Ziraat Fakültesi Dergisi 14 (1):83-88, 2019
ISSN 1304-9984, e-ISSN 2687-3419 Araştırma Makalesi

Acaricidal activity of *Fusarium subglutinans* 12A on *Tetranychus urticae* Koch (Acari: Tetranychidae

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The arrival date:01.03.2019, Acceptance date: 28.05.2019

Abstract: In this study, efficacy of different spore concentrations of *Fusarium subglutinans* 12A isolate on *Tetranychus urticae* Koch females was investigated. The experimental design was a complete randomized block and all trials were conducted in five replications. In the study, $1x10^4$, $1x10^6$ and $1x10^8$ spores/ml spore concentrations were applied to shell bean leaves that were prepared according to leaf disc method spraying in droplets at 1 atm pressure. Observations on mortality of females and also mycosis developing on dead individuals were conducted on the 3rd, 5th, and 7th days after application. According to the study results, mortality rates were higher than control at three spore concentrations, but they did not differ from each other (F 44,239; df 3; P> 0.05). Mycosis were not significant at three spore concentrations (F 2,387; df 2; P> 0.05). Moreover, it was determined that the time-dependent mortality rate after application of *Fusarium subglutinans* 12A isolate was the highest on the 7th day at all spore concentrations.

Keywords: Biological control, enthomopathogen fungi, two-spotted spider mite

Tetranychus urticae Koch (Acari: Tetranychidae) üzerinde *Fusarium subglutinans* 12A'nın akarisidal aktivitesi

Özet: Bu çalışmada, *Fusarium subglutinans* 12A izolatının farklı spor konsantrasyonlarının *Tetranychus urticae* Koch dişi bireyleri üzerindeki etkililiği araştırılmıştır. Denemeler, tesadüf parselleri deneme desenine göre 5 tekerrürlü olarak yürütülmüştür. Çalışmada, $1x10^4$, $1x10^6$ ve $1x10^8$ spor/ml olarak hazırlanan spor konsantrasyonları, 1 atm basınç altında püskürtme yapılarak yaprak disk yöntemine göre hazırlanan fasulye yapraklarına uygulanmıştır. Dişi bireylerde gerçekleşen ölüm oranları ve ölü bireylerdeki mikozis gelişimine ait gözlemler, uygulamadan sonraki 3., 5. ve 7. günlerde yapılmıştır. Çalışma sonuçlarına göre, uygulamalar sonrası belirlenen ölüm oranının her üç spor konsantrasyonunda da kontrolden istatistiki olarak farklı olduğu ancak, birbirleri arasında fark olmadığı belirlenmiştir (F 44,239; df 3; P> 0.05). Mikozis gelişim oranı için her üç spor konsantrasyonu arasındaki farkın istatistiki olarak önemli olmadığı bulunmuştur (F 2,387; df 2; P> 0.05). Ayrıca, zamana bağlı gerçekleşen ölüm oranının her bir spor konsantrasyonunda da en yüksek 7. günde gerçekleştiği saptanmıştır.

Anahtar kelimeler: Biyolojik kontrol, entomopatojen fungus, iki noktalı kırmızı örümcek

Introduction

Tetranychus urticae Koch (Acari: Tetranychidae) is polyphagous and one of the pests causing important economic losses worldwide (Leeuwen et al., 2006). It causes damage to agricultural crops by feeding on the plant tissues and transmitting plant viruses (Huffaker et al., 1969). The population density of two-spotted spider mite can increase rapidly at appropriate climatic conditions and host plant existence. Eggs and other stages are not affected by acaricide applications due to pest's intensive web on the plant when the adults begin to feed. These features make the pest difficult to control (Susurluk, 2008).

