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Pathogenicity of *Beauveria bassiana* (Balsamo) Vuillemin F7-1 on *Sitophilus oryzae* L. (Coleoptera: Curculionidae) Adults and the Effect of Ambient Humidity

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Abstract

Sitophilus oryzae causes considerable damage in stored grain. Considering sustainable agricultural practices biological control plays an important role in the control of this pest. In this study, the pathogenicity of a *Beauveria bassiana* isolate (F7-1) against *S. oryzae* adults and the effect of ambient relative humidity (RH) were investigated. For each experimental unit, fungal spores were applied to 40 gr wheat kernels within a 50 ml centrifuge tube. Thereafter, *S. oryzae* adults were placed in the tube. The tests were conducted at 26±2 °C temperature, 65±5% RH in darkness. The mortality of *S. oryzae* was recorded 7 and 14 later. For the pathogenicity test, spore concentrations were applied at 250, 500, 1000 and 1500 ppm. Increasing with the concentration, lethal effect reached to a maximum of 87.7%. According to the results of the experiment carried out to determine the effect of ambient relative humidity on fungal pathogenicity, adult mortality did not differ statistically at 65-75% RH. However, when RH was increased to 100%, the mortality reached to a statistically higher level of 91.6%. It is concluded that *B. bassiana* F7-1 has a considerable potential providing appropriate concentrations, and ambient humidity did not have a limiting effect except for extreme conditions.

Keywords: Rice weevil; Stored grain; Microbial control; Hypocreales

INTRODUCTION

Cereals and their products constitute an important share in nutrition in Türkiye, mosly due to the fact that the climate zone in which Türkiye is located is suitable for the cultivation of wheat and other cereals (Dizlek, 2012). According to FAO, wheat was cultivated on 6623061 ha area in Türkiye in 2021 (Anonymous, 2023). Harvested wheat needs to be stored and protected due to its consumption over a long period of time. During this post-harvest process, many abiotic and biotic factors have a significant impact on product quality and quantity. Unless appropriate precautions are not taken considering storage conditions and period, 10-20% loss can increase as high as 100% (Yıldırım et al., 2009). One of the biotic factors affecting stored products is insect pests. Rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), a primary pest, can cause significant losses in wheat warehouses. In order to prevent this, biological, chemical and physical control methods can be applied after monitoring and decision-making processes (Hagstrum, 2014). Due to various negative effects of chemical insecticides, which are frequently used against stored product pests, many studies have been carried out on physical, mechanical and biological control methods as alternatives to insecticides (Yıldırım, 2000; Vincent et al., 2001; Lucas and Riudavets, 2002).

Chemical control is still the most widely used method in stored product insect pest management, but these chemicals have increased problems related with human health, environmental pollution, resistance of pests to pesticides and disruption of the natural balance (Bora and Özaktan, 1998). In addition to efforts to reduce these problems, sustainable agricultural practices, organic farming and activities for the conservation of biodiversity emphasize the use of alternative control methods instead

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of chemical insecticides. Therefore, the importance of biological control and studies in this direction have gradually increased. Among biological control agents, entomopathogenic fungi are considered as promising agents and studies on their development against stored grain pests were reviewed by Batta and Kavallieratos (2018). It was determined that damage and losses in stored products can be reduced with entomopathogenic fungus applications (Mantzoukas et al., 2022).

In the selection of entomopathogenic agents for insect control, initially two features are quite important; high efficacy against targeted pest and tolerance to environmental factors. Among the environmental factors, especially ambient humidity has a significant effect on entomopathogenic fungus activity (Demirci et al., 2011). Ambient humidity affects not only the germination of the fungal spores and crucial for initiation of infection, but also conidiation on dead insects and consequently dissemination of infection (Acheampong et al., 2020). Prior to studies on the utilization of entomopathogenic fungi, efficacy studies at a range of ambient humidity are required (Mishra et al., 2013). In this study, the effect of various concentrations of *B. bassiana* isolate F7-1, selected among 33 *Beauveria* isolates considering its pathogenicity (Şahin, 2015), on *S. oryzae* adults and the effect of ambient humidity on the performance of *B. bassiana* F7-1 were determined.

