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Effects of *Beauveria bassiana* on Quantity of F_1 Larvae and Larval Mortality of *Tribolium confusum* (Coleoptera: Tenebrionidae)

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ABSTRACT

In this study, larval mortality due to *Beauveria bassiana* infections and the number of larvae produced in the presence of *B. bassiana* spores were investigated. Larvae were kept in wheat treated with spores and mortalities were assessed. In a long-term experiment, number of larvae and mortality was recorded after 3 months. Within 7 days, larval mortality was 55% at 250 ppm and over 75% at higher concentrations. The mortality reached 100% on day 14 in all fungus treatments. After the adults were kept for 14 and 28 days, 6.3 and 12.0 larvae in treatments and 1 and 6.3 larvae in control units were counted, respectively. In the long-term experiment, number of larvae was 10.3, 42.0 and 260.3 in the control, 500 ppm and 1000 ppm treatments, respectively. Larval mortalities were %97.4 in 500 ppm and 100% in 1000 ppm treatments. The results show that although *B. bassiana* treatment increases the number of *T. confusum* larvae as the concentration increases, *B. bassiana* treatment also causes high levels of larval mortalities depending on concentration and time, suggesting that, *B. basssiana* treatment can have a role in controlling *T. confusum* populations.

Research Article

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Beauveria bassiana'nın *Tribolium confusum* (Coleoptera: Tenebrionidae) F_1 Larva Sayısına ve Larva Ölüm Oranına Etkisi

ÖZET

Bu çalışmada, *Beauveria bassiana* enfeksiyonu neticesinde larva ölümleri ve *B. bassiana* sporlarının bulunması durumunda yeni nesil larva sayıları belirlenmiştir. Larvalar spor ile muamele edilmiş buğdayda bekletilmiş ve ölümler kaydedilmiştir. Uzun süreli denemede, larva sayısı ile ölüm oranları 3 ay sonra tespit edilmiştir. Yedi günde larva ölümü 250 ppm'de %55 ve daha yüksek konsantrasyonlarda %75'in üstünde bulunmuştur. Uygulamalardaki ölüm 14 günde %100'e ulaşmıştır. Erginlerin 14 ve 28 gün sporlu buğdayda bekletilmesi sonucunda 6.3 ve 12 larva belirlenirken kontrolde bu sayı sırası ile 1 ve 6.3 olmuştur. Uzun süreli denemede, kontroldeki, 500 ppm ve 1000 ppm uygulamalarındaki larva sayıları sırası ile 10.3, 42.0 ve 260.3 olarak tespit edilmiştir. 500ppm'de 97.4% ve 1000ppm'de %100 larva ölümü kayıt edilmiştir. Sonuçlar, spor konsantrasyonu artışı ile birlikte *B. bassiana* uygulamasının *T. confusum* larva sayısında artışa neden olduğunu; ve ancak konsantrasyon ve süreye bağlı olmakla birlikte *B. bassiana* uygulamasının yüksek larva ölümlerini sağladığını göstermiş olup, *B. bassiana* uygulamasının *T. confusum* popülasyonları ile mücadelede rol alabileceğini işaret etmektedir.

