Türk. Biyo. Mücadele Derg. 2020, 11(1):71-81
DOI: 10.31019/tbmd.626923 ISSN 2146-0035-E-ISSN 2548-1002

Orijinal araştırma (Original article)

Investigation of the effects of ambient humidity on the combined application of *Beauveria bassiana* (Balsamo) Vuillemin and diatomaceous earth against the lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)[1]

Hasan USLU[1*], Mehmet Kubilay ER[2]

Ortam neminin, *Beauveria bassiana* (Balsamo) Vuillemin ve diatomlu toprağın Ekin kambur biti *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)'ya karşı kombine uygulaması üzerine etkinliğinin araştırılması

Öz: Entomopatojen fungus (EF) ve diyatom toprağının (DE) depolanmış tahıl zararlılarına karşı tek başlarına veya birlikte etkili olduğu bilinmektedir. Bu çalışmada, bir Türk DE (ACN) ile birleştirilmiş iki yerel *Beauveria bassiana* (Balsamo) Vuillemin izolatı (5-4, 1-1) seçilen ortam koşullarının birleşik uygulamaların etkinliği üzerindeki etkisini ortaya çıkarmak için üç nispi nemde (%45, %55, %65) ve iki sıcaklık değerinde (25ºC, 30ºC) Ekin kambur biti *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) erginlerine karşı test edilmiştir. Denemeler için 40 g buğday alan 50 ml kapasiteli santrifüj tüpleri kullanılmıştır. Kontrol için sadece buğday kullanılmıştır. EF ve DE ayrı ayrı 300 ppm (w/w) konsantrasyonunda ve birlikte 300ppm+300ppm konsantrasyonunda kullanılmıştır. Böcek ölümleri 7-14 gün sonra kaydedilmiştir. Elde edilen sonuçlara göre, *B. bassiana*'nın DE ile birlikte uygulanması, test edilen nispi nem seviyelerinde *R. dominica*'ya karşı aynı derecede etkilidir ve bu yüzden etkinlik kaybı olmadan bir dizi ortam koşulunda kullanılabilir.

Anahtar sözcükler: *Beauveria bassiana,* diatom toprağı, nispi nem, *Rhyzopertha dominica*

Abstract: Entomopathogenic fungi (EF) and diatomaceous earth (DE) are effective alone or in combination against stored-grain insects. In this study, two Turkish isolates of *Beauveria bassiana* (Balsamo) Vuillemin and a Turkish DE were tested separately and in combination at three relative humidities (45%, 55%, 65%) and two temperatures (25ºC, 30ºC) to determine their efficacy against adults of the lesser grain borer *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). For the bioassays, 50 mL centrifuge tubes containing 40 g of wheat were used. For the controls, wheat alone was used. The EFs and DE were used at 300 ppm (w/w) individually and 300 ppm+300 ppm in combination. Insect mortality was recorded after 7 and 14 days. The application of *B. bassiana* with DE was equally effective against *R. dominica* at the three levels of RH used. Therefore, the further testing of combinations of entomopathogenic fungi and diatomaceous earths in grain storages under a range of ambient condition is recommended.

Key words: *Beauveria bassiana,* diatomaceous earth, relative humidity, *Rhyzopertha dominica*

[1] Kahramanmaras Sutcu Imam University, Graduate School of Natural and Applied Sciences, Plant Protection Department, Kahramanmaraş, Turkey, ORCID ID: 0000-0002-8072-9189
[2] Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Plant Protection Department, Kahramanmaraş, Turkey, ORCID ID: 0000-0003-1568-8656
* Sorumlu Yazar (Corresponding author) e-mail: h.uslu@yahoo.com
Alınış (Recieved): 30.09.2019 Kabul ediliş (Accepted): 05.12.2019

Introduction

Cereals comprise an important part of the agricultural production sector in Turkey. These products are subject to damage by stored-product pests which affect the quantity, nutritional value and other commercial properties of the products. The control of insect pests of stored products mostly depends upon chemical insecticides (Karaca and Ayyıldız, 2018). Although they can kill the pests in a short time, these chemicals are hazardous to the environment and human health (Altıkat et al, 2009). In addition, the wide use of insecticides also causes pests to gain resistance to these chemicals (Çakır and Yamanel, 2005). Due to all such problems, alternative methods are continually under investigation.

