http://dergipark.gov.tr/trkjnat
Trakya University Journal of Natural Sciences, 18(1): 33-38, 2017
ISSN 2147-0294, e-ISSN 2528-9691
DOI: 10.23902/trkjnat.285656

Research Article/Araştırma Makalesi

DETERMINATION OF CONTROL POTENTIALS AND ENZYME ACTIVITIES OF *Beauveria bassiana* (BALS.) VULL. ISOLATES AGAINST *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE)

Serkan ÖRTÜCÜ[1*], Nurcan ALBAYRAK İSKENDER[2]

[1]Department of Molecular Biology and Genetics, Faculty of Science, Erzurum Technical University, Erzurum
[2] Department of Nursing, Faculty of Health Sciences, Artvin Coruh University, Artvin
*Corresponding author: e-mail: serkan.ortucu@erzurum.edu.tr

Received (Alınış): 12 January 2017, Accepted (Kabul): 15 March 2017, Online First (Erken Görünüm): 8 April 2017, Published (Basım): 15 June 2017

Abstract: Broad host range of entomopathogenic fungi may have a commercial advantage for biological control industry. In addition, all entomopathogenic fungi rely on a combination of different enzymes to penetrate their hosts and these enzymes play an important role in the host range of fungal isolates. The aim of this study is to determine the potential of three *Beauveria bassiana* (Bals.) Vull. isolates in control of *Tetranychus urticae* Koch (Acari: Tetranychidae) and to determine their lipase, chitinase, and protease activities. For this purpose, *B. bassiana* (PaF04, PaF09 and PaF76) isolates, which was previously demonstrated to have entomopathogenic activity against Coleoptera, Lepidoptera, and Hymenoptera were chosen. *T. urticae* was used as the test organisms. Three different suspensions ($1x10^6$, $1x10^7$ and $1x10^8$ conidia ml^{-1}) were prepared in Tween 80. 2ml suspension of a single dose was sprayed onto down sides of bean leaf discs using hand sprayer. Mortality rates of *T. urticae*, in terms of LT50 values, were recorded daily for 5 days. Enzyme activities were investigated by petri plate assays and clear zones formed around the colonies indicated enzymatic activities. All tested fungal isolates were pathogenic to the *T. urticae* causing mortality between 64.2±5.6% and 100% on the 5^{th} day after the application. LT50 values varied between 3.16±0.2 and 3.72±0.5 days. Mortality caused by PaF04 isolate was found to be significantly different ($p<0.05$). In conclusion, PaF isolates of *B. bassiana* have been determined to have potential to suppress *T. urticae*, in addition to their potential in control of Coleoptera, Lepidoptera, and Hymenoptera. The present results showed that the isolates used, especially PaF04, have a broad host range.

Key words: *Beauveria bassiana*, broad host range, *Tetranychus urticae*, enzyme activity.

Beauveria bassiana (Bals.) Vull. İzolatlarının *Tetranychus urticae* Koch'a (Acari: Tetranychidae) Karşı Kontrol Potansiyellerinin ve Enzim Aktivitelerinin Belirlenmesi