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INTRODUCTION

Stored grain pests have worldwide distribution with economic importance and their control is usually by means of chemical insecticides. Due to various problems caused by these chemicals, alternative management tactics have been under investigation and using entomopathogenic fungi is an alternative promising control approach reported against some important pests such as *Rhyzopertha dominica*, *Oryzaephilus surinamensis* and *Sitophilus* species. Entomopathogenic fungi have also been studied against *Tribolium* species with less encouraging results mainly for suppression of adult populations. Barra et al. (2013), Kavallieratos et al. (2006), Stephou et al. (2012) tested fungal isolates against *T. confusum* adults. Wakil and Schmitt (2014), Batta (2008), Shafighi et al. (2014), Sugandi and Awaknavar (2014), Padin et al. (2002), Zamani et al. (2013), Khashaveh et al. (2011), Rice and Cogburn (1999) used entomopathogenic fungi for *T. castaneum* adults. It is well established in the literature that *Tribolium* adults are quite resistant to *Beauveria bassiana*, widely reported as a promising entomopathogenic fungus species against other stored grain pests. The larval stages of *Tribolium* species was found more susceptible and moderate infection levels were achieved by Akbar et al. (2004, 2005), Wakil et al. (2014), Lord (2007, 2009, 2010) for *T. castaneum* larvae, while Michalaki et al. (2006, 2007) for *T. confusum* larvae. Although controlling *Tribolium* species by using entomopathogenic fungi does not seem to be sufficient alone considering the current knowledge, these fungi could well affect these pests when applied for the management of other stored grain pests. *Tribolium* species are commonly found to exist together with primer stored grain pest species against which mostly *B. bassiana* was shown to present opportunities for developing mycoinsecticides. In this study, *T. confusum* larval mortality due to *B. bassiana* infections and the number of larvae produced in the presence of *B. bassiana* spores on grains were investigated to find possible effects of the fungus on the pest's population growth.

MATERIALS and METHODS

Tribolium confusum and *Beauveria bassiana* cultures

T. confusum larvae were obtained from a laboratory culture originally established from samples collected in Kahramanmaraş, Turkey. The culture has been maintained on flour with addition of inactive brewer's yeast at 25±2°C and 65±5% relative humidity in darkness. After leaving adults for three days in clean food, the adults were removed and larvae were gathered one week later to use in the experiments. When adults were required, emerged adults of 7-10 days old were utilized.

B. bassiana isolate used in this study is a single spore culture of *B. bassiana* 155657, which was originally isolated from a *T. castaneum* adult (Er et al., 2016). The fungus was initially grown on Potato Dextrose Agar (PDA), and thereafter its spores were produced on rice following procedure of Barış (2016). Obtained *B. bassiana* spores were tested for viability prior to each experiment. A dilute suspension of spores in 0.2% Tween 80 was prepared and spread on PDA and incubated 24 hours at 25±2 °C in darkness. 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%.

Short-Term Incubation Tests

For each treatment 15 larvae were placed in a 50ml centrifuge tube including 40 g wheat grains homogenously mixed with required number of spores to produce spore concentrations of 250, 500, 750, 1000 ppm (w/w). For control, clean wheat without fungal spores was used. All the experimental tubes were placed in a humidity chamber made up of a sealed plastic container (42x28x17cm) with saturated $NaNO_2$ solution (Winston and Bates, 1960). The experiment had four replications and conducted at 25±2 °C and 65±5% relative humidity in darkness. After 7 and 14 days of incubation larval mortalities were assessed.

In the second test, number of larvae produced was found after leaving adults in 1000 ppm treated wheat grains. For each treatment 20 adults were placed in a 1 L glass jar including 200 g wheat grains homogenously mixed with required number of spores to produce a concentration of 1000 ppm (w/w). For control, clean wheat without fungal spores was used. All the jars were placed in humidity chambers as described above. The experiment had four replications and conducted at the same conditions with the first experiment. After 14 and 28 days of incubation the number of larvae in each jar was recorded.

Long-Term Incubation Test

In a long incubation experiment, 1 kg bulgur (wheat product) was used for each treatment in a 3 L glass jar. After mixing *B. bassiana* spores to obtain 1000 ppm concentration (w/w), 100 adults were released and incubated for 3 months at 25±2 °C and 65±5% relative humidity in darkness in a conditioned growth room. Clean bulgur without fungal spores was used for control unit. The experiment was carried out with three replications. Number of larvae and larval mortality in each unit were recorded at the end of the experiment.

Statistical Analysis of Data

The data were subjected to one way ANOVA followed by Duncan's multiple comparison test ($P \leq 0.05$). When there were only two treatments to compare, 't' test ($P \leq 0.05$) was applied. Mortality data were arcsine transformed prior to the statistical analysis. All statistical analyses were conducted by using SPSS statistics program.