The use of entomopathogenic fungi (EF) is an alternative control technique against stored-product pests. The hyphae of pathogenic fungi develop in the insect's epidermis and continue to proliferate in the body and hemolymph, causing the death of the insect (Deacon, 1983).

In many studies, promising results have been obtained with the use of EF in laboratory tests against stored-product pests (Michalaki et al, 2006; Mahdneshin et al, 2009; Wakil et al, 2010; Wakil et al, 2011; Şahin and Er, 2014; Sewify et al, 2014; Er et al, 2015). There are commercially available EF products (Um et al., 2017). Diatomaceous earth (DE) is another alternative to insecticides that are used against stored-product pests. DE is non-toxic to mammals and does not leave residues in commodities and is in the GRAS (generally recognized as safe) category, according to the Environment Protection Agency of the USA, and thus it is possible to use DE as a food additive (FDA, 1995). Diatomaceous earth causes insects to dehydrate and die by absorbing the oils and fats from the cuticle of the insect's exoskeleton. Its sharp edges are abrasive, which speeds up the process. It remains effective as long as it is kept dry and preserved (Bunch, et al, 2013). Turkish DE that could be used commercially were reported by Doğanay et al (2014). Studies have shown that commercially available DEs are highly effective against stored product pests (Athanassiou et al., 2003; Athanassiou et al, 2005; Beriş et al, 2010). Furthermore, it has been shown that the combined use of EFs and DEs has a high efficacy against stored product pests at low concentrations (Vassilakos et al, 2006; Athanassiou et al., 2007a; Athanassiou et al, 2008; Riasat et al, 2011; Sabbour et al, 2012; Shafighi et al, 2014).

In this laboratory study, the efficacy of the combined use of the EF, *Beauveria bassiana* (Balsamo) Vuillemin, and a DE, against *Rhyzopertha dominica* (F.), was investigated under three different relative humidities (RHs).

Materials and Methods

Fungal culture

The EF (*B. bassiana*) cultures were started with spores isolated from *R. dominica* collected in Adana Province in Turkey. Specifically, spores of the two Turkish isolates of *B. bassiana* (5-4, 1-1) used in testing were mass produced (Barış, 2016). After adding 1.5 g CaCO3 and CaSO4 per bag for getting rice as uniform to polyethylene bags containing rice, the bags were sterilized in an autoclave at 121°C for 20 minutes. When the completion of the autoclaving process was done, the polyethylene bags were allowed for 2 hours until they reached room temperature. Ten mL of spore suspension at $2x10^7$ spores/mL was added to each polyethylene bag and mixed with the rice for inoculation purposes. The polyethylene bags were then sealed with a 30 cm wide bag sealer. Thereafter, the bags were kept for 14 days at 25±2°C and 12:12 hour photoperiod for fungal growth and sporulation. Following that procedure, the polyethylene bags were opened and kept at 25±2°C until the contents were dry. After that, the fungi spores were separated from the rice grains by sieving through a 500 µm sieve. The obtained spores were kept at + 4°C until used for testing.

Insect culture

The *R. dominica* culture was obtained from samples collected from grain storages in Kahramanmaraş, Turkey. Wheat was used for the culturing of *R. dominica*. For getting plenty of *R. dominica*, many adults were placed in 1 L glass jars with 250 g of wheat, and for oviposition purposes were kept for three days at 30±°C and 65±5% RH in continuous darkness. The eggs were then separated from the wheat and added to new jars which containing clean wheat. In this way, the continuity of the culture was ensured during the study period. One week old females and males from this culture were used for testing.

Diatomaceous earth

Diatomaceous earth (coded CAN) was collected from DE reserves located in central Anatolia, Turkey.

