EFFICACY OF ENTOMOPATHOGENIC FUNGUS *BEAUVERIA BASSIANA* ISOLATES AGAINST THE TWO-SPOTTED SPIDER MITE, *TETRANYCHUS URTICAE* KOCH (ACARI: TETRANYCHIDAE)

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Abstract. A total of 17 isolates of entomopathogenic fungus (*Beauveria bassiana*) were tested against adult females of the two-spotted spider mite (*Tetranychus urticae* Koch) under laboratory conditions. Spore suspension was prepared from 15 days old culture of the isolates on PDA medium. The fungal surface was scraped using a sterile loop with 10 ml of sterile distilled water having 0.02% Tween 80. Spore concentration of the filtrate was determined using a Neubauer Hemocytometer. This served as a stock suspension. In the pathogenicity tests 5×10^6 conidia ml^{-1} was used. Inoculation was performed by spaying spor suspension directly on the adult female mites. Inoculated mites were transferred on bean leaf disks in sterile petri dishes and incubated under room conditions at 25 ± 2°C and 65 ± 5% RH for ten days. The mite sprayed with sterile distilled water having 0.02% Tween 80 were used as control. Mortality was noted daily. Dead mites were kept separately in humid sterile Petri dishes for another 10 days to determine the mycosis rate. Three isolates with higher mortality or mycosis rates (F-12, F-53, and F-56) were selected for dose-mortality tests. Spore suspension of the isolates at five different concentrations, 1×10^4, 1×10^5, 1×10^6, 1×10^7, and 1×10^8 conidia ml^{-1}, was prepared and tested for its efficacy on adult females of two-spotted spidermite. The experiments were carried out with 3 replications and 10 adults were used for each replicate. In single dose trial, all isolates caused mortality to *T. urticae* adults. Different mortalities resulting from different isolates of *B. bassiana* were observed with the concentration of 5×10^6 conidia ml^{-1} and varied from 32.5-72.5% at the end of 72 h incubation period. Mycosis ranged between 2.5% and 40.0%. In dose-mortality trial, isolate F53 caused the highest mortality percentage of *T. urticae* adults, followed by F-12 and F-56. The effectiveness of these isolates was not significantly different at the concentration of 1×10^8 conidia ml^{-1}. Concentration of conidia affected the mortality of the mites differently ($P < 0.05$). The results showed that mortality and mycosis were dose dependent which increased with enhancing the spore concentration of the isolates. Mortality ranged between 43.3% and 83% on isolate F-53 from 24 h to 72h incubation period at concentration of 1×10^8 conidia ml^{-1}. Followed by isolates F-12 and F-56 with 78.3% and 76.7% mortality rates, respectively. Maximum percent mycosis of 43.33% was recorded on 10th day after treatment with the concentration of 1×10^8 conidia ml^{-1}; in contrast to this minimum percent mortality of 3.33% was obtained by the application of the lowest concentration 1×10^4 conidia ml^{-1} at F-53 isolate. The present results demonstrated that the entomopathogenic fungi isolates of *B. bassiana* could be used as an alternative for the control *T. urticae*.

Keywords: *entomopathogen, Beauveria, biological control, mites*

Introduction

In the world's agricultural areas one of the cosmopolitan pest is the two-spotted spider mite, *Tetranychus urticae* Koch. It causes the reduction of yield by sucking the plant cell sap and affect the plantgrowth and yield of infested plants (Meyer, 1996). Acaricides application is the common control practice of *T. urticae* so far. Indiscriminate use of acaricides not only has caused the environmental pollution but