MATERIALS and METHODS

Insect culture

Rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionidae) culture in the laboratory of Plant Protection Department of Kahramanmaraş Sütçü İmam University was used. 'Elbistan yazlığı' whole soft wheat (*Triticum aestivum* L.) with 11-13% moisture content was used for the culture of *S. oryzae*. After placing 250 g of clean whole soft wheat kernels in a 1 l capacity glass jar, mixed gender adults were placed inside and the jar was covered with gauze and kept in a climate room at 26±2 ºC temperature and 65±5% relative humidity under completely dark conditions. After three days of oviposition, the adults were removed in jars. When the new generation of adults emerged, they were separated with the help of sieves and transferred to other glass jars containing clear wheat kernels to initiate new cultures. Care was taken to ensure that the adults used in the experiments were of the same age of 7-10 days.

Fungal culture

Beauveria bassiana isolate F7-1 was initially obtained from soil sample taken from a pistachio orchard and kept in fungal culture collection in Plant Protection Laboratory at Kahramanmaraş Sütçü İmam University. The fungal spores used in the experiments were obtained from cultures that completed sporulation after growning on PDA (potato dextrose agar) at 26±2 °C and 16/8 hours light/dark conditions for about one month. As described in Athanassiou and Steenberg (2007), these cultures were kept open overnight to reduce moisture, before collecting spores by vacuuming. They were kept on silica gel at +4 °C for a maximum of two days until use.

Experimental design

The wheat grain to be used for the experiments were sieved and kept at -18 ºC for 10 days to eliminate any possible previous pest infestation. Before used in the experiments the wheat was get to room temperature. For the mortality test with various concentrations of *B. bassiana* F7-1 on *S. oryzae* adults, 50 ml centrifuge tubes containing 40 g of wheat were used as experimental units and the top of the tubes were covered with gauze to ensure ventilation. After adding the required amount of fungal spores for designated concentration to each tube, they were mixing for 20 minutes on a mechanical horizontal shaker. Thereafter, twenty 7-10 days old mixed gender *S. oryzae* adults were placed into the

tubes. In the control treatments, the setup was the same without fungal spores. Spore concentrations of 250, 500, 1000, 1500 ppm were used in the experiment. The study was carried out in a completely dark climate room at 26±1 °C temperature and 65±5% relative humidity. The experiment was established according to randomized plot experimental design with three replications and the experiment was repeated three times. Live/dead insect counts were made at the end of one and two weeks, and dead ones were removed from the test in the first count. Prior to the experiment, the germination rate of fungal spores was determined by examining under a light microscope at 40× magnification 24 hours after the spores were spread on PDA medium. The test was carried out under the same conditions as the fungal cultures. The germination rates were determined by counting at least 100 spores, and those with germ tubes at least as long as the spore length were considered as germinated. The process was repeated three times. The germination rate of the spores used in the experiment was 96-98%.

The test to determine the effect of ambient relative humidity on the performance of *B. bassiana* F7-1 was carried out in the same way and under the same conditions as the previous experiment described above. The units prepared with 1000 ppm spore concentration were kept at 65%, 75% and 100% relative humidity conditions during the experiment. 1000 ppm was chosen so that any increase as well as decrease in mortality can be detected under different relative humidity conditions. While 65% relative humidity was provided within the climate room, saturated NaCl solution for 75% humidity and pure water (500 ml each) for 100% relative humidity were ensured in plastic containers (22×20×23 cm) used as humidity chambers The lid of the plastic container was closed and sealed with a flexible tape to prevent air exchange.

Statistical analysis

The mortality rates obtained at 7 and 14 days after the treatment were corrected using Abbott's formula (Abbott, 1925) and then arcsine transformation (Zar, 1996) was applied. Data were subjected to one-way and two-way analysis of variance. Differences between treatments were analyzed by Duncan's test at 5% significance level. Where only two treatments were required to be compared, independent samples t test was used. Statistical tests were performed using SAS computer program (Proc GLM; SAS Ins., 2009).