Table 1. Description of the diatomaceous earth used with *Beauveria bassiana* against *Rhyzopertha dominica*

DE Code	SiO_2 (%)	Median particle size* (µm)
ACN	73.80	14.297

*The analyses were carried out in the Analysis Laboratories of the General Directorate of Mineral Research and Exploration, Turkey

Experimental procedure

The germination rate of the *B. bassiana* spores was evaluated before each experiment. A dilute suspension of conidia in sterile, distilled water including 0.01% Tween 80, was spread on PDA and incubated for 24 h at 25±2°C in darkness. One hundred spores were checked then under a light microscope (Olympus BX51) and those with a germination tube with a length at least equal to the diameter of the spore

were recorded as germinated. The germination rate of spores used in all applications was 96-100%.

Centrifuge tubes of 50 mL capacity were used for the bioassays and each tube had 40 g of wheat added to it. Three hundred ppm EF+300ppm DE were added to each tube and shaking for 5 minutes by hand. Finally, 20 insects were placed in each tube. The same procedure was used to test the EF and DE individually. For the control units, wheat alone was used. Tests were performed at 25ºC and 30ºC at 45%, 55% and 65% RH. Potassium nitrite (KNO_2), calcium nitrate ($Ca(NO_3)_2$) and sodium nitrite ($NaNO_2$) saturated salt solutions were used to achieve the required RH levels (Greenspan, 1977). Dead insects were taken into moisture circle which is in petri dishes, fungal growth on the insects was monitored and mortalities were evaluated according to fungal growth on adults. Insect mortalities were recorded after 14 days for the statistical analyses.

Statistical analysis

After the mortality rates were corrected by using Abbott's formula (Abbott, 1925), the statistical analyses were performed. After arcsine transformation was used to fit the data, the 7th and 14th-day mortality rates were subjected to variance analysis (one-way ANOVA) by using the SPSS 24 statistics program (SPSS Inc., 2015). Differences between treatments were determined by using the Duncan multiple comparison test at the 5% significance level. In the tests with two treatments, the t-test was applied to determine the differences between mean mortalities.

Results and Discussion

The 7-day mortality rates for *R. dominica* adults obtained at three different relative humidities at 25^{o}C are given in Table 2. According to the one-way ANOVA results, there was a significant difference between the effects of the applied agents on the death rates of adult *R. dominica* at 25^{o}C ($F_{2.36}$=50.766, P<0.001); moreover, it was determined that ambient humidity affects the mortality rate ($F=_{2,36}$=4.572, P=0.017) but the interaction between the two factors was not significant ($F_{4,36}$=2.034, P=0.110). While there was no significant difference for RH for the *B. bassiana* 5-4 isolate and ACN combination, the activity of *B. bassiana* 1-1 was statistically lower at 65% RH. The 14-day mortality rates of *R. dominica* adults obtained with the three different RH treatments at 25^{o}C are given in Table 3. According to the one-way ANOVA results, there was a significant difference between the effects of the applied agents on the death rates of adult *R. dominica* ($F=_{2,36}$=30.664, P<0.001), ambient humidity did not significantly affect the mortality rate ($F_{2,36}$=2.378, P=0.107), and the interaction between the two factors was significant ($F_{4,36}$=0.281, P=0.030). In addition, isolate 5-4 caused a significantly higher mortality level than ACN in all three RH environments.

Table 2. Corrected mortality at 7 days for the application of a Turkish diatomoceous earth (ACN) and two isolates of *Beauveria bassiana* (5-4 and 1-1) to *Rhyzopertha dominica* adults at 25ºC at three ambient relative humidities

Treatments	Corrected mortality (%)* ± SEM			F and P values
	45%	55%	65%	
300 ppm 5-4 EP	59.55±3.57 Aa	63.15±6.14 Aa	64.54±4.12 Aa	$F_{2.12}$=0.296 P=0.749
300 ppm 1-1 EP	56.09±4.74 ABa	66.81±3.57 Aa	44.78±3.27 Bb	$F_{2.12}$=7.936 P=0.006
300 ppm ACN	13.73±4.71 Ab	31.68±6.42 Ab	21.59±4.81 Ac	$F_{2.12}$=2.661 P=0.111
Control	13.00±2.55	9.00±4.30	4.00±1.87	
F and P values	$F_{2.12}$=21.883 P<0.001	$F_{2.12}$=11.298 P=0.002	$F_{2.12}$=22.462 P<0.001	

*Differences between the means were determined according to the Duncan test at the 5% significance level. Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means are significantly different from each other (n=5).

