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Uygulama Sonrası Zararlı Bulaşmasından Depolanmış Buğdayın Korunması Bakımından *Beauveria bassiana* Etkinliğinin Kalıcılığı

Huriye Yasemin KORKMAZ[1]✉, Mehmet Kubilay ER[2]
[1]Department of Plant Protection, Graduate School of Natural and Applied Sciences, University of Kahramanmaraş Sütçü İmam, Kahramanmaraş, Türkiye, [2]Department of Plant Protection, Faculty of Agriculture, University of Kahramanmaraş Sütçü İmam, Kahramanmaraş, Türkiye
[1]https://orcid.org/0000-0002-7586-1812, [2] https://orcid.org/ 0000-0003-1568-8656
✉: hkorkmaz@ksu.edu.tr]

ÖZET

Bu çalışma, üç yerel *Beauveria bassiana* izolatının spor etkinlik sürelerinin belirlenmesi amacıyla, yaygın olan depolanmış ürün zararlılarından *Sitophilus oryzae* L. (Coleoptera: Curculionidae) *Rhyzopertha dominica* F. (Col.: Bostrichidae) ve *Oryzaephilus surinamensis* L. (Col.: Silvanidae) erginlerine karşı uygulanarak yürütülmüştür. Biyolojik testler için buğday danelerine 1000 ppm (w/w) konsantrasyonunda *B. bassiana* sporu karıştırılmış ve 1, 15 ve 28 gün sonra 20'şer ergin salımı yapılmıştır. *Sitophilus oryzae* ile yürütülen testlerde 3 izolat için genel olarak; 7. ve 14. gün ölüm oranı başlangıçta %33.3-41.6 ve %68.3-76.6 olup 28 gün sonraki salımlarda %6.6-18.3 ve %13.3-21.6'ya düşmüştür. *Rhyzopertha dominica* başlangıç ölüm oranları 7. ve 14. gün için %46.6-50.0 ve %93.3-95.0'dır ve 28 gün sonraki salımlar sonucunda %10.0-18.3 ve %16.6-28.3 olmuştur. *Oryzaephilus surinamensis* başlangıç ölümleri ise 7. ve 14. gün için %41.6-46.6 ve %70.0-85.0'den 28 gün sonraki salımda %8.3-15.0 ve %13.3-20.0'a düşmüştür. Buğdayda sporların bekleme süresi uzadıkça, tüm fungus izolatları ve böcek türleri için her inkübasyon süreci sonrasında ölüm oranları önemli derecede düşmüştür. İnkübasyon süreçlerinin tümünde tüm izolatlar için benzer ölüm oranları belirlenmiş ve etkinlik kayıplarının benzer olduğu gözlenmiştir. Tüm sonuçlar *B. bassiana* izolatlarında zamana bağlı aktivite kaybından sorumlu faktörlerin belirlenmesi ve bu veriler doğrultusunda önlem alınması gerektiğini göstermektedir.

Entomoloji

Araştırma Makalesi

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Anahtar Kelimeler
Biyolojik mücadele
Mikrobiyal mücadele
Entomopatojen fungus
Beauveria bassiana
Depolanmış ürün zararlıları

Persistence of *Beauveria bassiana* Efficacy on Wheat Grains to Protect Stored-Grains from Post-Treatment Pest Infestations

ABSTRACT

This study was carried out to determine the post-treatment efficacy of three local *Beauveria bassiana* isolates by applying them against adults of three common stored-product pests, *Sitophilus oryzae* L. (Coleoptera: Curculionidae) *Rhyzopertha dominica* F. (Col.: Bostrichidae) and *Oryzaephilus surinamensis* L. (Col.: Silvanidae).

For the biological tests, wheat grains were mixed with fungal spores at 1000 ppm (w/w) concentration then 20 adults were released 1, 15 and 28 days after the treatments. For the tests carried out with *S. oryzae*; the mortality rate was initially 33.3-41.6% and 68.3-76.6%; and declined to 6.6-18.3% and 13.3-21.6% on the 7th and 14th day, respectively, when released 28 days later. The initial mortality of *R. dominica* was 46.6-50.0 % and 93.3-95%; and became 10-18.3% and 16.6-28.3% when released 28 days later. *Oryzaephilus surinamensis* mortality on the 7th and 14th day was initially 41.6-46.6% and 70.0-85.0%, and decreased to 8.3-15% and 13.3-20.0%, respectively, when released 28 days later. Mortality rates at the end of each incubation period, for all testing isolates and insect species, were significantly reduced when waiting time of spores on wheat was prolonged. For all incubation periods, similar mortality rates were obtained from all

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isolates and similar efficiacy loss was observed. All the results together indicate that the responsible factors for time-dependent loss of activity in *B. bassiana* isolates should be determined, and in line with these data, precautions need to be taken.