RESULTS and DISCUSSION

The results of the experiment carried out to determine the effects of different concentrations of *B. bassiana* isolate F7-1 on *S. oryzae* adult mortality are presented in Table 1. It is found that as the fungal spore concentration increases, mortality rates also increase. As a result of the two-way analysis of variance, the concentration and exposure time had statistically significant effects on mortality rates (Concentration $F_{4,80}$= 191.50, P<0.0001; exposure time $F_{1,80}$= 89.90, P<0.0001; interaction $F_{4,80}$= 3.30, P<0.05). At the end of the 7th day, all concentrations caused statistically different mortality from each other, but according to the 14th day results, there was no statistical difference between 1000 ppm and 500 ppm in terms of the mortality they caused. The fact that it caused more than 50% mortality at 500 ppm after 14 days of the treatment and a very high mortality at 1500 ppm shows that this isolate is promising for the control of *S. oryzae*.

Table 1. *Mortality rates of Sitophilus oryzae adults on the 7th and 14th day after application of various concentrations of Beauveria bassiana F7-1 spores under three different ambient humidities*

Spore concentration	Mean mortality ± SEM (%)		t and P values
	7thday	14thday	
1500 ppm	75.5±4.5 Ab*	87.7±2.6 Aa	t(16)= 2.42, P=0.028
1000 ppm	46.6±3.8 Bb	66.1±1.3 Ba	t(16)= 4.77, P<0.0001
500 ppm	25.5±2.4 Cb	57.2±3.0 Ba	t(16)= 8.03, P<0.0001
250 ppm	16.1±2.1 Db	42.2±4.1 Ca	t(16)= 5.55, P<0.0001
Control	2.7±0.8 Ea	6.1±1.1 Da	t(16)= 2.08, P=0.054
ANOVA P and F values	$F_{4,40}$= 81.95 P<0.0001	$F_{4,40}$= 118.30, P<0.0001	

*One-way analysis of variance and Duncan test (P≤0.05) were used for spore concentrations. Mortalities on two days were compared by t tests (P≤0.05). Different capital letters in the same column and different lower case letters in the same row are statistically different from each other.

The results of the experiment on the effect of ambient relative humidity on the performance of *B. bassiana* F7-1 are shown in Table 2 and Table 3 for the 7th day and 14th day, respectively. As can be seen from Table 1, mortality rates were almost the same in the first 7 days at 65% and 75% RH, while an increase was noticed at 100% humidity. As a result of the analysis of variance performed on the data obtained at the end of the 7th day, it was determined that humidity had a statistically significant effect on mortality rates (Humidity $F_{2,48}$ =22.98, P<0.0001, Concentration $F_{1,48}$ = 589.27, P<0.0001; and interaction $F_{2,48}$ = 6.34, P<0.01). ANOVA results were similar to those of 7th day data; humidity had a statistically significant effect on mortality rates (Humidity $F_{2,48}$ = 13.79, P<0.0001, concentration $F_{1,48}$ = 740.36, P<0.0001; interaction $F_{2,48}$= 8.62, P<0.001). According to the results at the end of the 14th day, there was no statistical difference between 65% and 75% RH in terms of mortality caused by the fungus. However, 100% RH resulted in a statistically significant high insect mortality.

Table 2. *Mortality rates of Sitophilus oryzae adults 7 days afterapplication of 1000 ppm Beauveria bassiana F7-1 spore concentration under three different ambient humidities*

Treatments	Mean mortality ± SEM (%)			ANOVA F and P values
	% 65	% 75	% 100	
B. bassiana F7-1	46.6±3.8 Ab*	47.2±2.3 Ab	77.7±4.1 Aa	$F_{2,24}$= 24.14 P<0.0001
Control	0.5±0.5 B	2.2±0.8 B	4.4±1.3 B	-
t and P values	t(16) =15.665 P<0.0001	t(16)=14.194 P<0.0001	t(16) =13.416 P<0.0001	

*One-way analysis of variance and Duncan test (P≤0.05) were used for relative humidities. Mortalities of treatment and control were compared by t tests (P≤0.05). Different capital letters in the same column and different lower case letters in the same row are statistically different from each other.