Table 3. Corrected mortality at 14 days after the application of a Turkish diatomoceous earth (ACN) and two isolates of *Beauveria bassiana* (5-4 and 1-1) against *Rhyzopertha dominica* adults at 25ºC at three ambient relative humidities

Treatments	Corrected mortality (%) ± SEM			F and P values
	45%	55%	65%	
300 ppm 5-4 EP	89.65±3.89 Aa	93.89±3.66 Aa	88.56±4.38 Aa	$F_{2.12}$=2.285 P=0.98
300 ppm 1-1 EP	78.43±7.54 Aab	83.45±4.28 Aa	65.61±3.74 Ab	$F_{2.12}$=0.524 P=0.98
300 ppm ACN	52.68±10.52 Ab	53.59±9.17 Ab	43.40±6.16 Ac	$F_{2.12}$=2.285 P=0.605
Control	14.0±2.44	9.00±4.30	6.0±2.91	
F and P values	$F_{2.12}$=6.008 P<0.001	$F_{2.12}$=12.218 P<0.001	$F_{2.12}$=16.724 P<0.001	

*Differences between the means were determined according to the Duncan test at the 5% significance level. Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means are significantly different from each other (n=5).

The 7 and 14 day mortality rates obtained with the application of the combinations of ACN and *B. bassiana* isolates against *R. dominica* adults at 25ºC and three different relative humidities are given in Tables 4 and 5. There were no differences in the 7th day mortalities obtained from the applications in the different RH treatments. However, at day 14, the application of the combination of 1-1+ACN caused a significantly lower level of mortality at 65% RH. Although the effect of the combinations was generally not significantly different on days 7 and 14, a significant difference was found between mortalities on day 7 at 65% RH (Table 4).

Table 4. Corrected mortality rates 7 days after the application of combinations of Turkish diatomaceous earth (ACN) and isolates of *Beauveria bassiana* (5-4 and 1-1) against *Rhyzopertha dominica* adults at 25ºC and three ambient relative humidities

Treatments	Corrected mortality (%) ± SEM			F and P values
	45%	55%	65%	
300 ppm 5-4 EP+300 ppm ACN	72.16±4.84 Aa	82.23±3.67 Aa	74.81±5.99 Aa	$F_{2.12}$=1.234 P=0.331
300 ppm 1-1 EP+300 ppm ACN	68.35±5.43 Aa	64.91±6.69 Aa	51.83±4.99 Ab	$F_{2.12}$=2.241 P=0.149
Control	13.0±2.54	9.0±4.30	4.0±1.87	
T and P values	T_8=0.505 P=0.627	T_8=2.231 P=0.56	T_8=2.988 P=0.017	

*Differences between the means were determined with the Duncan test at the 5% significance level. Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means are significantly different from each other (n=5).

Table 5. Corrected mortality rates 14 days after the application of combinations of Turkish diatomaceous earth (ACN) and two isolates of *Beauveria bassiana* against *Rhyzopertha dominica* adults at 25ºC and three ambient relative humidities

Treatments	Corrected mortality (%) ± SEM			F and P values
	45%	55%	65%	
300 ppm 5-4 EP+300 ppm ACN	92.86±2.87 Aa	97.61±1.47 Aa	91.62±4.86 Aa	$F_{2.12}$=0.891 P=0.436
300 ppm 1-1 EP+300 ppm ACN	96.47±1.44 Aa	92.12±4.09 Aa	79.99±3.26 Ba	$F_{2.12}$=5.551 P=0.020
Control	14.0±2.44	9.0±2.44	6.0±2.91	
T and P values	T_8=0.505 P=0.627	T_8=0.505 P=0.627	T_8=0.505 P=0.627	

*Differences between the means were determined with the Duncan test at the 5% significance level. Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means are significantly different from each other (n=5).