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INTRODUCTION

Cereals are among the most important food sources. Grain production in Turkey was 37.187.508 tons in 2020 (Anonymous, 2021). The climatic conditions and inproper storage facilities in Türkiye often create favorable environments for the development of insect pests. Stored-cereals are commonly exposed to stored-product insect pests. These pests are responsible for qualitative and quantitative loss due to feeding activities reducing weight, nutrient content and germination as seeds while contaminating cereals due to their existance and waste accumulation, consequently decreasing market value of the commodity (Sewify et al., 2014). Among pests of stored-cereals, the rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), saw toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) and the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrychidae) are considered notorious species worldwide (Kaur et al., 2014). Damage increases rapidly when several generations develop during the storage period. There are some insecticides registered for use against these pests. However, as a result of excessive pesticides usage, significant problems arise including insecticide resistance, hazards to human, livestock and environment. Scientists have been seeking alternative pest management strategies for stored-product pests to avoid such adverse effects of insecticides. Entomopathogenic fungi can control stored-grain insect pests effectively according to Batta (2016). Around the World, fungal isolates have been tested against various stored-product pest species both under controlled conditions and in field experiments. High mortality rates from applications were reported by several authors who tested *B. bassiana* on various insects (Heviefo et al. 2020). Batta (2003) mentioned that different fungal isolates of the same species can vary in infectivity to a given host species. Wakil et al. (2020) suggested that a mixture of biological control agents and natural or chemical substances exhibiting insecticidal properties has a potential to provide high protection of stored-cereals for short or long storage periods. Batta and Kavallieratos (2018) as a result of their work emphasized that obtaining new and effective isolates of entomopathogenic fungi for biological control of stored-product pest insects and improving their formulations are important requirements for success. Another area requiring more studies is their post-application status. This study was carried out to determine the post-treatment efficacy of three local *B. bassiana* isolates against three coleopteran pests (*R. dominica, S. oryzae* and *O. surinamensis*) of stored grains.

MATERIAL and METHODS

Insect cultures

Insect cultures were maintained in the laboratory, Department of Plant protection at University of Kahramanmaraş Sütçü İmam. *Oryzaephilus surinamensis* cultures were maintained on wheat with oat flakes, the *S. oryzae* and *R. dominica* cultures were maintained on wheat grains at 25±2°C and 65±5% relative humidity in darkness. Adults were left in clean food for three days for oviposition. After this period all adults removed and cultures incubated for the emergence of new generation adults. Seven-ten days old adults were used for testing.

Fungal cultures

Beauveria bassiana isolates were previously obtaied from stored-grain pests collected from grain storages in Adana and Kahramanmaraş province in Turkey. Isolate identifications; carried out using morphological and molecular techniques. Three *B. bassiana* isolates (1-1, 22-1, 42-1) used in this study were quite successful in terms of mortality and reduction in new generation emergence of three coleopteran pests (*R. dominica*, *S. oryzae* and *O. surinamensis*). Potato Dextrose Agar (PDA) was used for growing fungal isolates in Petri dishes (Ø = 90 mm). After sealing the Petri dishes with Parafilm they were incubated at 26°C ± 1°C in a dark incubator for 10 days. The cultures that completed sporulation were left open one night to reduce moisture. Then, the conidia of the fungi were gathered by vacuming. They were kept at +4°C on silica gel in Eppendorf tubes until used. Germination test was performed for fungal spores before experiments. Fungal spores were diluted in 0.01% Tween 80 solution and spread on PDA medium. After 24 hours at 25°C the spores were examined under a microscope (×40) and those with a

germ tube equal or longer than the spore were considered germinated. The spores used in the experiments had germination rate of 96-98%.