Özet: Entomopatojen fungusların geniş konukçu çeşitliliği biyolojik mücadele endüstrisi için ticari bir avantaj olabilir. Bununla birlikte, entomopatojen fungusların tümü konukçuya nüfuz etmek için çeşitli enzimlerinin kombinasyonlarını kullanır ve bu enzimler fungal izolatların konukçu çeşitliliğinde önemli bir rol oynar. Bu çalışmanın amacı, üç *Beauveria bassiana* (Bals.) Vull. izolatının *Tetranychus urticae* Koch'a (Acari: Tetranychidae) karşı kontrol potansiyellerinin ve lipaz, kitinaz ve proteaz enzim aktivitelerinin belirlenmesidir. Bu amaçla, daha önce Coleoptera, Lepidoptera ve Hymenoptera'ya karşı entomopatojenik aktivite gösteren *B. bassiana* (PaF04, PaF09 ve PaF76) izolatları seçilmiştir. Test organizması olarak, *T. urticae* kullanılmıştır. Üç farklı dozda süspansiyon ($1x10^6$, $1x10^7$ ve $1x10^8$ konidi ml^{-1}) Tween 80'de hazırlanmıştır. 2ml tek doz spor süspansiyonu el spreyi kullanılarak fasülye yaprak disklerinin alt yüzüne püskürtülmüştür. *T. urticae*'nın ölüm oranları 5 gün boyunca günlük olarak kaydedilmiştir. Enzim aktiviteleri petri plate yöntemi ile araştırılmış ve koloni çevresinde oluşan açık zonlar, enzimatik aktivitenin varlığını göstermiştir. Test edilen tüm fungus izolatları uygulamadan 5 gün sonra, 64,2±5,6% ve 100% arasında mortaliteye yol açarak *T. urticae*'ye karşı patojenite göstermiştir. LT50 değerleri 3,16±0,2 ve 3,72±0,5 gün arasında değişmiştir. PaF04 izolatının neden olduğu mortalite önemli derecede farklı bulunmuştur ($p<0,05$). Sonuçta, *B. bassiana* PaF izolatlarının Coleoptera, Lepidoptera ve Hymenoptera'yı kontrol potansiyellerinin olduğu kadar *T.* urticae'yı baskılama potansiyeline de sahip oldukları belirlenmiştir. Mevcut sonuçlar, kullanılan izolatların, özellikle de PaF04'ün geniş bir konukçu aralığına sahip olduğunu göstermiştir.

Anahtar kelimeler: *Beauveria bassiana*, geniş konukçu aralığı, *Tetranychus urticae*, enzim aktivitesi.

Introduction

Entomopathogenic fungi are considered to be one of the promising biological control agents (St Leger *et al.* 1996). *Beauveria bassiana* (Bals.) Vull., a fungal species naturally growing in soils and acting as a parasite on various arthropod species, has a potential to be used against both insect pests and plant pathogens. However,

no data was reported about phytopathogenic activity of this fungal species (Clarkson & Charnley 1996, Ownley *et al.* 2004, 2008).

The two spotted spider mite, *Tetranychus urticae* Koch is a worldwide economically important major pest of agriculture (Numa Vergel *et al.* 2011) and has been a serious problem due to insecticide use. The use of entomopathogen fungi has been an increasingly popular option to control mites and suppress their populations (Zhang 2003). In recent years, a growing interest about using *Beauveria* spp. in mite control applications occurred (Shi & Feng, 2004, Alves *et al.* 2005, Bugeme *et al.* 2008). Laboratory studies revealed that *B. bassiana* as an effective pathogen of *T. urticae* (Irigaray *et al.* 2003, Bugeme *et al.* 2009, Draganova & Simova 2010, Geroh *et al.* 2015).

B. bassiana is known to have a potential to control more than 70 insect pests, but most of the studies evaluating infection have focused on phytophagous hosts, most of which are Arthropoda members (Feng *et al.* 1994, Roy *et al.* 2006, van Lenteren 2012). *Beauveria* genus was shown to be affective against members of the insect orders Coleoptera, Lepidoptera, and Hymenoptera (Hatting *et al.* 2004).

The commercial production of mycoinsecticides has been a successful and environmentally safe alternative to insecticides. However, acceptance of these products has been limited when compared with conventional chemical products because they are not acting on pest species as fast as chemical products (Leger & Screen 2001, Sevim 2015). Narrow host range of mycoinsecticides is also one of the factors limiting their commercial use (Ownley *et al.* 2004). However, a mycoinsecticide including entomopathogenic fungi with a broad host range may have a commercial advantage over others (Brodeur 2012).

B. bassiana has an extremely broad host range and uses a combination of cuticle-degrading enzymes for degradation of and penetration into the host cuticle (Butt 2002, Meyling *et al.* 2009, Ortiz-Urquiza & Keyhani 2013). These enzymes including lipase, chitinase and protease may determine not only the virulence but also the host range of fungal isolates (Gupta *et al.* 1994, Butt 2002).