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also created resistance to the pesticide in mites (Ay and Gurkan, 2005; Gerson and Weintraub, 2012). Therefore resistance development to the acaricides and the concerns about public health and environmental issues have impelled the researchers for alternative control measures. In this context, incorporation of entomopathogenic fungi as biocontrol agents in *T. urticae* management program is being explored. Entomopathogens as biocontrol agents have several advantages, comparing with synthetic acaricides such as, low cost, high efficacy, safe to environment and benificial organisms (Lacey et al., 2001). So, various researchers have tested different entomopathogenic fungi including *Beauveria bassiana* against Tetranychid mites in *invitro and invivo* trials (Shi and Feng, 2004; Chandler et al., 2005; Shi et al., 2008 Ujian and Shahzad, 2007; Anand and Tiwary, 2009). The aims of all these studies were to develop myco-acaricides for use in spider mites control programs. There are few microbial pesticides have been developed for the control of *T. urticae* (Faria and Wraight, 2007) although most isolates tested have been pathogenic to *T. urticae* and provided different levels of control on its population (Irigaray et al., 2003; Wekesa et al., 2006).

The biocontrol potential of entomopathogenic fungi usually vary among fungal species and isolates. Therefore, most virulent fungal isolates against specific mite species can be identified and manipulated. The present study were therefore, aimed to evaluate potential use of the local entomopathogenic *B. bassiana* isolates for controlling of the two- spotted spider mite (*T. urticae*) in *in vitro* conditions.

Materials and methods

Two-spotted spider mite culture

Two-spotted spider mites (*Tetranychus urticae*) were obtained from a colony of a continuous culture in the entomology Laboratory, Faculty of Agriculture, Department of Plant Protection, Tokat, Turkey. The initial culture originated from two-spotted spider mite collected from Tokat district, Turkey in 2013. The two-spotted spider mite population was reared and maintained on garden bean (*Phaseolus vulgaris* L.) at 25 ± 2 °C and 65 ± 5% RH, and 16:8 (L:D) photoperiod. To obtain fixed-age females for the bioassays, quiescent deutonymphs were collected from the two-spotted spider mite cultures and put on leaf discs. The newly emerged females were used for the experiments (Wekesa et al., 2006).

Fungal isolates, culturing and preparation of conidial suspension

Seventeen *B. bassiana* isolates were obtained from Tokat Gaziosmanpasa University, Department of Plant Protection culture collections. The virulence of isolates were tested on *T. urticae* female adults. The fungal isolates used in this study were obtained from soil in Kelkit Valley in Middle Black Sea Region, Turkey. *Beauvaria bassiana* isolates were identified morphologically and molecularly and used in previous studies (Yanar et al., 2014; Kepenekçi et al., 2017). Fungi were grown for 4 weeks at 25 ± 2 °C on Sabouraud Dextrose Agar (SDA) (Difco) under natural light. Conidia were harvested by surface scraping 15-day-old culture plates. Subsequently the spore suspension was filtered through several layers of cheesecloth to remove mycelium. Inocula were suspended in 10-mL sterile distilled water containing 0.02% Tween 80 in 50 ml falcon tube. The concentration of conidia in the sample suspensions was determined using a

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Neubauer Haemocytometer and adjusted to the final concentration required in the experiment by diluting the conidial suspension with 0.02% Tween 80 in water. Viability of conidia was determined before each bioassay by spread plating 0.1 mL of conidial suspension titrated at 5×10^6 conidia mL^{-1} on SDA plates. Sterile microscope cover slips were placed on each plate and plates were incubated at 26 ± 2 °C and examined after 15–18 h. Germination percentage was determined from 100 spore counts at 40× magnification.

Single-dose screening bioassay

Total of 17 *Beauveria bassiana* isolates were screened against *Tetranychus urticae* under laboratory conditions. Conidial suspension (300 µl) of each isolate (5×10^6 conidia /ml) with 0.02% Tween 80 solution were sprayed directly on 10 adult mites in 1.5 ml plastic cage by using a hand sprayer. One day after sprayed inoculation, the mites were transferred on 25 mm diameter bean leaf disc placed on moist cotton in the 90 mm in diameter glass petri dish using a single hair brush. The petri dishes were placed in an incubator at 25 ± 2 °C, 65 ± 5% RH, and 16:8 (L:D) photoperiod. Each treatment was replicated 3 times. The experiments were repeated two times. Control was sprayed with 300 µl of sterile distilled water containing 0.02% Tween 80. Two-spotted spider mites were checked daily for mortality, and all cadavers were placed at >90% relative humidity in an incubator at 25 ± 2 °C, 65 ± 5% RH until condition was observed. Mycosis was confirmed by microscopic examination of the hyphae and spores on the surface of the cadavers.