Table 3. *Mortality rates of Sitophilus oryzae adults 14 days after application of 1000 ppm Beauveria bassiana F7-1 spore concentration under three different ambient humidities*

Treatments	Mean mortality ± SEM (%)			ANOVA F and P values
	% 65	% 75	% 100	
B. bassiana F7-1	66.1±1.3 Ab*	75.5±1.9 Ab	91.6±2.6 Aa	$F_{2,24}$= 24.53 P<0.0001
Control	2.7±0.8 B	5.0±1.4 B	5.0±1.4 B	-
t and P values	t(16)=19.475 P<0.0001	t(16)=16.533 P<0.0001	t(16)=14.421 P<0.0001	

*One-way analysis of variance and Duncan test (P≤0.05) were used for relative humidities. Mortalities of treatment and control were compared by t tests (P≤0.05). Different capital letters in the same column and different lower case letters in the same row are statistically different from each other.

According to the results of the experiment on the relationship between fungal spore concentration and insect mortality rate, it was revealed that the mortality rate increased with increasing spore concentration. According to the 14th day results, while there was no statistical difference between 1000 ppm and 500 ppm, at the highest concentration tested (1500 ppm) 87.7% mortality was recorded. Vassilakos et al. (2006) also used different concentrations (2500, 5000, 10000ppm) and observed that *S. oryzae* mortality increased as the concentration increased. In the study by Ramswamy et al. (2009 two different concentrations were used and 500 ppm resulted in higher insect mortality than 250 ppm. The results obtained in this study confirm the results reported in the literature. Sewify et al. (2014) evaluated the pathogenic effect of *B. bassiana* and found a concentration of 0.35×10^{7} conidia.gr^{-1} and found a 64% mortality effect on *S. oryzae*. Bello et al. (2000) recorded 74.17% *S. oryzae* mortality with the combination of *B. bassiana* ARSEF 5500 + *M. anisopliae* ARSEF 2974 + 3 ppm fenitrothion. Although a high mortality rate (80%) was reached in the study conducted by Rice et al. (1999), the fact that immersion method (using suspension of 2×10^{8} conidia.ml^{-1}) was chosen for application in their experiment and the evaluation was carried out on the 21st day enabled them to reach a higher mortality rate. In the study of Vassilakos et al. (2006), 95% mortality of *S. oryzae* adults was obtained at a high concentration of 2500 ppm of *B. bassiana*. Considering the results of all these studies, the results obtained in present study show that *B. bassiana* F7-1 is as effective against *S. oryzae* as the isolates reported in the literature, and even higher than those at similar conditions.

In the experiment carried out to evaluate the relationship between ambient humidity and the efficacy of isolate F7-1, significant increase in mortality rate was detected only at the highest humidity (100%). Although the mortality rate at 75% RH was higher than that at 65% humidity, no statistical difference was found, and the treatment at 100% humidity caused statistically higher mortality (96.6%). In a research by Sheeba et al. (2000), the experiment was conducted at 70% RH and mortality rate was found as 75%. In Batta's (2003) study, 73.3-86.7% mortality was reached in *S. oryzae* at 75% RH by using additives (furnace ash, chalk powder, charcoal and wheat flour) to increase *M anisopliae* efficacy. As a result of decreasing the RH from 75% to 55%, an increase in mortality rate was found for *Rhyzopertha dominica* (Wakil et al., 2011) and *Tribolium confusum* (Michalaki et al., 2006).

All the results together indicate that the effect of the fungus on storage pests does not increase in direct proportion to increasing RH. However, in this study, when the RH was increased to 100%, the mortality rate also increase.

CONCLUSION

It was concluded that the *B. bassiana* isolate F7-1 examined in present study is a potential agent for the microbial control of *S. oryzae*, and that except in extreme relative humidity environments, differences in ambient humidity did not make a significant difference in the ability of *B. bassiana* F7-1 to control *S. oryzae* adults. This result indicates that ambient humidity will not be a limiting factor if *B. bassiana* F7-1 can be used against *S. oryzae* in future studies..

CONFLICTS of INTEREST

The authors declare there is no conflict of interest.

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