Experimental design

Wheat grains were homogenously mixed with *B. bassiana* spores at 1000 ppm (w/w) concentration and 40 g was placed in each centrifuge tube (50ml capacity). One, 15 and 28 days after the treatment of wheat grains, twenty adults were release into each tube. As controls, pests were released simultaneously into clean grains without fungal spores. All were incubated under the same conditions at 25±2°C, 65±5% relative humidity in darkness. The experiment had 3 repetitions and mortality rates were assessed on the 7th and 14th days after insect releases.

Statistical analysis

Adult mortality rates were corrected by using Abbott's formula (Abbott, 1925) before Arcsine transformation. The data were subjected to one-way ANOVA, and Duncan multiple comparison test at 5% significance level to determine differences between treatments. The statistical analysis were performed by using SPSS15.0.

RESULTS and DISCUSSION:

This study was to investigate post-treatment efficacy of three *Beauveria bassiana* isolates against adults of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Oryzaephilus surinamensis* under laboratory conditions. The results for *S. oryzae, R. dominica* and *O. surinamensis* were presented in Table 1, 2 and 3, respectively. They confirmed the pathogenic characteristic of the *B. bassiana* isolates (1-1, 22-1, 42-1) to the tested stored-grain pests. The mortality effects of the testing isolates were statistically the same in majority of the experimental units (Tables 1,2,3). The only exceptions were 7th day mortalities of *S. oryzae* for insect release 15 days after fungus application (AFA) ($F_{2,6}$=6.45, P< 0.05) and 14th day mortalities of *R. dominica* for insect release 28 days AFA ($F_{2,6}$=12.30, P<0.01). In both cases, isolate 42-1 killed significantly lower insects than the other two *B. bassiana* isolates. The mortality of *S. oryzae* adults was 33.3-41.6% and 68.3-76.6% on the 7th and 14th day, respectively, when the insects were released one day AFA. The mortalities declined gradually with extended time gap between fungus application and insect release. The mortality was 6.6-18.3% and 13.3-21.6% on the 7th and 14th day, respectively, when the insects were released 28 days AFA. Mortality rates segragated for each time gap between fungus application and insect release, particularly more clear for the results obtained on 14th day post-release. The trend was the same for the other two tested insect species. The mortality of *R. dominica* adults was 46.6-50.0% and 93.3-95.0% on the 7th and 14th day, respectively, when the insects were released one day AFA. These mortality rates became 10.0-18.3% and 16.6-28.3% when the time gap was increased to 28 days. *Oryzaephilus surinamensis* adult mortality on 7th and 14th day was initially 41.6-46.6% and 70.0-85.0%, and decreased to 8.3-15.0% and 13.3-20.0% for 1 and 28 days AFA, respectively.

All the obtained data showed clearly that mortality rates at the end of both incubation periods were significantly reduced with prolonged time between treatment of wheat with fungal spores and release of the insects. This was the case for all three fungal isolates and for all pest species tested (Tables 1- 3). Each two-weeks of time gap caused mostly a reduction of 35-60%; in one case, an almost 70% decline in *R. dominica* mortality between 15th and 28th days for isolate 42-1.

One of the most important factors in pathogenicity is the virulence of the pathogen, and each isolate has a specific innate capacity (Soetopo, 2004). Er et al. (2016), in the search for biological agents, reported that wild fungi comprise genetic and adaptive diversity. They show utility of selecting single spores from wild fungal isolates for studies towards developing more virulent microbial control agents. As a result of the trials carried out with *S. oryzae* to determine the rate of decrease in the number of individuals in following generation, Korkmaz (2017) determined that only isolate 1-1 caused a decrease of 100% amongst several isolates. Spore viability is another important factor that influences the efficacy of entomopathogenic fungi, and thus maintaining viability of spores is as important as the virulence of the fungus against targeted pest (Glare et al., 2012, Moore et al.,2000; Batta, 2004). Aregger (1992) demonstrated that viability loss of *B. bassiana* spores varies among isolates. However, mortality effect of fungal isolates is not solely dependent on their virulence and their spore viability, but also on the conditions in which they are applied.

Ambient temperature and humidity, aeration, light, air as well as the condition of the host itself have significant effects on the pathogenicity of entomopathogenic fungi (Padmini and Padmaja 2010). Kim et al (2019) studied the effect of temperature on storing *B. bassiana* for a long-term and found that *B. bassiana* can tolerate a wide range of temperatures between 4°C and 30°C. However, in long-term storage of entomopathogenic fungi, fungal isolate, ambient conditions during its production and storage influence the viability (Faria et al., 2009; Blanford et al., 2012). Therefore, it seems that stability of insecticides based on entomopathogenic fungi is a major obstacle for commercialization. Several studies have been conducted for stabilization of isolates under a range of temperature and

Table 1. Mortality rates on days 7 and 14 following release of *Sitophilus oryzae* adults on wheat treated with 1000 ppm *Beauveria bassiana* spores.