Dose-mortality bioassays

Three isolates exhibited intense sporulation, higher mortality or mycosis rates in single dose bioassays were used in this test. Conidia of these isolates were prepared as mentioned above and the conidia viability was also assessed before application. Five different concentrations (1×10^4, 1×10^5, 1×10^6, 1×10^7, 1×10^8 conidia ml^{-1}) of conidial suspension in 0.02% Tween 80 solution were prepared for each isolates and sprayed on the mites as mentioned above and 0.02% Tween 80 solution was used as a negative control. All the experimental procedure remained the same as described earlier.

Data analysis

Data were corrected for mortality in the control using Abbott's Formula (Abbott, 1925). The percent mortality of each fungus was subjected to ANOVA test and the means were compared by the Tukey's test, using SPSS 17.0 software program (SPSS, 2008). Dose mortality data was analyzed using POLO_PC (Leora, 1994) probit and logit analysis software according to Finney (1971). All calculations of half maximal lethal concentration (LC_{50}) values, lethal concentration ratios and the tests of the equality and parallelism of slopes were carried out with POLO-PC program.

Results

In sigle dose trial, among the 17 *B. bassiana* isolates tested, few of them were highly pathogenic to *T. urticae* adults, but the mortalities of mites caused by most of them were significantly higher than that of the negative control at the end of 72 h post-inoculation ($P < 0.05$). The mortality level of the control in this study was low which indicated that

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the mortality due to fungal infection. The maximum mortality in the control was 6.7% at the end of 72 h incubation period. All of the isolates were pathogenic to *T. urticae*. After 72 h, percentage mortality at 5×10^6 conidia ml^{-1} varied from 32.5% to 72.5% (*Table 1*). Isolate F-56 showed the highest mortality (72.5 ± 4.79%), followed by the isolates F-53 (70.0 ± 5.77%) and F-12 (68.8 ± 4.34%) at the dose of 5×10^6 conidia mL^{-1} (*Table 1*).

Table 1. *Efficacy of entomopathogenic fungal isolates (5×10^6 conidia mL^{-1}) on Tetranychus urticae*

Treatments	24 h	48 h	72 h
	Mortality %		
Control	6.7±1.67* a	6.7±3.33 a	6.7±3.89 a
F-2	27.5±2.50 b	28.8±2.26 b	32.5±5.59 b
F-4	34.3±5.71 b	40.0±8.73 bc	50.0±6.55 bc
F-7	32.9± 4.74 b	34.3±4.28 b	58.6±7.38 bc
F-12	55.6±7.09 c	60.0±6.01 c	68.8±4.34 d
F-15	28.8± 3.98 b	47.5±4.53 bc	61.3±6.10 c
F-42	32.5± 3.13 b	42.5±7.26 bc	57.5±8.40 bc
F-53	40.0± 3.33 bc	53.3±8.82 bc	70.0±5.77 d
F-56	44.0 ±4.27 bc	49.0±7.37 bc	72.5±4.79 d
F-73	22.5 ±2.50 b	55.0±9.57 bc	63.0±5.17 c
F-76	26.7 ±6.67 b	46.7±8.82 bc	50'0±5.78 bc
F-97	32.5± 4.79b	47.5±8.54 bc	62.5±4.79 c
F-108	30.0 ±2.67 b	37.5±3.66 bc	61.3±6.39 c
F-123	26.7 ±2.11 b	35.0±3.41 ab	43.3±6.15 b
F-127	46.7± 3.33 bc	60±5.77 bc	56.7±3.33 bc
F-145	28.9± 3.51b	43.33±5.53 bc	64.4±4.44 c
F-156	36.7± 3.33 b	56.7±8.82 bc	57.3±8.82 bc
F-159	45.6± 5.30 bc	47.4±4.75 bc	55.4±7.66 b

*The data are presented as the mean ± SE. The same letters in the same column represent no significant differences between the groups at the $P < 0.05$ level by Tukey's HSDTest

The percentages of mycosed mite were 40% for isolate F-73, 11.25% for isolate F-56, and 5% for isolate F-53 and F-12 at 5×10^6 conidia concentration ml^{-1} single dose treatment (*Fig. 1*).