This research was funded by TUBITAK 2209-A program

One of the most important problems in the control of *T. urticae* is acaricide resistance. The first resistance problem in chemical management was determined against the organic phosphorous insecticides in the European and American greenhouses at the beginning of 1950's (Jeppson et al., 1975; Cranham and Helle, 1985) and it has developed resistance to most of the pesticides in recent years (Leeuwen and Tirry, 2007). Moreover, these pesticides are also known to have other adverse effects such as effects on non-target organisms, contamination in groundwater and residue in products. Alternative control methods have been investigated and prefered due to these reasons. Entomopathogenic fungi are preferred in control of pests due to several reasons such as host specificity, ease of mass production and minimal effect on non-target organisms (Shahid et al., 2012). It is known that spider mites are infected by entomopathogenic fungi that belong to the order Entomophthorales. Numerous studies that related to efficacy of entomopathogenic fungi against different developmental stages of the two spotted spider mite have been studied. In this studies, it was determined that most entomopathogenic fungi that were used caused mortality on *T. urticae* (Chandler et al., 2005; Doğan et al., 2017; Zhang et al., 2016; Maniania et al., 2008; Saranya et al., 2013; Draganova and Simova, 2010). Entomopathogenic fungus *Fusarium subglutinans* (Ascomycota: Nectriaceae) was isolated previously from different aphid species (Gerin, 1998; Erkılıç et al., 1999; Satar et al., 2000). In this study, the lethal effect of three different spore concentrations ($1x10^4$, $1x10^6$ and $1x10^8$ spores/ml) of *F. subglutinans* 12A isolated from *Aphis gossypii* Glover in Adana-Karataş were investigated on adult females of *T. urticae*.

Materials and Method

The main materials of the study are new adult female individuals of *T. urticae*, 12A isolate of *F. subglutinans* ($1x10^4$, $1x10^6$ and $1x10^8$ spores/ml). The plant used in this study was shell beans. Other materials were laboratory tools used in their production and entomopathogenic fungus application. *F. subglutinans* 12A was obtained from *A. gossypii* in cotton fields of Adana-Karataş. Adult females of *T. urticae* which were obtained from colonies in climate-controlled rooms (25±1°C, 60-70% RH and 16:8 h D:L) were used in the experiments.

In the study, for preparation of *F. subglutinans* spore concentration, the fungus was grown in petri dishes on potato dextrose agar at 25°C for 7 days. After 7 days, 5 ml of sterile water was added to each petri dish to collect the spores. The spore suspension in sterile distilled water was adjusted to a concentration of $1x10^4$, $1x10^6$ and $1x10^8$ spores/ml after spore count using a haemocytometer. In the control, distilled water was used. The experimental design was a complete randomized block design and all trials were conducted in five replications. The experiment was conducted in glass petri dishes (9 cm diameter) containing shell bean leaf discs (5 cm diameter) on filter papers, to feed the individuals. In each petri treatment, 10 newly emerged adult females were used. The Petri dishes were kept in climate-controlled rooms (25±1°C, 60-70% RH, and 16: 8 h D: L). Before the treatment, female individuals were deprived for food for 1 hour. In the study, spore suspensions at $1x10^4$, $1x10^6$ and $1x10^8$ spores/ml were applied to shell bean leaves prepared according female individuals to leaf disc method spraying in droplets at 1 atm pressure. Observations on mortality of females and mycosis developing on dead individuals were conducted on the 3rd, 5th and 7th days after application. Obtained data from were analyzed using Tukey's test after One-way ANOVA. For statistical analyses, IBM® SPSS® Statistics (Version 20.0, IBM Corp., Armonk, NY, USA.) was used. As for significance level, $P< 0.05$ was accepted as statistically significant.

Results

In this study, lethal effect of different spore concentrations of *F. subglutinans* 12A on *T. urticae* females was investigated. Mean mortality rates were different than control in three spore concentrations, but

they did not differ from each other (F 44,239; df 3; P> 0.05). Besides that, mycosis rates were not significantly different at three spore concentrations (F 2,387; df 2; P> 0.05) (Table 1).