Çizelge 1. Buğdayın 1000 ppm fungus sporu ile muamelesinden 1, 15, 28 gün sonra Sitophilus oryzae erginlerinin düzeneklere bırakılmasını izleyen 7. ve 14. günlerdeki ölüm oranları

Isolate no. (İzolat no)	7th day mortality rates (%) ± SEM *(7. gün ölüm oranı(%) ± S.Hata)*			F ve P values *(F ve P değeri)*
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	41.6 ± 0 Aa	23.3 ± 1.6Ab	18.3 ± 1.6Ab	$F_{2,6}$=29.17 P<0.001
22-1	33.3 ± 3.3Aa	21.6 ± 6ABb	18.3 ± 1.6Ab	$F_{2,6}$=51.43 P<0.001
42-1	36.6 ± 4.4Aa	16.6 ± 7.2Bab	6.6 ± 3.3Ab	$F_{2,6}$=6.88 P<0.05
Control(Kontrol)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
F and P values *(F ve P değeri)*	$F_{2,6}$= 1.27 P= 0.3462	$F_{2,6}$=6.45 P< 0.05	$F_{2,6}$=4.14 P=0.0742	
Isolate no. (İzolat no)	14th day mortality rates (%) ± SEM *(14. gün ölüm oranı(%) ± S.Hata)*			F ve P values *(F ve P değeri)*
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	76.6 ± 3.3Aa	41.6 ± 1.6Aab	18.2 ± 3.1Ab	$F_{2,6}$=84.39 P<0.0001
22-1	70.0 ± 2.8Aa	38.3 ± 4.4Ab	21.6 ± 1.6Ac	$F_{2,6}$=69.43 P<0.001
42-1	68.3 ± 3.3Aa	30 ± 2.8Ab	13.3 ± 1.6Ac	$F_{2,6}$=91.52 P<0.0001
Control *(Kontrol)*	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
F and P values *(F ve P değeri)*	$F_{2,6}$= 1.97 P=0.2203	$F_{2,6}$= 3.55 P= 0.0962	$F_{2,6}$= 3.42 P=0.1019	

*Different capital letters in each column and different lowercase letters in each line constitute statistically different groups according to Duncan multiple comparison tests (P ≤ 0.05).

Table 2. Mortality rates on days 7 and 14 following release of *Rhyzopertha dominica* adults on wheat treated with 1000 ppm *Beauveria bassiana* spores

Çizelge 2. Buğdayın 1000 ppm fungus sporu ile muamelesinden 1, 15, 28 gün sonra Rhyzopertha dominica erginlerinin düzeneklere bırakılmasını izleyen 7. ve 14. günlerdeki ölüm oranları

Isolate no. (İzolat no)	7th day mortality rates (%) ± SEM *(7. gün ölüm oranı(%) ± S.Hata)*			F and P values (F ve P değeri)
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	50.0 ± 5.0Aa	33.3 ± 3.3Aab	18.3 ± 6.0Ab	$F_{2,6}$=7.06 P<0.05
22-1	50.0 ± 5.7Aa	28.3 ± 4.4Ab	18.3 ± 4.4Ab	$F_{2,6}$=10.46 P<0.05
42-1	46.6 ± 1.6Aa	35.0 ± 5.0Aa	10.0 ± 2.8Ab	$F_{2,6}$=28.03 P<0.001
Control(Kontrol)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
F and P values (F ve P değeri)	$F_{2,6}$= 0.11 P=0.2203	$F_{2,6}$= 0.56 P= 0.5990	$F_{2,6}$= 1.20 P= 0.3637	
Isolate no. (İzolat no)	14th day mortality rates (%) ± SEM *(14. gün ölüm oranı(%) ± S.Hata)*			F and P values (F ve P değeri)
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	93.3 ± 6.6Aa	60.0 ± 2.8Ab	28.3 ± 1.6Ac	$F_{2,6}$=29.03 P<0.001
22-1	95.0 ± 5.0Aa	46.6 ± 4.4Ab	23.3 ± 1.6Ac	$F_{2,6}$=46.97 P<0.001
42-1	95.0 ± 2.8Aa	53.3 ± 6.0Ab	16.6 ± 1.6Bc	$F_{2,6}$=62.91 P<0.0001
Control(Kontrol)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	
F and P values (F ve P değeri)	$F_{2,6}$= 0.04 P=0.9638	$F_{2,6}$=2.08 P= 0.2062	$F_{2,6}$=12.30 P<0.01	