Dose response studies were conducted with effective isolates F-53, F-56 and F-12. In dose studies with F-53, F-56 and F-12 mortality percentages of *T. urticae* adult at 72 h post-inoculation varied between the different concentrations within the isolates ($P < 0.05$). Almost similar mortalities resulting from different isolates of *B. bassiana* were observed with the dose of 1×10^7 conidia mL^{-1} (*Tables 2, 3* and *4*). Isolate F-53 obviously caused the highest mortality of *T. urticae*, followed by isolate F-12 at the end of 72 h incubation with the dose of 1×10^8 conidia mL^{-1} (*Tables 2* and *4*). However, the effectiveness of isolates F-53, F-12 and F-56 were not significantly different at a dose of 1×10^8 conidia ml-1. Also, mortalities of *T. urticae* adults resulting from the two highest doses (1×10^7 and 1×10^8 conidia mL-1) for each of the isolates, were significantly

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different ($P < 0.05$) (*Tables 2*, *3* and *4*). The mortality of control was significantly less than the each dose of the isolates tested ($P < 0.05$) (*Tables 2* and *3*).

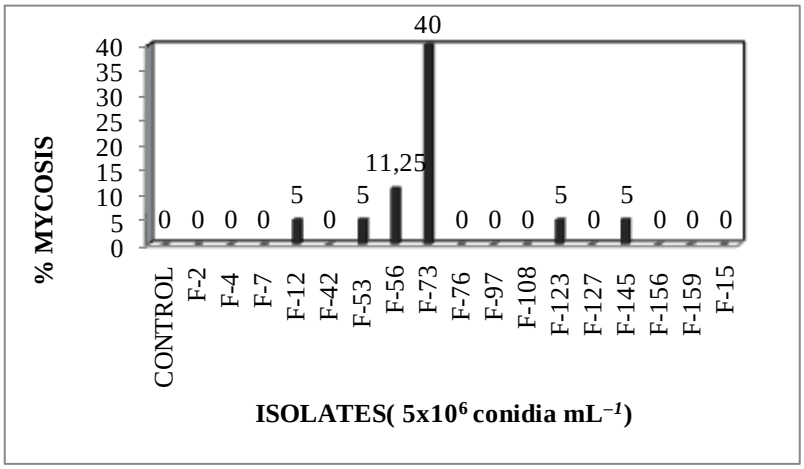

Figure 1. *Mycosis rates of entomopathogenic fungal isolates (5×10^6 conidia mL^{-1}) on Tetranychus urticae*

Table 2. *Efficacy of F-53, F-56 and F-12 entomopathogenic fungal isolates dose treatment on Tetranychus urticae*