The 7-day mortality rates of adult *R. dominica* obtained from the treatments at 30ºC under three different RH conditions are given in Table 6. According to the one-way ANOVA results, there was a significant difference between the effects of the applications on the mortality rate of adult *R. dominica* ($F_{2,36}$=72.124, P<0.001). On the other hand, RH did not affect mortality ($F=_{2,36}$=0.693, P=0.507). Also, the interaction between the two applications was not significant ($F_{4,36}$=0.764, P=0.556). In addition, in all three RH environments, there was no significant difference between the effects of the two fungal isolates. Separately, the mortalities caused by both isolates were significantly higher than for ACN on day 7.

The day 14 mortality rates at 30ºC are presented in Table 7. One-way ANOVA demonstrated that the two *B. bassiana* applications and ambient humidity did not have a significant effect on mortality ($F_{2,36}$=43.590, P<0.001; $F_{2,36}$=3.499, P=0.041, respectively)and the interaction between them was not significant ($F_{4,36}$=1.224, P=0.318).

Table 6. Corrected mortality of *Rhyzopertha dominica* adults 7 days after the application of a Turkish diatomoceous earth (ACN) and two isolates of *Beauveria bassiana* at three ambient relative humidities at 30ºC

Treatments	Corrected mortality (%) ± SEM			F and P values
	45%	55%	65%	
300 ppm 5-4 EP	49.13±4.74 Aa	57.63±8.15 Aa	54.0±5.09 Aa	$F_{2.12}$=0.508 P=0.614
300 ppm 1-1 EP	57.54±5.12 Aa	53.52±4.68 Aa	45.0±3.53 Aa	$F_{2.12}$=2.032 P=0.174
300 ppm ACN	7.0±4.89 Ab	10.31±3.33 Ab	8.0±2.54 Ab	$F_{2.12}$=0.558 P=0.586
Control	7.0±3.74	3.0±1.22	0±0	
F and P values	$F_{2.12}$=25.564 P<0.0001	$F_{2.12}$=19.165 P<0.0001	$F_{2.12}$=32.668 P<0.0001	

*Differences between the means were determined with the Duncan test at the 5% significance level. Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means that are significantly different from each other (n=5).

Table 7. Corrected mortality of *Rhyzopertha dominica* adults at 14 days after the application of a Turkish diatomoceous earth (ACN) and two isolates of *Beauveria bassiana* at three ambient relative humidities at 30ºC

Treatments	Corrected mortality rates (%)* ± SEM			F and P values
	45%	55%	65%	
300 ppm 5-4 EP	70.99±5.02 Aa	83.15±5.61 Aa	69.80±4.57 Aa	$F_{2.12}$=2.284 P=0.144
300 ppm 1-1 EP	83.85±1.19 Aa	81.15±5.46 Aa	83.40±4.30 Aa	$F_{2.12}$=0.055 P=0.947
300 ppm ACN	26.63±8.63 Ab	49.21±9.94 Aa	25.64±6.59 Ab	$F_{2.12}$=2.439 P=0.129
Control	8.0±3.39	4.0±1.0	4.0±1.87	
F and P values	$F_{2.12}$=23.217 P<0.0001	$F_{2.12}$=5.567 P=0.019	$F_{2.12}$=27.433 P<0.0001	

*Differences between the means were determined with the Duncan test at the 5% significance level. Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means that are significantly different from each other (n=5).

R. dominica adult mortalities after the applications of different combinations of a Turkish DE and *B. bassiana* isolates at three different relative humidities and 30ºC are given in Table 8 (day 7 mortalities) and Table 9 (day 14 mortalities). There was no significant difference in the effects of both combinations in all RH environments

on both day 7 and day 14. Corrected mortality rates 7 days after the application of combinations of local diatomaceous earth ACN and isolates of *Beauveria bassiana* (5-4 and 1-1) against *R. dominica* adults at 30°C and at three ambient relative humidities.