Table 1. Mean mortality and mycosis rates of *Tetranychus urticae* at different spore concentrations of *Fusarium subglutinans* 12A

Treatments	Number of individuals	Group averages for mortality rate (%) ±Standard Error	Group averages for mycosis rate (%) ±Standard Error
10^4 spores/ml	50	2.4098± 0,094 a	2.2667± 0,371 a
10^6 spores/ml	50	2.3158± 0,086 a	2.0000± 0,323 a
10^8 spores/ml	50	2.4206± 0,100 a	3.1333± 0,445 a
Control	50	1.0259± 0,123 b	

Means with different letter in the same column differ significantly

The time-dependent mean mortality rates after application of entomopathogenic fungus were highest at 7th days at 3 different spore concentrations and 7th day mean mortality rates were found same at 10^6 spores/ml and 10^8 spores/ml (P> 0.05) (Table 2).

Table 2. Time-dependent mean mortality rates of *Tetranychus urticae* treated with *Fusarium subglutinans* 12A

Time	Number of individuals	Spore Concentrations		
		10^4 spores/ml Mean mortality rates (%)	10^6 spores/ml Mean mortality rates (%)	10^8 spores/ml Mean mortality rates (%)
3rd day	50	4.20b	4.00b	4.60b
5th day	50	5.00b	6.20a	5.80ab
7th day	50	7.20a	7.60a	7.60a

Means with different letter in the same column differ significantly

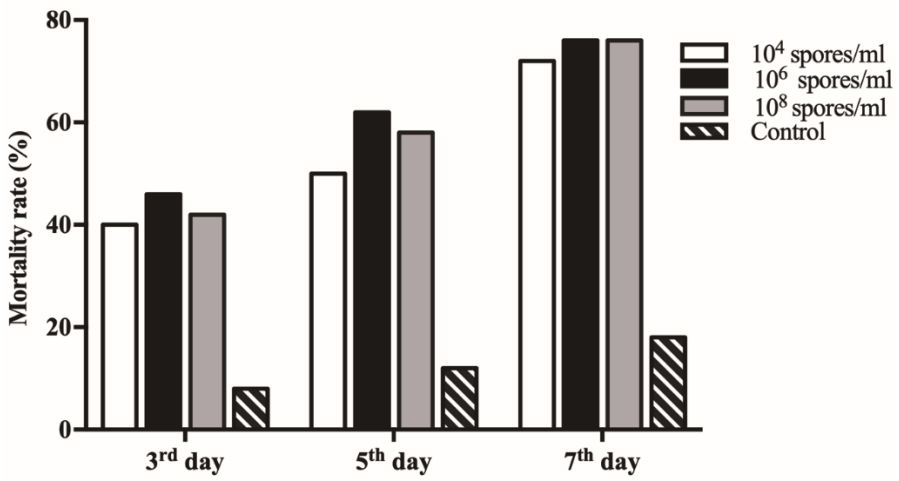

Figure 1. Mortality rates of *Tetranychus urticae* at different spore concentrations of *Fusarium subglutinans* 12A

Mortality rate varied between 72-76% on the 7th day of the study at three spore concentrations of *F. subglutinans* 12A. The highest mortality rates was recorded as 46%, 62%, 76% at 10^6 spores/ml on the 3rd, 5th, 7th day, respectively. The highest mycosis rates was 11%, 15%, 21% at 10^8 spores/ml on the 3rd, 5th, 7th day, respectively. Mortality rate reached 18% on the 7th day in the control unit.