Some studies indicated that strains of entomopathogenic fungi can be more virulent to pests than the same or related host species that they were isolated from (Goettel *et al.* 1990, Ekesi *et al.* 2000, Shaw *et al.* 2002). Besides, pathogens that are not associated with a host in nature can be tested for their pathogenicity (Wekesa *et al.* 2005).

In the present study, we selected three native *B. bassiana* isolates, which were previously demonstrated to be active against Coleoptera (Albayrak İskender *et al.* 2013), Lepidoptera (Albayrak İskender *et al.* 2012), and Hymenoptera (Albayrak İskender *et al.* 2017), and evaluated their pathogenicity potentials towards *T. urticae* (Acari) considering their extremely broad host range.

Materials and Methods

Biological material and history of isolates

PaF04, PaF09 and PaF76 isolates of *B. bassiana* were used in this study. These isolates, previously isolated from *Pristiphora abietina* (Christ) larvae, were shown to be highly pathogen on different insect species and are deposited within the GenBank database with accession numbers KT962854, KT962855, and KT962857 respectively. A history of each isolates was given in Table 1. *T. urticae* was reared, as the target organisms, on bean plants at 25±1°C and 60±5% relative humidity (RH) with a 12:12h (L:D) photoperiod.

Conidial suspension and viability

The isolates were cultured in Potato Dextrose *Agar* (PDA) containing 2% yeast extract and kept for two weeks at 25°C for conidial production. Conidia were suspended in sterile distilled water, a surfactant (0.2ml/l Tween 80) was added to reduce clumping of the conidia, and the suspension was vortexed to produce a homogenous state (Belczewski & Harmsen 2000). The prepared suspensions were then filtrated through three layers of muslin to eliminate hyphae and unsuspended conidia. The spore concentrations were determined using a haemacytometer and suspensions were prepared in logarithmic series from $1x10^6$ to $1x10^8$ (Eken & Hayat 2009).

Conidial viability was tested in Sabouraud Dextrose Agar (SDA) *plates*. Droplets were taken from suspensions in sterile conditions and spread over the SDA medium. After 24h, percent germination was determined from 100 spore counts on each plate (Ekesi *et al.* 2000). Only conidia with a germ tube as long as the conidium's width were considered to be germinated. Spore suspensions were sealed with Parafilm and kept at 4°C in a refrigerator until use (Safavi *et al.* 2010).

Bioassays

Effect of conidial concentration on pathogenity

Three different preparations, $1x10^6$, $1x10^7$ and $1x10^8$, each supplied with 0.02% Tween 80, were tested. 2ml suspension of a single dose was sprayed onto down side of bean leaf discs (30mm diameter) using a hand sprayer. The leaf discs were air dried and placed in Petri dishes (Quesada-Moraga *et al.* 2006). Distilled water containing 0.02% Tween 80 was used as control. Twenty vigorous adult mites arbitrarily taken from the reared population were transferred to each disc by means of a fine soft brush (Kady *et al.* 2007). After exposure, all petri dishes were sealed with Parafilm and the lids were provided with holes of 6 mm diameter for proper ventilation.

All petri dishes were incubated at 25±1°C and 65±5% RH. Mortality was recorded on daily basis for a period of 5 days. Dead mites were surface sterilized in 70% ethanol, dried and transferred to Petri dishes lined with moist filter paper for 10 days to observe mycosis. Mortality caused by fungi was confirmed by microscopic examination (Cherry *et al.* 2005).

Table 1. History of the isolates used in this study.