Table 8. Corrected mortality of *Rhyzopertha dominica* adults 7 days after the application of a Turkish diatomoceous earth (ACN) and two isolates of *Beauveria bassiana* at three ambient relative humidities at 30°C

Treatments	Corrected mortality (%) ± SEM			F and P values
	45%	55%	65%	
300 ppm 5-4 EP+300 ppm ACN	76.76±4.83 Aa	77.26±4.96 Aa	68.0±4.89 Aa	$F_{2.12}$=1.043 P=0.382
300 ppm 1-1 EP+300 ppm ACN	79.84±2.08 Aa	74.10±5.27 Aa	72.0±3.0 Aa	$F_{2.12}$=1.127 P=0.356
Control	7.0±3.74	3.0±1.22	0±0	
T and P values	T_8=0.501 P=0.630	T_8=0.450 P=0.665	T_8=0.219 P=0.832	

*Differences between the means were determined with the Duncan test at the 5% significance level. Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means are significantly different from each other (n=5).

Table 9. Corrected mortality rates 14 days after the application of combinations of a Turkish diatomaceous earth ACN and isolates of *Beauveria bassiana* (5-4 and 1-1) against *Rhyzopertha dominica* adults at 30°C and at three ambient relative humidities

Treatments	Corrected mortality (%) ± SEM			F and P values
	45%	55%	65%	
300 ppm 5-4 EP+300 ppm ACN	92.73±3.91 Aa	91.57±4.27 Aa	86.57±3.72 Aa	$F_{2.12}$=0.993 P=0.399
300 ppm 1-1 EP+300 ppm ACN	93.42±1.94 Aa	88.52±6.74 Aa	88.51±1.09 Aa	$F_{2.12}$=0.556 P=0.588
Control	8.0±3.39	4.0±1.0	4.0±1.87	
T and P values	T_8=0.219 P=0.832	T_8=0.238 P=0.818	T_8=0.278 P=0.788	

*Differences between the means were determined with the Duncan test at the 5% significance level. Different uppercase letters in the same row and different lowercase letters in the same column indicate that the means are significantly different from each other (n=5).

When the effect of RH on the efficacies of the treatments against *R. dominica* was determined, lower effects of the *B. bassiana* isolate1-1 on 7th day and 1-1+ACN on the 14th day were observed at 65% RH and 25°C. In general, the efficacies of treatments at both temperatures (25°C and 30°C) were not different in the RH range tested.

The relative humidity is one of the crucial factors in the determination of the efficacy of both fungus and DE (Moore et al, 2000). It has been reported that DEs are not very effective against stored product insects at high RH values (Fields and Korunic, 2000; Vayias and Athanassiou, 2004). In the previous studies, it is understood that the decrease in the effectiveness of the combination in high humidity, environments were due in particular to the effect of RH on the ACN in the combination. Stathers et al. (2004) reported that RH changes the effect of DE on insects and thus our findings support theirs. Athanassiou et al. (2014) also reported that RH affects the efficacy of DE. In their application of 200 ppm DE, 99.4% mortality was achieved at 55% humidity after 14 days, while it was 86.1% at 75% humidity. The results of both studies indicate that high RH decreases the effectiveness of DE.

In conclusion, ambient humidity in the tested range did not change the level of effectiveness of combined applications of EF and DE on the pest insect. Our results demonstrate that *B. bassiana* can be used successfully in conjunction with DE against *R. dominica* under laboratory conditions. It is important to test these two factors in combination in a range of environments to better understand their interaction, with the goal of achieving greater efficacy of treatment. Separately, the search for a fungal strain that is equally or more effective against *R. dominica* at low RH levels should be prioritised.

Acknowledgments

This study, which was accepted as an abstract for oral presentation at the 7th International Entomopathogens and Microbial Control Congress held from 11 to 13 September 2019 in Kayseri, Turkey is a partial summary of the first author's master thesis and was supported by Kahramanmaras Sutcu Imam University Scientific Research Projects Unit (2016/3-38 YLS).

References

Abbott, W.S., 1925. A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology*, 18: 265-267.

Altıkat, A., T. Turan & F. Ekmekyapar 2009. Türkiye'de Pestisit Kullanımı ve Çevreye Olan Etkileri. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 40 (2): 87-92.