RESULTS and DISCUSSION

Short-Term Incubation

After leaving the larvae 7 days in fungus treated wheat grains, larval mortalities were between 55.00±0.85% and 81.67±0.75% (Figure 1), the lowest being at 250ppm. The mortality in control unit was 6.67±0.58%. According to ANOVA there were statistical differences ($F_{4,15}=23.663$; $P<0.001$). The mortalities in all fungus treated units were statistically higher than the one in control unit (Figure 1). Michalaki et al. (2006) tested *Metarhizium anisopliae* against *T. confusum* larvae and found about 40% mortality at the concentration of 8×10^{10} spores/kg in 7 days. In another study (Michalaki et al., 2007), in the same incubation period *Isaria fumosorosea* (formerly *Paecilomyces fumosoroseus*) caused less than 20% mortality at the maximum tested concentration of 400ppm. In bioassays conducted on *T. castaneum* larvae, 900 ppm and 2700 ppm treatments with *B. bassiana* spores resulted in 40% and 62.7% mortalities in 8 days (Akbar et al., 2004). Wakil et al. (2014) used immersing method (1×10^6 spores/ml) and after 7 days of incubation found 85.81% mortality due to *B. bassiana*, 59.79%, 76.86% and 67.83% mortalities due to *Lecanicillium attenuatum*, *M. anisopliae* and *Purpureocillium lilacinum* (formerly *Paecilomyces lilacinus*) treatments. In the present study, while the mortalities in fungus treatments reached 100% on day 14, control mortality increased to 41.67±1.03% (Figure 1). Statistically, the difference was significant ($F_{4,15}=152.152$; $P<0.001$). Due to the high mortality in the control, not all mortalities in fungus treatments can be attributed to fungal infections. As larvae can not feed on whole wheat grains, the increase could be partially because of starvation related causes. Food deprivation was previously reported to increase larval mortality in *T. castaneum* due to *B. bassiana* infections (Lord, 2010). The only larval mortality results in two and three weeks after *I. fumosorosea* treatment were reported by Michalaki et al. (2007). They found close to 50% and 60% at 25°C and over 70% and 90% at 20°C after 14 and 21 days of incubation, respectively. Although organisms and methods varied in the mentioned previous studies above, the findings of the present study seem to fit to the range of expectations considering tested concentration and bioassay conditions.

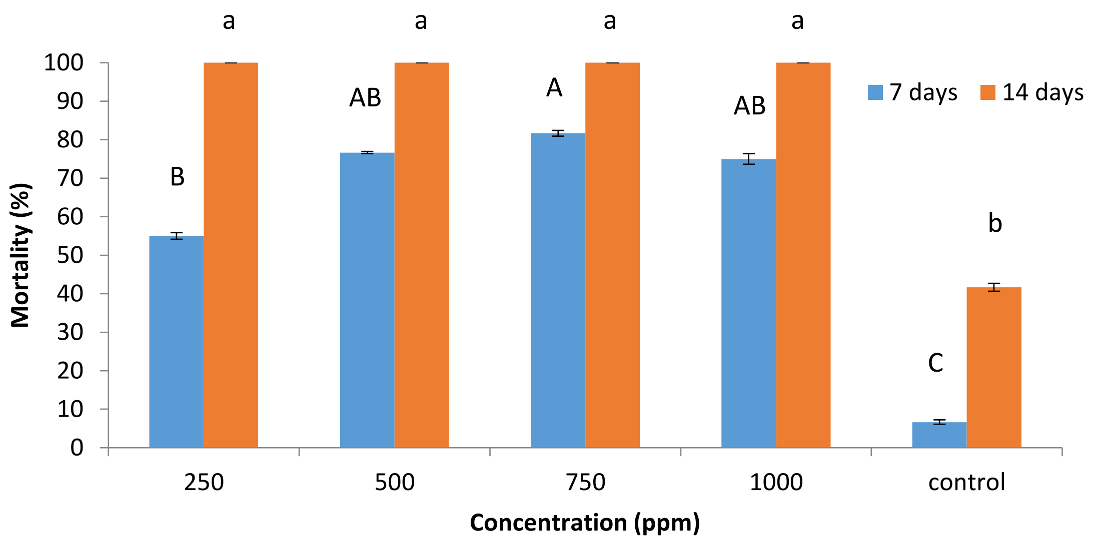

Figure 1. Mortality of *Tribolium confusum* larvae at various concentrations of *Beauveria bassiana* spores in wheat (different lower or upper case letters show statistical differences according to Duncan test at $P \leq 0.05$; bars represent standard errors; n=4)