Host	Order	Application	Dose (conidia/ml)	Mortality (%) (5th day after application)			Reference
				PaF04	PaF09	PaF76	
P. abietina larvae	Hymenoptera	Immersing	10^6	48.2±8.9b	80±6.4a	72.8±7.7ab	(Albayrak Iskender *et al.* 2017)
H. cunea larvae	Lepidoptera	Immersing	10^6	96.66±3.33a	90±5.77a	96.66±3.33a	(Albayrak Iskender *et al.* 2012)
D. micans larvae	Coleoptera	Immersing	10^9	100a	100a	100a	(Albayrak Iskender *et al.* 2013)

In-vitro enzyme plate assays

B. bassiana isolates were tested for qualitative analysis of chitinase, protease and lipase enzymes by measured clear zone. For analysis of the lipolytic activity, the medium described by Sierra (1957) was used. Olive oil was used as the lipid substrate. For analysis of the proteolytic activity, caxsein soluble medium with milk powder was used. For the chitinolytic activity, medium-5 as described by Abd-Aziz *et al.* (2008) was used with addition of agar. An agar disk (6mm in diameter) from 7-old day fungal culture was placed in the center of these agar plates and the inoculated plates were incubated at 28˚C for 10 days. Clear zones formed around the colonies indicated degradation of the substrate due to enzymatic activity. Zone formations in the protease activity were enhanced by flooding the plates with 10% glacial acetic acid.

Statistical analysis

The experimental design was a randomized complete block with three replicates, and each replicate consisted of 20 mites. The analysis of variance was conducted using one-way ANOVA test using SPSS 13.0 LT50 was determined graphically for mortality (Alves *et al.* 2005).

Results

This study determined pathogenicity of *B. bassiana* against *T. urticae* under laboratory conditions. In viability tests, 95–100% of the spores germinated and all tested fungal isolates were pathogenic to the *T. urticae*, causing a mortality rate between 64.2±5.6% and 100% after 5 days from application. Microscopic investigations confirmed mycosis for all dead mites, and *B. bassiana* was reisolated from all dead individuals. LT50 values varied between 3.16±0.2 and 3.72±0.5 days. Mortality caused by PaF04 isolate was found to be significantly different ($p<0.05$) between all preparations. For this isolate, the lowest *B. bassiana* concentration ($1x10^6$ conidia/ml) gave less control (80.7±4.2%) of the mites until the 5th day after inoculation. By comparison, the intermediate concentration ($1x10^7$ conidia/ml) caused a mortality with 92±3.5%, and the best results were obtained with the highest conidial concentrations ($1x10^8$ conidia/ml) with which a high control (100%) of the mites were achieved. PaF04 isolate was consistently more virulent than PaF09 and PaF76 isolates for all preparations. PaF09 isolate showed a virulence similar to that of PaF76 ($p<0.05$) except for $1x10^7$ conidia/ml. LT50 values of these three isolates changed between 3.16 and 3.72 days (Table 2).

Discussion

B. bassiana isolate PaF04 was consistently more virulent than PaF09 and PaF76 isolates for all preparations. Mortality of the target organism *T. urticae* was found to increase with time (data not shown) and was dependent on spore concentration. PaF04 isolate led to the shortest LT50 value with 3.16 days. This value was significantly ($P>0.05$) shorter than LT50 values obtained with PaF09 and PaF76 isolates. Albayrak İskender *et al.* (2012) compared the virulence of the same isolates against *H. cunea* larvae and found that their virulence was similar ($P>0.05$). Furthermore, Albayrak İskender *et al.* (2017) reported that these isolates were pathogenic against *P. abietina* larvae as well, but the levels of virulence were different among the isolates ($P>0.05$).

They also reported that PaF09 isolate was highly virulent to *P. abietina* larvae, and all PaF04, PaF09 and PaF76 isolates possessed a good potential against *H. cunea* and *D. micans* larvae. Nevertheless, in our study, only PaF04 isolate was shown to have a potential to control *T. urticae*. Our results which indicated a poor potential for PaF09 and PaF76 againts *T. urticae* migth

Table 2. Mortality of *T. urticae* within 5 days after application of fungal isolates and the enzyme activities of the isolates in terms of clear zone evaluations on agar plates ($p<0.05$±SE)*.