Athanassiou, C.G., B.J. Vayias, C.B. Dimizas, N.G. Kavallieratos, A.S. Papagregoriou & C. Th. Buchelos 2003. Insecticidal efficacy of diatomaceous earth against *Sitophilus* oryzae (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* du Val (Coleoptera: Tenebrionidae) on stored wheat: influence of dose rate, temperature and exposure interval. *Journal of Stored Products Research*, 41: 47-55.

Athanassiou, C.G., N.G. Kavallieratos, L.P. Economou, C.B. Dimizas, B.J. Vayias, S., Tomanović & M. Milutinović 2005. Persistence and efficacy of three diatomaceous earth formulations against *Sitophilus oryzae* (Coleoptera: Curculionidae) on wheat and barley. *Journal of Economic Entomology*, 98: 1404-1412.

Athanassiou, C.G. & T. Steenberg 2007. Insecticidal effect of *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreaes) in combination with three diatomaceous earth

formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Biological Control,* 40: 411-416.

Athanassiou, C.G., N.G. Kavallieratos, B.J. Vayias, J.B. Tsakiri, N.H. Mikeli, C.M. Meletsis & Ž. Tomanović 2008. Persistence and efficacy of *Metarhizium anisopliae* (Metschnikoff) Sorokin (Deuteromycotina: Hyphomycetes) and diatomaceous earth against *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae) on wheat and maize. *Crop Protection,* 27: 1303-1311.

Athanassiou, C.G., N.G. Kavallieratos, G.G. Peteinatos, S.E. Petrou, M.C. Boukouvala & Ž. Tomanović 2014, Influence of temperature and humidity on insecticidal effect of three diatomaceous earth formulations against larger grain borer (Coleoptera: Bostrychidae). *Journal of Economic Entomology,* 100(2): 599–603.

Barış, C., 2016. *Beauveria bassiana*'nın Katı ortam fermantasyonu ile üretiminde bazı tahılların kullanımı, yüksek lisans tezi. Kahramanmaraş Sütçü İmam Üniversitesi, Fen Bilimleri Enstitüsü, Bitki Koruma Anabilim Dalı.

Beriş, G., A.G. Ferizli & M. Emekçi 2010. Effects of diatomaceous earth on the mortality and progeny production of *Rhyzopertha dominica* (Coleoptera: Bostrychidae). *Tarım Bilimleri Dergisi,* 17: 85-94.

Bunch T. R., C. Bond, K. Buhl & D. Stone 2013. Diatomaceous Earth General Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services. http://npic.orst.edu/factsheets/degen.html.

Çakır, Ş. & Ş. Yamanel 2005. Böceklerde insektisitlere direnç. Gazi Üniversitesi Kırşehir Eğitim Fakültesi, 6(1): 21-29.

Deacon, J. W., 1983. Microbial Control of Pests and Diseases. New York., 31-41.

Doğanay İ.Ş., A.A. Işıkber, Ö. Sağlam & Y. Bilgili 2014. Efficiency of some Turkish diatomaceous earth deposits against stored-grain insects, *Sitophilus granarius* (L.) and *Rhyzopertha dominica* (F.). International Conference on Biopesticide (ICOP 7), p.91.

Er, M.K., A.A. Işıkber, H. Tunaz, F. Aydın, Ü.T. Özbalıkçıoğlu & A. Öz 2015. Mortality effects of selected native *Beauveria* isolates on three coleopteran pests of stored wheat under controlled conditions. Conference of the IOBC/WPRS (OILB/SROP) Working Group on Integrated Protection of Stored Products, Zagreb, Croatia June 28 - July 1, 2015. 87s.

Fields, P. & Z. Korunic, 2000. The effect of grain moisture content and temperature on the efficacy of diatomaceous earths from different geographical locations against stored-product beetles. *Journal of Stored Product Research,* 36: 1–13.

Greenspan, L., 1977. Humidity fixed points of binary saturated aqueous solutions. *Journal of Research of the National Bureau of Standards Section A-Physics and Chemistry,* 81: 89-96.