After the adults were kept for 14 and 28 days in fungus treated wheat grains, 6.3±0.33 and 12.0±1.00 larvae in treatments and 1.0±0.58 and 6.3±0.33 larvae in control units were counted, respectively (Figure 2). The number of larvae in 14 days and 28 days were statistically higher than related control units (for 14 days: $t=8$; d.f.=4; $P=0.001$, and for 28 days: $t=5.376$; d.f.=4; $P=0.006$). This indicates a significant increase in the production of larvae in fungus treated wheat grains. As such an outcome has not been reported previously, reasons need to be investigated in future studies.

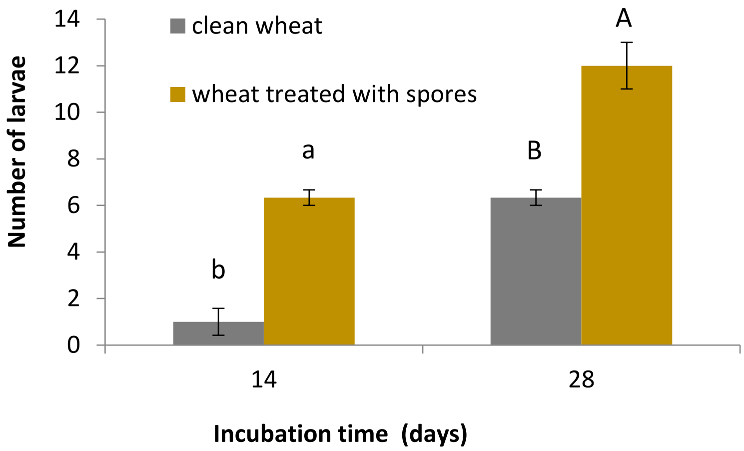

Figure 2. Number of *Tribolium confusum* larvae after incubation of 100 adults in wheat treated with 1000 ppm *Beauveria bassiana* spores (different lower or upper case letters show statistical differences according to 't' test at P≤0.05; bars represent standard errors; n=4)

Long-Term Incubation

In the long incubation experiment, after three months, while there were 10.3±1.45 larvae in the control unit, there were 42.0±2.65 larvae in 500 ppm and 260.3±11.20 larvae in 1000 ppm fungus treated wheat grains (Figure 3). The differences amongst them were statistically significant ($F_{2,6}$=413.109; P<0.001). The mortalities of these larvae were %97.4±1.52 in 500 ppm and 100% in 1000 ppm treatments, while there was not larval mortality in the control (Figure 4). According to ANOVA, there was significant differences ($F_{2,6}$=490.484; P<0.001). The mortalities in fungus treatments did not differ from each other; however, they were both statistically higher than the control mortality. Such long-term incubation experiments were reported previously for comparison. However, findings are in parallel with the results of the short-term incubation experiments reported here and as discussed earlier. The results of the long-term incubation experiment revealed more pronounced differences among treatments.

CONCLUSION

In this study, *T. confusum* larval mortality due to *B. bassiana* infections and the number of *T. confusum* larvae produced in the presence of *B. bassiana* spores were investigated. It can be concluded that *B. bassiana* treatment increases the number of *T. confusum* larvae as the concentration increases. *B. bassiana* treatments also cause high levels of larval mortalities depending on concentration and time. These findings suggest that treating wheat with *B. basssiana* either against this pest or against another one can have a role in suppressing *T. confusum* populations.