65±5%RH				LT50 (days)	Enzyme activity (mm)		
Conidial suspension					Lipase	Protease	Chitinase
Isolates	$1x10^6$	$1x10^7$	$1x10^8$				
PaF04	80.7±4.2^a	92±3.5^a	100^a	3.16±0.2	ND**	4.5±0.5^a	6±2^a
PaF09	67.6±6.3^b	83.4±2.2^b	89.8±3.3^b	3.52±0.3	2±0.4^c	5±0.5^a	1±0.5^c
PaF76	64.2±5.6^b	77.8±4.2^c	90.1±1.1^b	3.72±0.5	5±1^a	4±0.7^a	ND**

* The differences between the values indicated by the same letters (used as superscript) in the same column are not statistically significant ($p\leq0.05$), **ND: Not determinated.

be due to the experimental method employed, to the type of the host and host stage because all these parameters are know to affect the results of bioassays (Mascarin *et al.* 2013).

When the lipase and protease activities values of PaF76 isolate were compared, the clear zone for lipase activity was (5±1mm) appeared to be higher than its protease-originated clear zone (4±0.7mm). The obtained results indicated that mortality effect of PaF76 on *T. urticae* was too low. Extracellular lipases are involved in microbial virulence and play different roles in infection processes (Stehr *et al.* 2003). Khan *et al.* (2012) reported that lipase was the most important enzyme for virulence as compared to chitinase and protease. The differences of the hosts and isolates used might be the factors leading to differences in mortality results obtained (Clarkson & Charnley 1996). In addition, the developmental stage of the host organism might also be a reason of differences in mortality. PaF04 isolate possessed the highest chitinase activity. This isolate was consistently more virulent than PaF09 and PaF76 isolates in this study. Previous studies have shown that enzymatic activities based on chitinase and protease played important roles in mortality of pest in the presence of entomopathogens (St Leger *et al.* 1996, Campos *et al.* 2005). Similarly, Khan *et al.* (2012) reported that protease was found to be effective in the pathogenicity of *B. bassiana*. We therefore conclude that PaF04 isolate could be used as potential control agent on a varity of target organisms, considering its broad host range.

It is well known that host specificity has been a critical issue in biological control studies and the first step in the selection of biological control agent candidate. Furthermore, narrow host range can be a limiting factor for the commercialization of entomopathogens (Waage 2001, Brodeur 2012). Entomopathogens such as *B. bassiana* used in biological control has the capacity to exploit a wide range of hosts (Charnley & Collins 2007, Bugeme *et al.* 2008).

The three *B. bassiana* isoaltes used in the present study are fungal agents which have previously been to be pathogenic against various insects (for *P. abietina* larvae see Albayrak İskender *et al.* 2017, for *H. cunea* larvae see Albayrak İskender *et al.* 2012, and for *D. micans* larvae see Albayrak İskender *et al.* 2013). Here, we showed their pathogenicity potentials on the agricultural pest *T. urticae* based on qualitative analysis of their enzyme activities which could be related to pathogenicity (Feng & Johnson 1990). Our findings are important to fundamentally understand the host-fungus relationships and may also be useful for selection of these organisms for biological control applications. In conclusion, PaF isolates of *B. bassiana* were proven to have a potential to control Coleoptera, Lepidoptera, and Hymenoptera as well as *T. urticae*.