Karaca, İ. & T. Ayyıldız 2018. Bazı Biyolojik Preparatların *Sitophilus granarius* (Coleoptera: Curculionidae) Erginlerine Etkileri. *Journal of Natural and Applied Sciences,* 22(1): 671-678.

Moore, D., J.C. Lord & S.M. Smith 2000. Pathogens. In: Subramanyam, Bh., Hagstrum, D.W. (Eds.), Alternatives to Pesticides in Stored-Product IPM. Kluwer Academic Publishers, Dordreecht, pp. 193–227.

Michalaki, M.P., C.G. Athanassiou, N.G. Kavallieratos, Y.A. Batta & G.N. Balotis 2006. Effectiveness of *Metarhizium anisopliae* (Metschinkoff) Sorokin applied alone or in combination with diatomaceous earth against *Tribolium confusum* du Val. larvae: Influence of temperature, RH and type of commodity. *Crop Protection,* 25: 418-425.

Mahdneshin, Z., M. H. Safaralizadah & Y. Ghosta 2009. Study on the efficacy of Iranian isolates of *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin against *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). *Journal of Sciences*, 9(2): 170-174.

Riasat, T., W. Wakil, A. Muhammad & S. Shahbaz 2011. Effect of *Beauveria bassiana* mixed with diatomaceous earth on mortality, mycosis and sporulation of *Rhyzopertha dominica* on stored wheat. *Phytoparasitica*, 39(4): 325-331.

Stathers, T., M. Denniff & P. Golob 2004. The efficacy and persistence of diatomaceous earth admixed with commodity against four tropical stored product beetle pests. *Journal of Stored Products Research*, 40: 113-123.

Sabbour, M., S. Abd-El-Aziz & M. Sherief 2012. Efficacy of three entomopathogenic fungi alone or in combination with diatomaceous earth modifications for the control of three pyralid moths in stored grains. Journal of Plant Protection Research, 53(3): 359-363.

Sewify, G.H., H.A. El Shabrawy, M.E. Eweis, & H. Naroz Magda 2014. Efficacy of entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae*, for controlling certain stored product insects. *Egyptian Journal of Biological Pest Control*, 24(1): 191-196.

Shafighi, Y., M. Ziaee & Y. Ghosta 2014. Diatomaceous earth used against insect pests, applied alone or in combination with *Metarhizium anisopliae* and *Beauveria bassiana*. *Journal of Plant Protection Research*, 54(1): 62-66.

SPSS 2015. SPSS 24 for Windows User's Guide Release. Spss Inc, Chicago.

Şahin Z. & M.K. Er 2014. Effects of local *Beauveria bassiana* isolates on *Sitophilus oryzae* adults under controlled conditions. International Conference on Biopesticides 7 (ICOB7), Side-Antalya, Turkey, 19-25 October, 2014. 133s.

Um, M., I Galadima, F. Gambo & D. Zakaria 2018. A review on the use of entomopathogenic fungi in the management of insect pests of field crops. *Journal of Entomology and Zoology Studies*, 6(1): 27-32.

Vayias, B.J. & C.G. Athanassiou 2004. Factors affecting efficacy of the diatomaceous earth formulation SilicoSec against adults and larvae of the confused beetle *Tribolium confusum* Du Val (Coleoptera: Tenebrionidae). *Crop Protection* 23: 565–573.

Vassilakos, T.N., C.G. Athanassiou, N.G. Kavallieratos & B.J. Vayias, 2006. Influence of temperature on the insecticidal effect of *Beauveria bassiana* in combination with diatomaceous earth against *Rhyzopertha dominica* and *Sitophilus oryzae* on stored wheat. *Biological Control*, 38: 270-281.

Wakil, W., M. Ashfaq, M.U. Ghazanfar & T. Riasat 2010. Susceptibility of stored-product insects to enhanced diatomaceous earth. *Journal of Stored Products Research*, 46: 248-249.

Wakil, W., T. Riasat, M.U. Gazanfar, Y.J. Kwon & F.A. Shaseen 2011. Aptness of *Beauveria bassiana* and enhanced diatomaceous earth (DEBBM) for control of *Rhyzopertha dominica*. *Entomological Research*, 41(6): 233–241.