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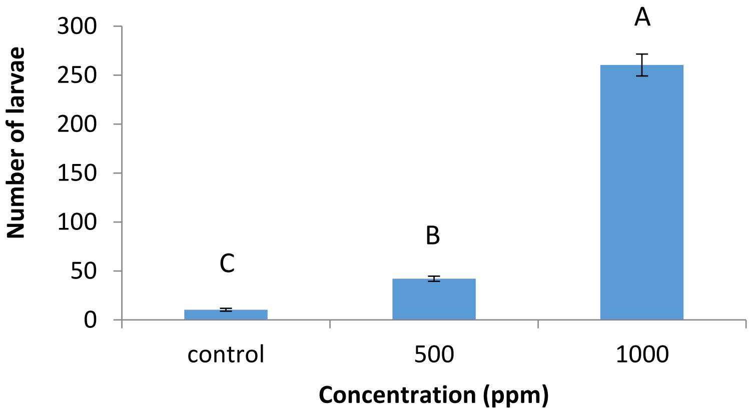

Figure 3. Number of *Tribolium confusum* larvae after incubation of 100 adults in wheat treated with 1000 ppm *Beauveria bassiana* spores (different letters indicate statistical differences according to Duncan test at P≤0.05; bars represent standard errors; n=4)

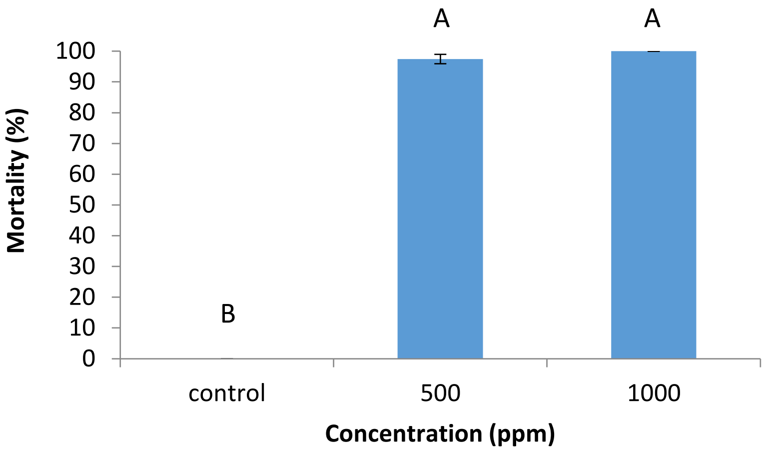

Figure 4. Mortality of *Tribolium confusum* larvae at various concentrations of *Beauveria bassiana* spores in wheat (different letters show statistical differences according to Duncan test at P≤0.05; bars represent standard errors; n=4)

REFERENCES

Akbar W, Lord JC, Nechols JR, Howard RW 2004. Diatomaceous Earth Increases the Efficacy of *Beauveria bassiana* Against *Tribolium castaneum* Larvae and Increases Conidia Attachment. Journal of Economic Entomology, 97: 273-280.

Akbar W, Lord JC, Nechols JR, Loughin TM 2005. Efficacy of *Beauveria bassiana* for Red Flour Beetle When Applied with Plant Essential Oils or in Mineral Oil and Organosilicone Carriers. Journal of Economic Entomology, 98: 683-688.

Barış C 2016. The Use of Some Cereals on The Production of *Beauveria bassiana* By Solid State Fermentation Technique. University of Kahramanmaraş Sütçü İmam, Graduate School of Natural and Applied Sciences, Department of Plant Protection, M.Sc. thesis, 33 pp, Turkey.

Barra R, Rosso L, Nesci A, Etcheverry M 2013. Isolation and Identification of Entomopathogenic Fungi and Their Evaluation Against *Tribolium confusum*, *Sitophilus zeamais*, and *Rhyzopertha dominica* in Stored Maize. Journal of Pest Science, 86: 217-226.

Batta YA 2008. Control of Main Stored-Grain Insects with New Formulations of Entomopathogenic Fungi in Diatomaceous Earth Dusts. International Journal of Food and Engineering, 4: 1-16.

Er MK, Tunaz H, Ücük C, Barış C, Işıkber AA 2016. Occurrence of Entomopathogenic Fungi on Insect Pests of Stored Wheat and Maize in Central and South Anatolia in Turkey. Turkish Journal of Entomology, 40: 249-263.