References

1. Abd-Aziz, S., Sin, T.L., Alitheen, N., Shahab, N. & Kamaruddin, K. 2008. Microbial degradation of chitin materials by *Trichoderma virens* UKM1. *Journal of Biological Sciences*, 8(1): 52–59.
2. Albayrak İskender, N., Ortucu, S., Aksu, Y. & Saral, A. 2017. Isolation, characterization and pathogenicity of fungi from *Pristiphora abietina* (Hymenoptera: Tenthredinidae). *Fresenius Environmental Bulletin*, 26(1a): 628–633.
3. Albayrak İskender, N., Ortucu, S., Kılıc, E. & Aksu, Y. 2013. Assessment of *Beauveria bassiana* strains isolated from various insects to control *Dendroctonus micans* (Kugel.) larvae, 4th International Participated Entomopathogens and Microbial Control Symposium, 11-14 September, Artvin, 97.
4. Albayrak İskender, N., Örtücü, S. & Aksu, Y. 2012. Pathogenicity of three isolates of the entomopathogenic fungi *Beauveria bassiana* to control *Hyphantria cunea* (Drury) (Lepidoptera:Arctiidae) larvae. *Kırgızistan-Türkiye Manas Üniversitesi Fen Bilimleri Dergisi*, 13: 15–21.
5. Alves, S.B., Tamai, M.A., Rossi, L.S. & Castiglioni, E. 2005. *Beauveria bassiana* pathogenicity to the citrus rust mite *Phyllocoptruta oleivora*. *Experimental and Applied Acarology*, 37(1–2): 117–122.
6. Belczewski, R. & Harmsen, R. 2000. The effect of non-pathogenic phylloplane fungi on life-history traits of urticae (Acari: Tetranychidae). *Experimental and Applied Acarology*, 24(4): 257–270.
7. Brodeur, J. 2012. Host specificity in biological control: Insights from opportunistic pathogens. *Evolutionary Applications*, 5(5): 470–480.
8. Bugeme, D.M., Knapp, M., Boga, H.I., Wanjoya, A.K. & Maniania, N.K. 2009. Influence of temperature on virulence of fungal ısolates of *Metarhizium anisopliae* and *Beauveria bassiana* to the two-spotted spider mite *Tetranychus urticae*. *Mycopathologia*, 167(4): 221–227.
9. Bugeme, D.M., Maniania, N.K., Knapp, M. & Boga, H.I. 2008. Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates to *Tetranychus evansi*. *Experimental and Applied Acarology*, 46(1–4): 275–285.
10. Butt, T.M. 2002. Use of Entomogenous Fungi for the Control of Insect Pests, 111–134. in: (Kempken, F.), *Agricultural Applications*, Springer Berlin Heidelberg, Berlin, 388.
11. Campos, R.A., Arruda, W., Boldo, J.T., Silva, M.V., de Barros, N.M., de Azevedo, J.L., Schrank, A. & Vainstein, M.H. 2005. *Boophilus microplus* infection by *Beauveria amorpha* and *Beauveria bassiana*: SEM analysis and regulation of subtilisin-like proteases and chitinases. *Current Microbiology*, 50(5): 257–261.
12. Charnley, A.K. & Collins, S.A. 2007. Entomopathogenic fungi and their role in pest control, 159–187. in (Kubicek,

C.P. & Druzhinina, I.S.), *Environmental and Microbial Relationships*, Heidelberg: Springer, Berlin, 350.

13. Cherry, A.J., Abalo, P. & Hell, K. 2005. A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) to control Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) in stored cowpea. *Journal of Stored Products Research*, 41(3): 295–309.

14. Clarkson, J.M. & Charnley, A.K. 1996. New insights into the mechanisms of fungal pathogenesis in insects. *Trends in Microbiology*, 4(5): 197–203.

15. Draganova, S.A. & Simova, S.A. 2010. Susceptibility of *Tetranychus urticae* Koch. (Acari: Tetranychidae) to isolates of entomopathogenic fungus *Beauveria bassiana*. *Pesticides and Phytomedicine*, 25(1): 51–57.

16. Eken, C. & Hayat, R. 2009. Preliminary evaluation of *Cladosporium cladosporioides* (Fresen.) de Vries in laboratory conditions, as a potential candidate for biocontrol of *Tetranychus urticae* Koch. *World Journal of Microbiology and Biotechnology*, 25(3): 489–492.

17. Ekesi, S., Maniania, N. K. & Lwande, W. 2000. Susceptibility of the legume flower thrips to Metarhizium anisopliae on different varieties of cowpea. *BioControl*, 45(1):79–95.

18. Feng, M.G. & Johnson, J.B. 1990. Relative virulence of six isolates of *Beauveria bassiana* on *Diuraphis noxia* (Homoptera: Aphididae). *Environmental Entomology*, 19: 3(3): 785–790.