Treatments	24 h	48 h	72 h
	Mortality %		
Control	6.67±3.33*a	6.67±3.33a	6.67±3.33a
F-53 $0.01X10^6$	23.33±3.33abc	30.00±5.77bcde	30.00±5.77bc
F-53 $0.1X10^6$	23.33±3.33abc	33.33±3.33bcde	33.33±3.33bcd
F-53 $1X10^6$	23.33±3.33abc	26.67±3.33abcd	33.33±3.33bcd
F-53 $1X10^7$	43.33±3.33d	50.00±0.00e	53.33±3.33d
F-53 $1X10^8$	43.33±3.33d	50.00±0.00e	53.33±3.33d
F-56 $0.01X\ 10^6$	16.67±3.33ab	20.00±0.00abc	30.00±0.00bc
F-56 $0.1X10^6$	23.33±3.33abc	23.33±3.33abc	30.00±0.00bc
F-56 $1X10^6$	33.33±3.33bc	36.67±6.67bcde	43.33±3.33cd
F-56 $1X10^7$	36.67±3.33cd	40.00±5.77cde	43.33±3.33cd
F-56 $1X10^8$	36.67±3.33cd	46.67±3.33de	46.67±3.33cd
F-12 $0.01X10^6$	16.67±3.33ab	16.67±3.33ab	16.67±3.33ab
F-12 $0.1X10^6$	16.67±3.33ab	26.67±3.33abcd	30.00±5.77bc
F-12 $1X10^6$	26.67±3.33bcd	33.33±6.67bcde	43.33±6.67cd
F-12 $1X10^7$	26.67±3.33bcd	33.33±6.67bcde	43.33±6.67cd
F-12 $1X10^8$	30.00±0.00bcd	36.67±3.33bcde	43.33±3.33cd

*The data are presented as the mean ± SE. The same letters in the same column represent no significant differences between the groups at the $P < 0.05$ level by Tukey's HSDTest

The percentage of mycosed mite was between 3.33 and 43.33% for isolate F-53. It was between 8.33 and 40% for isolate F-56, and between 5 and 23.33% for isolate F-12.

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For all the isolates tested, mycosis rates were higher at 1×10^8 conidia concentration mL^{-1} (*Table 3*).

Table 3. *Mycosis rates of F-53, F-56 and F-12 entomopathogenic fungal isolates different doses on Tetranychus urticae*

Treatments	Dozlar conidia mL^{-1}				
	0.01×10^6	0.1×10^6	1×10^6	1×10^7	1×10^8
	Mycosis %				
F-12	5*a	15b	18.33b	18.33a	23.33a
F-53	3.33a	10a	10a	40c	43.33b
F-56	16.67b	16.67b	16.67b	30b	40b

The same letters in the same column represent no significant differences between the groups at the $P < 0.05$ level by Tukey's HSDTest

For 72 h mortality rates were evaluated for LC_{50} values with POLO PC program. Results showed that LC_{50} values of F-12, F-53 and F-56 were 9.12×10^7, 6.6×10^7 and 4.3×10^7 respectively. LC_{50} values fiducial limits (95%) of F-12, F-53 and F-56 were2.49-42.16, 1.89-27.65 and 1.4-9.25 respectively (*Table 4*).

Table 4. *LC_{50} values and fiducial limits of different ontomopatogenic Beauvaria bassiana isolates for two-spotted spider mite at the end of 72 h*

Beauvaria bassiana isolates	LC_{50} values	Fiducial limits for LC_{50}	Slope±SE	Chi-square
F-12	9.12×10^7	2.49-42.16	0.49±0.04	211.36
F-53	6.6×10^7	1.89-27.65	0.36±0.04	124.61
F-56	4.3×10^7	1.4-9.25	0.37±0.04	127.38

Discussion

Control programmes of spider mites are aimed at maintaining the *Tetranychus spp.* population at low levels throughout the growing season. Therefore, the main target in integrated spider mite management could be the adult female mites. The studies indicated that the acaricidal activities of most of the entomopathogenic fungi towards *T. urticae* can be attributed to disruption of mite development through their penetration and subsequent nutrient uptake (Zhang et al., 2014; Shi and Feng, 2009). The results were presented and discussed below in the light of earlier studies. In single dose trial, all the tested *B. bassiana* isolates pathogenic to *T. urticae* adults and the mortalities of mites caused by them were significantly higher than that of the negative control at the end of 72 h post-inoculation ($P < 0.05$). The mortality rate of the control was low (6.67%) at the end of 72 h incubation period which indicated that the mortality due to fungal infection. The percentage mortality at 5×10^6 conidia mL^{-1} varied from 32.5% to 72.5% after 72 h. Isolate F-56 showed the highest mortality (72.5 ± 4.79%), followed by the isolates F-53 (70.0 ± 5.77%) and F-12 (68.8 ± 4.34%) at the dose of 5×10^6 conidia ml^{-1} (*Table 1*). Also, sporulations of these isolates were higher on PDA media at the end of 10 days incubation period. The results obtained in the present study have similarity with those of other scientists that used entomopathogen fungi against mites (Wekesa, et al., 2006; Fayyadh et al., 2005; Slavimira and Simova, 2010; Gatarayiha et al., 2012).