Kavallieratos NG, Athannassiou CG, Michalaki MP, Batta YA, Rigatos HA, Pashalidou FG, Balotis GN, Tomanovic Z, Vayias BJ 2006. Effect of the Combined Use of *Metarhizium anisopliae* (Metschinkoff) Sorokin and Diatomaceous Earth for the Control of Three Stored-Product Beetle Species. Crop Protection, 25: 1087-1094.

Khashaveh A, Ghosta Y, Safaralizadeh MH, Ziaee M 2011. The Use of Entomopathogenic Fungus, *Beauveria bassiana* (Bals.) Vuill. in Assays with Storage Grain Beetles. Journal of Agricultural Science and Technology, 13: 35-43.

Lord JC 2007. Enhanced Efficacy of *Beauveria bassiana* For Red Flour beetle With Reduced Moisture. Journal of Economic Entomology, 100: 1071-1074.

Lord JC 2009. Efficacy of *Beauveria bassiana* for Control of *Tribolium castaneum* with Reduced Oxygen and Increased Carbon Dioxide. J. Appl. Entomol., 133: 101-107.

Lord JC 2010. Dietary Stress Increases The Susceptibility of *Tribolium castaneum* to *Beauveria bassiana*. Journal of Economic Entomology, 103: 1542-1546.

Michalaki MP, Athanassiou CG, Kavallieratos NG, Batta YA, Balotis GN 2006. Effectiveness of *Metarhizium anisopliae* (Metschinkoff) Sorokin Applied Alone or in Combination With Diatomaceous Earth Against *Tribolium confusum* du Val. Larvae: Influence of Temperature, Relative Humidity and Type of Commodity. Crop Protection, 25: 418-425.

Michalaki MP, Athanassiou CG, Steenberg T, Buchelos C Th 2007. Effect of *Paecilomyces fumosoroseus* (Wise) Brown and Smith (Ascomycota: Hypocreales) Alone or in Combination With Diatomaceous Earth Against *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Biological Control, 40: 280-286.

Padin S, Bello GD, Fabrizio M 2002. Grain Loss Caused by *Tribolium castaneum, Sitophilus oryzae* and *Acanthoscelides obtectus* in Stored Durum Wheat and Beans Treated With *Beauveria* *bassiana*. Journal of Stored Products Research, 38: 69-74.

Rice WC, Cogburn RR 1999. Activity of the Entomopathogenic Fungus *Beauveria bassiana* (Deuteromycota: Hyphomycetes) Against Three Coleoptera Pest of Stored Grain. Journal of Economic Entomology, 92(3): 691-694.

Shafighi Y, Ziaee M, Ghosta Y 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.

Stephou VK, Tjamos SE, Paplomatas EJ, Athanassiou CG 2012. Transformation and Attachment of *Beauveria bassiana* Conidia on The Cuticle of *Tribolium confusum* and *Sitophilus oryzae* in Conjunction with Diatomaceous Earth. Journal Pest Science, 85: 387-394.

Sugandi R, Awaknavar JS 2014. The Pathogenicity of Entomopathogenic Fungus, *Metarhizium anisopliae* (Metschinkoff) Sorokin against Three Major Storage Grain Beetles. Journal of Experimental Zoology, 17 (2): 775-780.

Wakil W, Ghazanfar MU, Yasin M 2014. Naturally Occurring Entomopathogenic Fungi Infecting Stored Grain Insect Species in Punjab, Pakistan. Journal of Insect Science, 14(1). DOI: 10.1093/jisesa/ieu044.

Wakil W, Schmitt T 2014. Field Trials on the Efficacy of *Beauveria bassiana*, Diatomaceous Earth and Imidacloprid for the Protection of Wheat Grains From Four Major Stored Grain Insect Pests. Journal of Stored Products Research, 64: 160-167.

Winston PW, Bates DH 1960. Saturated Solutions for The Control of Humidity in Biological Research. Ecology, 41:232-237.

Zamani Z, Aminaee MM, Khaniki GB 2013. Introduction of *Beauveria bassiana* as a Biological Control Agent for *Tribolium castaneum* in Kerman Province. Archives of Phytopathology and Plant Protection, 46: 2235-2243.