19. Feng, M.G., Poprawski, T.J. & Khachatourians, G.G. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. *Biocontrol Science and Technology*, 4(1): 3–34.

20. Geroh, M., Gulati, R. & Tehri, K. 2015. Determination of lethal concentration and lethal time of entomopathogen *Beauveria bassiana* (Balsamo) Vuillemin against *Tetranychus urticae* Koch. *International Journal of Agriculture Sciences*, 7(5): 523–528.

21. Goettel, M.S., Poprawski, T.J., Vandenberg, J.D. & Roberts, D.W. 1990. Safety to non target invertebrates of fungal biocontrol agents, 209–231. in (Laird, M., Lacey, L. & Davidson, E.), *Safety of Microbial Insecticides*, CRC Press, Boca Raton, 272.

22. Gupta, S.C., Leathers, T.D., Elsayed, G.N. & Ignoffo, C.M. 1994. Relationships among enzyme activities and virulence parameters in *Beauveria bassiana* infections of *Galleria mellonella* and *Trichoplusia ni*. *Journal of Invertebrate Pathology*, 64(1): 13–17.

23. Hatting, J.L., Wraight, S.P. & Miller, R.M. 2004. Efficacy of *Beauveria bassiana* (Hyphomycetes) for control of Russian wheat aphid (Homoptera: Aphididae) on resistant wheat under field conditions. *Biocontrol Science and Technology*, 14(5): 459–473.

24. Kady, G.A, Sharabasy, H.M., Mahmoud, M.F., Bahgat, I.M. & Said, P. 2007. Toxicity of two potential bio-insecticides against moveable stages of *Tetranychus urticae* Koch. *Journal of Applied Sciences*, 3(11): 1315–1319.

25. Khan, S., Guo, L., Shi, H., Mijit, M. & Qiu, D. 2012. Bioassay and enzymatic comparison of six entomopathogenic fungal isolates for virulence or toxicity against green peach aphids Myzus persicae. *African Journal of Biotechnology*, 11(77): 14193–14203.

26. Leger, R.S. & Screen, S. 2001. Prospects for strain improvement of fungal pathogens of insects and weeds, 219–237. in: (Butt, T., Jackson, C. & Magan, N.), *Fungi as Biocontrol Agents Progress, Problems and Potential*, CABI, Wallingford, 416.

27. van Lenteren, J.C. 2012. The state of commercial augmentative biological control: plenty of natural enemies, but a frustrating lack of uptake. *BioControl*, 57(1): 1–20.

28. Mascarin, G.M., Kobori, N.N., Quintela, E.D. & Delalibera, I. 2013. The virulence of entomopathogenic fungi against *Bemisia tabaci* biotype B (Hemiptera: Aleyrodidae) and their conidial production using solid substrate fermentation. *Biological Control*, 66(3): 209–218.

29. Meyling, N., Lubeck, M., Buckley, E., Eilenberg, J. & Rehner, S. 2009. Community composition, host range and genetic structure of the fungal entomopathogen *Beauveria* in adjoining agricultural and seminatural habitats. *Molecular Ecology*, 18(6): 1282–1293.

30. Numa Vergel, S.J., Bustos, R.A., Rodríguez, C.D. & Cantor, R.F. 2011. Laboratory and greenhouse evaluation of the entomopathogenic fungi and garlic–pepper extract on the predatory mites, *Phytoseiulus persimilis* and *Neoseiulus californicus* and their effect on the spider mite *Tetranychus urticae*. *Biological Control*, 57(2): 143–149.

31. Ortiz-Urquiza, A. & Keyhani, N. 2013. Action on the surface: entomopathogenic fungi versus the insect cuticle. *Insects*, 4(3): 357–374.

32. Ownley, B.H., Griffin, M.R., Klingeman, W.E., Gwinn, K.D., Moulton, J.K. & Pereira, R.M. 2008. *Beauveria bassiana*: endophytic colonization and plant disease control. *Journal of Invertebrate Pathology*, 98(3): 267–270.