*Different capital letters in each column and different lowercase letters in each line constitute statistically different groups according to Duncan multiple comparison tests (P ≤ 0.05).

Table 3. Mortality rates on days 7 and 14 following release of *Oryzaephilus surinamensis* adults on wheat treated with 1000 ppm *Beauveria bassiana* spores

Çizelge 3. Buğdayın 1000 ppm fungus sporu ile muamelesinden 1, 15, 28 gün sonra Oryzaephilus surinamensis erginlerinin düzeneklere bırakılmasını izleyen 7. ve 14. günlerdeki ölüm oranları

Isolate no. (İzolat no)	7thday mortality rates (%) ± SEM *(7. gün ölüm oranı(%) ± S.Hata)*			F and P values (F ve P değeri)
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	46.6 ± 3.3 Aa	26.6 ± 1.6 Ab	15 ± 5.7 Ab	$F_{2,6}$=11.37 P < 0.01
22-1	46.6 ± 4.4 Aa	23.3 ± 1.6 Aa	8.3 ± 4.4 Ab	$F_{2,6}$=13.41 P < 0.01
42-1	41.6 ± 3.3 Aa	11.6 ± 6 Ab	8.3 ± 1.6 Ab	$F_{2,6}$=7.82 P < 0.05
Control(Kontrol)	0.0 ± 0.0	0.0±0.0	0.0 ± 0.0	
F and P values (F ve P değeri)	$F_{2.6}$= 0.60 P=0.9638	$F_{2,6}$=2.61 P= 0.1527	$F_{2,6}$= 0.67 P=0.5456	
Isolate no. (İzolat no)	14thday mortality rates (%) ± SEM *(14. gün ölüm oranı(%) ± S.Hata)*			F and P values (F ve P değeri)
	Released 1 day after treatment *(1 gün sonra salım)*	Released 15 days after treatment *(15 gün sonra salım)*	Released 28 days after treatment *(28 gün sonra salım)*	
1-1	83.3 ± 3.3Aa	39.6 ± 1.7Ab	20.0 ± 2.8Ac	$F_{2,6}$=96.06 P < 0.0001
22-1	85 ± 6.6Aa	36.2 ± 6.2Ab	20.0 ± 0Ab	$F_{2,6}$=18.66 P < 0.01
42-1	70.0 ± 6.6Aa	29.3 ± 4.5Ab	13.3 ± 1.6Ac	$F_{2,6}$=31.14 P < 0.001
Control(Kontrol)	0.0 ± 0.0	3.3 ± 1.6	0.0 ± 0.0	
F and P values (F ve P değeri)	$F_{2.6}$= 1.39 P=0.3190	$F_{2,6}$= 1.38 P= 0.3411	$F_{2,6}$=4.15 P=0.0738	

*Different capital letters in each column and different lowercase letters in each line constitute statistically different groups according to Duncan multiple comparison tests ($P \leq 0.05$).

ultraviolet light, and for obtaining thermotolerant fungal cultures (Kim et al., 2011; Santos et al., 2011; Shin et al., 2017).

In the present study, the tested three *B. bassiana* isolates expressed virulence against the pest species, but reduction in viability of their spores for long periods was their weakness. The factors responsible for the reduction of *B. bassiana* efficacy during the time after application should be determined and taken into account while spore production or application. Should they be developed as biocontrol agents as they are, their application needs to be repeated as required in time. According to Jackson (1997) and Rangel et al. (2015), it is possible to find more virulent and tolerant fungal isolates by screening. They also emphasize the importance of optimization of nutritions and physical manipulations while growing fungi for conidial vigor. St. Leger and Wang (2010) proposed using genetic engineering to increase the resistance of fungi to environmental factors.

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Conflict of Interest

The author declares that there is no conflict of interest in the study.

Author's Contributions

The contribution of the authors is equal.

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