Discussion

In previous studies efficacy of different entomopathogenic fungi were investigated on *T. urticae,* which is an important pest in agricultural production. Draganova and Simova (2010) determined that isolates 426 Bb, 444 Bb and 445 Bb of the entomopathogenic fungus *Beauveria bassiana* (Bals.-Criv.) Vuillemin (Deuteromycotina: Hyphomycetes) were highly virulent against the two-spotted spider mite. Saranya et al. (2013) also found that the isolate Bb101 of *B. bassiana* was highly virulent to *T. urticae* amongst nine different fungal isolates of *B. bassiana*, *Metarhizium anisopliae* (Metschnikoff) Sorokin (Ascomycota*:* Sordariomycetes), *Isaria fumosoroseus* (Deuteromycotina: Hyphomycetes), *Hirsutella thompsonii* Fisher (Ascomycota: Sordariomycetes) and *Cladosporium cladosporioides* (Fresen.) G.A. de Vries (Ascomycota: Dothideomycetes). Chandler et al. (2005) reported that *M. anisopliae*, *Hirsutella* spp. and *Lecanicillium* (*Verticillium*) *lecanii* (Zimm.) Zare & Gams (Sordariomycetes: Hypocreales) could cause significant mortalities when compared with the control treatment. Additionally, it was reported that mortality effects of these microorganisms could vary depending on plant species and environment (greenhouse, open field conditions or laboratory). Similar findings were reported for the fungus that was used in the present study. $1x10^7$ spores/ml concentration of *F. subglutinans* caused 16% and 45.5% mortality on *A. gossypii* fed on cotton and eggplant, respectively, and 12.9% on *Myzus persicae* (Hemiptera: Aphididae) fed on eggplant (Satar et al., 2000). Moreover, it is known that concentration of conidia and time between application and evaluation also affect mortalities of pests. Different mortalities were reported at different conidium concentrations and different post-treatment times using *B. bassiana* on *T. urticae*. The mortality was 4.06% three days after application of 1×10^4 conidia/ml, while it was 82.81% eight days after application of 1×10^8 conidia/ml of *B. bassiana* EUT116 (Seiedy et al., 2010). Additionaly, entomopathogenic fungi can be effective on early life stages of *T. urticae*. Zhang et al. (2016) reported that, entomopathogenic fungus *Isaria cateniannulata* (isolate 08XS-1) caused 100% death on eggs and larvae while it was 70% on adults 10 days after application. Likewise, 70% death was reported for *Paecilomyces fumosoroseus* (Deuteromycotina: Hyphomycetes) and *M. anisopliae* on adult females of *T. urticae* (Shi et al., 2009). Moreover, Doğan et al. (2017) found that adult mortality was >80% for *M. brunneum* (strains ARSEF 4556 and V275), *L. lecanii* UPH-0241 and *B. bassiana* UPH-1103 while *M. flavoviride* UPH-0288 caused 67% mortality on *T. urticae*. The present study has also significant results about *F. subglutinans* 12A isolate on two-spotted spider mite. According to our results, mean mortality rates were significantly higher than control ($F_{44,239}$; df 3; $P < 0.05$) and the treatment death rates did not differ from each other at all the spore concentrations ($F_{44,239}$; df 3; $P > 0.05$). Similar results were reported on adult females of *F. occidentalis* where death rates did not differ at all tested spore concentrations ($P > 0.05$) (Demirözer et al., 2016).

Conclusion

In this study, effects of different spore concentrations of *F. subglutinans* 12A on *T. urticae* females was investigated. It was found that mean mortality rates

were higher than control at all three spore concentrations and mean mortality rates of fungus treated mites did not differ from each other (F 44,239; df 3; P> 0.05). Mortality and mycosis rates were recorded as 76% and 21% at 10^8 spores/ml of *F. subglutinans* 12A on the 7^{th} day. It should be considered that remarkable effects of *F. subglutinans* 12A on different pest species would be advantageous in pest control studies. Moreover, mycosis observed three days after the first spore application would increase the effect on the pest population. Obtained results in our study show that *F. subglutinans* 12A has promising results on pest control but detailed studies needed in different production conditions such greenhouse and open fields.

Acknowledgement

We thank to TUBİTAK 2209-A programme for the financial support. We thank to Alican YELKEN and Ali Rıza ÇELİK for their contributions in this study.

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