33. Ownley, B.H., Pereira, R.M., Klingeman, W.E., Quigley, N.B. & Leckie, B.M. 2004. Beauveria bassiana, a dual purpose biocontrol organism, with activity against insect pests and plant pathogens, 255–269. in: (Lartey, R. & Caesar, A.), *Emerging Concepts in Plant Health Management*, Research Signpost, Kerala, 298.

34. Quesada-Moraga, E., Maranhao, E.A.A., Valverde-García, P. & Santiago-Álvarez, C. 2006. Selection of Beauveria bassiana isolates for control of the whiteflies *Bemisia tabaci* and *Trialeurodes vaporariorum* on the basis of their virulence, thermal requirements, and toxicogenic activity. *Biological Control*, 36(3): 274–287.

35. Roy, H.E., Steinkraus, D.C., Eilenberg, J., Hajek, A.E. & Pell, J.K. 2006. Bizarre interactions and endgames: entomopathogenic fungi and their arthropod hosts. *Annual Review of Entomology*, 51(1): 331–357.

36. Irigaray, F.J., Marco-Mancebón, V. & Pérez-Moreno, I. 2003. The entomopathogenic fungus *Beauveria bassiana* and its compatibility with triflumuron: effects on the twospotted spider mite *Tetranychus urticae*. *Biological Control*, 26(2): 168–173.

37. Safavi, S.A., Kharrazi, A., Rasoulian, G.R. & Bandani, A.R. 2010. Virulence of some isolates of entomopathogenic fungus, *Beauveria bassiana* on *Ostrinia nubilalis* (Lepidoptera: Pyralidae) larvae. *Journal of Agricultural Science and Technology*, 12(1): 13–21.

38. Sevim, A. 2015. Entomopatojenik fungusların genel biyolojileri ve Türkiye'de zararlı böceklerin mücadelesinde kullanılma potansiyelleri. *Erzincan Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 8(1): 115-147.

39. Shaw, K.E., Davidson, G., Clark, S.J., Ball, B.V., Pell, J.K., Chandler, D. & Sunderland, K.D. 2002. Laboratory bioassays to assess the pathogenicity of mitosporic fungi to *Varroa destructor* (Acari: Mesostigmata), an ectoparasitic mite of the honeybee, *Apis mellifera*. *Biological Control*, 24(3): 266–276.

40. Shi, W. & Feng, M. 2004. Lethal effect of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces fumosoroseus* on the eggs of *Tetranychus cinnabarinus* (Acari: Tetranychidae) with a description of a mite egg bioassay system. *Biological Control*, 30(2): 165–173.

41. Sierra, G. 1957. A simple method for the detection of lipolytic activity of micro-organisms and some observations on the influence of the contact between cells and fatty substrates. *Antonie van Leeuwenhoek*, 23(1): 15–22.

42. St Leger, R., Joshi, L., Bidochka, M.J. & Roberts, D.W. 1996. Construction of an improved mycoinsecticide overexpressing a toxic protease. *Proceedings of the National Academy of Sciences of the United States of America*, 93(13): 6349–6354.

43. Stehr, F., Kretschmar, M., Kröger, C., Hube, B. & Schäfer, W. 2003. Microbial lipases as virulence factors. *Journal of Molecular Catalysis B: Enzymatic*, 22(5): 347–355.

44. Waage, J. K. 2001. Indirect ecological effects in biological control: the challenge and the opportunity, 1–12, in: (Wajnberg, E., Scott, J. & Quimby, P.), *Evaluating Indirect Ecological Effects of Biological Control*, CAB International, Wallingford, 261.

45. Wekesa, V.W., Maniania, N.K., Knapp, M. & Boga, H.I. 2005. Pathogenicity of *Beauveria bassiana* and *Metarhizium anisopliae* to the tobacco spider mite *Tetranychus evansi*. *Experimental & applied acarology*, 36(1–2): 41–50.

46. Zhang, Z. 2003. *Mites of Greenhouses: Identification, Biology and Control*. CABI Publishing, Oxon, 240.