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Gatarayiha et al. (2012) reported that mortality caused by 62 *B. bassiana* isolates on *T. urticae* adults ranged between 0.5 and 92.8%; and 23 isolates resulted in more than %50 mortality. The variation in virulence among the different fungal isolates to *T. urticae* may be associated with the enzymes produced by each isolate. De La Rosa et al. (1997) suggested that the differences in virulence of entomopathogenic fungal isolates are probably associated with the presence of enzymes that influence the penetration process of the fungus. Secondary metabolites produced by entomopathogen fungi for example, toxins such as beauvericin, present in *B. bassiana*, which vary in could also contribute to the observed variation in virulence (Roberts and St. Leger, 2004). At 5×10^6 conidia concentration mL^{-1} single dose treatment, the mycosis rates were 40, 11.25, 5, and 5% for isolates F-70, F-56, F53, and F-12, respectively (*Fig. 1*).

Dose response studies were made with effective isolates F-53, F-56 and F-12. Isolate F-53 obviously caused the highest mortality of *T. urticae*, followed by isolate F-12 at the end of 72 h incubation with the dose of 1×10^8 conidia mL^{-1} (*Tables 2* and *4*). However, the effectiveness of isolates F53, F-12 and F-56 were not significantly different at a dose of 1×10^8 conidia ml-1. Also, mortalities of *T. urticae* adults resulting from the two highest doses (1×10^7 and 1×10^8 conidia mL^{-1}) for each of the isolates, were significantly different ($P < 0.05$) (*Tables 2, 3* and *4*). The mortality of control was significantly less than the each dose of the isolates tested ($P < 0.05$; *Tables 2* and *3*). The results observed in present study are in accordance with Gatarayiha et al. (2012) in which different strains of *B. bassiana* were used against *T. urticae* adults at different concentrations. The highest concentration (1×10^8 spores mL^{-1}) showed the maximum mortality percentage on *T. urticae*. Similarly, Oliveira et al. (2002) worked with *B. bassiana* isolates at 1×10^8 conidia ml-1 and the red mite *Oligonychus yothersi*, recorded 77.00% to 98.00% mortality. All tested fungi proved to be pathogenic to *T. urticae* adults. A similar level of control by *B. bassiana* isolates against tetranychid mites in laboratory trials was observed by Barreto et al. (2004) and Wekesa et al. (2006).

Results showed that LC_{50} values of F-12, F-53 and F-56 were 9.12×10^7, 6.6×10^7 and 4.3×10^7 respectively. LC_{50} values fiducial limits (95%) of F-12, F-53 and F-56 were 2.49-42.16, 1.89-27.65 and 1.4-9.25 respectively (*Table 4*). Furthermore, the dose-mortality studies suggested that *B. bassiana* isolate F-56 is efficient in controlling the adult mites as having the lowest LC50 (4.3×10^7 conidia/mL^{-1}) value showing the most virulent isolate among all tested isolates. These results are in conformity with those of Shi and Feng (2009) who used *B. bassiana, P. fumosoroseus* and *M. anisopliae* isolates and got 73.1, 75.4 and 67.9% mortality of mites after 10 days of spraying.

Conclusion

In conclusion, the two-spotted spider mite (*T. urticae)* was found to be susceptible to the isolates F-12, F-53 and F-56 of the entomopathogenic fungus *B. bassiana* at two higher doses (1×10^7 and 1×10^8 conidia mL^{-1}). Further studies will be conducted to determine the efficacy of these isolates under greenhouse conditions.

Conflict of interests. The authors declare no conflict of interests.

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