.05±0.51	4.57-6.97	4.35±0.95	17.96	0.0001	High
	PPRC-29	71.63±1.5^{b}	7.33±0.37	4.40-9.09	2.41±0.68	10.88	0.0029	Moderate
	EE	68.43±7.2^{bc}	7.79±0.09	5.59-10.57	2.07±0.89	4.16	0.0213	Moderate
	PPRC-19	65.65±5.5^{bcd}	7.62±0.45	4.98-8.67	5.15±1.99	5.91	0.0151	Moderate
	PPRC-66	63.74±4.3^{cde}	7.79±0.55	6.29-8.49	7.12±2.16	9.65	0.0010	Moderate
	PPRC-27	60.77±2.4^{de}	8.52±0.45	5.90-9.48	5.62±2.37	5.40	0.0200	Moderate
	PPRC-2	48.00±3.4^{f}	10.67±0.33	9.55-51.47	5.66± 2.63	4.59	0.0322	Weak
	PPRC-61	45.43±2.5^{f}	11.81±0.31	9.10-24.70	2.12±0.88	6.74	0.0094	Weak
	Control	0±0.0^{g}						

*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 relationship between concentration of isolates and mortality

There were significant differences in mortality among isolates inoculated at different concentrations. Mortality of mites in the untreated control which were treated only with 0.01% Tween 80 was very low during the entire study period. The very little mortality of mites in the control groups could be due to the effect of sprayed water for which they are sensitive. The majority of the spider mites in the control group has actively fed on the leaf disc and was able to reproduce.

Mortality of spider mites was dose-dependent and increased with the increase in concentrations of all the tested isolates (Table 4). At the lower concentration, the isolate MM caused 40.8%mortality followed by 9614 (37%) and the remaining two isolates caused less than 30% mortality six days after treatment. The trends of mortality by the different strains were similar at eight days after the treatments application. However, mortalities by the four isolates, MM, 9614, PPRC 6 and 9609, increased and reached to90.0%, 90.0%, 90.0% and 82.4%, receptively, at the highest concentration of 1×10^8 conidia ml^{-1}. Similar results were reported on *T. urticae*with *B.bassiana* (Irigaray *et al*. 2002; Wekesa et al. 2006).

Lethal concentrations to kill 50 percent of the treated two spotted spider mites for the isolates 9614 and MM were 1.37×10^5 and 1.9×10^5,respectively. PPRC 6 had the highest LC_{50} (2.78×10^5) followed by 9609(2.29×10^5) (Table 3). The study showed that the isolates 9614 and MM are effective at lower concentrations.

Tadele and Pringle (2004) reported that *B.bassiana* isolate BB-01 and *M.anisopliae*isolate PPRC-4 at high conidia concentration of 1×10^8 ml^{-1} caused high mortality and reduction in food consumption by second and third instar larvae of *Chilopartellus*than the lower concentrations. Such types of dose dependent mortality were also observed on tsetse flies (*Glossina* spp.) by *B.bassiana* and *M.anisopliae*(Kaaya and Munyinyi 1995) and *Scolytusscolytus* larvae by *B.bassiana* (Barson, 1977).

These reports agree with the current study conducted as isolates with high concentration took shorter time to kill spider mites. Sinishaw (1998) similarly reported aslow median lethal time for the highest conidial concentration on *Schistocercagregaria*, and high mortality reaching to 100% was achieved at 1×10^8 conidia ml^{-1}. Shi et al. (2008) also showed that the LT_{50}s of fungal isolates to carmine spider mite was shortened at higher concentrations.

Table 3: LC_{50} of *M. anisopliae*(MM and PPRC-6) and *B. bassiana*(9609 and 9614) isolates.

Fungal Isolates	LC_{50}	95% Fiducial Limit	Slope (±SE)	X^2	P- value
9609	2.29×10^5	1.04×10^5- 4.19×10^5	0.62±0.07	77.33847	0.0001
9614	1.37×10^5	8.99×10^4 – 6.25×10^5	0.33±0.056	35.86762	0.0001
MM	1.9×10^5	8.21×10^4 – 2.8×10^5	0.51±0.071	51.24892	0.0001
PPRC-6	2.78×10^5	1.1×10^5 – 3.53×10^5	0.613±0.071	74.65067	0.0001

Table 4: Percent mortality of two spotted spider mites treated with different conidial concentrations (conidia ml^{-1}) of *B.bassiana* and *M. anisopliae*

Concentration (ml^{-1})	Mortality (±SE)* 6 days after treatment				Mortality (±SE)* 8 days after treatment			
	MM	9614	PPRC 6	9609	MM	9614	PPRC 6	9609
1×10^5	40.87±1.63^{h}	36.99±3.69^{i}	23.86±6.19^{k}	27.36±2.22^{jk}	61.99±3.311^{e}	54.16±3.29^{b}	50.38±1.63^{f}	49.15±3.69^{f}
1×10^6	68.02±2.63^{de}	62.00±4.07^{fg}	54.16±4.35^{hg}	49.15±7.16^{h}	72.06±1.41^{cd}	68.02±8.49^{b}	68.02±2.63^{de}	63.51±4.07^{e}
1×10^7	76.14±5.06^{cd}	72.46±2.63^{de}	72.06±4.11^{de}	65.65±3.30^{ef}	79.12±7.87^{b}	76.14±7.27^{b}	76.14±5.06^{bc}	72.46±2.63^{cd}
1×10^8	90.01±0.0^{a}	87.07±2.31^{ab}	82.36±3.11^{abc}	79.12±1.15^{bcd}	90.01±0.0^{a}	90.01±0.0^{a}	90.01±0.0^{a}	82.36±3.7^{b}
Control	0±0.0^{l}				0±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.

Conclusion and Recommendations

The two spotted spider mites have become a serious problem, especially on flowers growing under greenhouse conditions, because of the continuous use of pesticides resulting in resistance among mite population. On the other hand, the costof controlling mites with acaricides is very high.

Ethiopian isolates of *B.bassiana* and *M. anisopliae* tested in the present study caused higher levels of mortality and showed the potential for the management of the two spotted spider mites. The result indicated that all isolates were able to kill the two spotted spider mites which were significantly different from the control. However, there were significant differences in the virulence and LT_{50} among the different entomopathogenic fungi at the same conidial concentration (1×10^8 conidia ml^{-1}). Eight days after treatment, MM, 9614, 9609, PPRC 6caused the highest mortality of 90.01%, 90.01%, 86.52% and 86.11% and other isolates were categorized as intermediate and weakly virulent. Therefore, these isolates, especially highly virulent isolates can be included in the management of the two spotted spider mites.

Integrating the use of entomopathogenic fungi in the management of the two spotted spider mites is an alternative to the heavy use of and reliance only on synthetic pesticides especially, for such pest having so wide host range in different families of plants. It avoids resistance development among mite populations and creates a competitive market advantage by reducing the associated miticides costs. Moreover, entmopathogens are safe to the environment and the workers. However, further research works in techniques for mass production, appropriate formulation to keep the quality, large scale application are needed. The variations in virulence among the few Ethiopian entomopathogenic isolates against the two spotted spider mites strongly suggests the possibility of obtaining more potent isolates if screening is conducted on larger collections. Therefore 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 aim of developing virulent entomopathogen products which are economical for production and use.

Future studies need to focus on mass production of these isolates, evaluationsin the field and greenhouse, and their compatibility with other management options including biocontrol agents, for example predators and parasitoid and bio pesticides.

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