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Toxicity of Some Entomopathogenic Fungi Combined with Boric Acid Againts Adults of American Cockroach *Periplaneta americana* (L.) (Blattodea: Blattidae)

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ABSTRACT

In this study, the pathogenicity of some entomopathogenic fungi on American cockroach *Periplaneta americana* (L.) adults and the effect of the most effective fungus isolate combined with boric acid on the virulence of the fungus were investigated in laboratory conditions. According to the results obtained from the experiments, the most effective isolate was found as *Beauveria bassiana* 5-4 and when it was used on insects, it caused 0%, 66.6% and 93.3% mortality at the dose of 0.1×10^9 conidia cm^{-2} at 72, 96 and 120 hours, respectively. This fungus also caused 0%, 20% and 20% mortality, respectively, at dose of 0.05×10^9 conidia cm^{-2} in the same time period. When boric acid was used on insects, it caused 6.6%, 40% and 66.6% mortality at dose of 1 mg cm^{-2} at 72, 96 and 120 hours, respectively. When 0.1×10^9 conidia cm^{-2} dose of *B. bassiana* 5-4 was used together with 1 mg cm^{-2} dose of boric acid, 33.3%, 60% and 93.3% mortality occurred at 72, 96 and 120 hours, respectively. When 0.05×10^9 conidia cm^{-2} dose of *B. bassiana* 5-4 was used together with 1 mg cm^{-2} dose of boric acid, 33.3%, 60% and 86.6% mortality occurred at 72, 96 and 120 hours, respectively. The results showed that the combined use of boric acid and *B. bassiana* 5-4 increased the virulence of the fungus at short exposure times (72 and 96 hours), but did not significantly affect the virulence of the fungus at long exposure times (120 hours and above).

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Bazı Entomopatojen Fungusların Borik Asitle Birlikte Kullanımının Amerikan Hamamböceği *Periplaneta americana* (L.) (Blattodea: Blattidae) Erginlerine Karşı Toksisitesi

ÖZET

Bu çalışmada, laboratuvar koşullarında bazı entomopatojen fungusların Amerikan hamamböceği *Periplaneta americana* (L.) erginlerine patojenitesi ve en etkili fungus izolatının borik asit ile birlikte kullanımının fungusun virülensliği üzerindeki etkisi araştırılmıştır. Testlerden elde edilen sonuçlara göre en etkili izolat *Beauveria bassiana* 5-4 olmuştur ve böcekler üzerinde tek başına kullanıldığında 72, 96 ve 120 saatlerde 0.1×10^9 konidi cm^{-2} dozunda sırasıyla %0, %66.6 ve %93.3 ölüm meydana getirmiştir. Bu fungus aynı zaman aralığında 0.05×10^9 konidi cm^{-2} dozunda sırasıyla %0, %20 ve %20 ölüm meydana getirmiştir. Borik asit böcekler üzerinde tek başına kullanıldığında 72, 96 ve 120 saatlerde 1 mg cm^{-2} dozunda sırasıyla %6.6, %40 ve %66.6 ölüm meydana getirmiştir. *B. bassiana* 5-4'ün 0.1×10^9 konidi cm^{-2} dozu borik asidin 1 mg cm^{-2} dozu ile birlikte kullanıldığında 72, 96 ve 120 saatlerde sırasıyla %33.3, %60 ve %93.3 ölüm meydana getirmiştir. *B. bassiana* 5-4'ün 0.05×10^9 konidi cm^{-2} dozu borik asidin 1 mg cm^{-2} dozu ile birlikte kullanıldığında sırasıyla 72, 96 ve 120 saatlerde %33.3, %60 ve %86.6 ölüm meydana getirmiştir. İstatistiksel analizler borik asit ve *B. bassiana* 5-4'ün kombine kullanımının kısa maruz kalma sürelerinde (72 ve 96 saat) fungusun virülanslığını artırdığını ancak uzun maruz kalma sürelerinde (120 saat ve üzeri) fungusun virülanslığına önemli bir etki etmediğini göstermiştir.

Entomoloji

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INTRODUCTION

Cockroaches are an ancient and highly successful form of insects. They have been in existence since Pennsylvanian (Upper Carboniferous) times (Cochran & WHO, 1999). The fossil record indicates that they achieved an optimum body form early in their evolutionary history and have remained a very stable group since that time (Moore et al., 1952).

They feed on human food and feces, so they pose a threat to human health. Because they have contact with various surfaces, they are carriers of many diseases. It has been determined that cockroaches are naturally contaminated with 40 different bacterial species that are pathogenic for invertebrates and other living things (Roth & Willis, 1957, 1960; Burgess et al., 1973a, 1973b, 1974; Artyukhina & Evokimov, 1973; Ulewicz & Zawistowski, 1973; Klowden & Greenberg, 1976; Ash & Greenberg, 1980; Cornwell & Mendes, 1981). Cockroaches are associated with many medical problems (Baumholtz et al., 1997). It has been determined that they carry viral and bacterial pathogens that cause poisoning, toxicosis, pneumonia, dysentery and diarrhea in humans (Fotedar et al., 1991). Laboratory studies have revealed that cockroaches may carry certain viruses (Roth & Willis, 1957). Some examples are the Coxsackie virus and several strains of poliomyelitis. The presence of several types of acids, which are tryptophan derivatives with mutagenic or carcinogenic effects, has been detected in the feces of some cockroach species (Mullins & Cochran, 1973).

Zahraei-Ramazani et al. (2018), reported that 90% of the American cockroach *Periplaneta americana* (L.) (Blattodea: Blattidae) population was successfully controlled using chemical pesticides, but this method also threatened non-target organisms, causing the pest to develop resistance and resulted in the formation of residues that cause environmental pollution (Nicolopoulou-Stamati et al., 2016). The increasing resistance of cockroaches to synthetic insecticides and their undesirable effects on humans has increased the necessity for new and sustainable developments to control them (Hubner-Campos et al., 2013). In this direction, the use of environmentally friendly microbial pesticides plays an important role in pest management programs.

Entomopathogenic fungi, which are used in the microbial control of pests, have advantages such as high efficiency and no residues in the environment (Mantzoukas & Eliopoulos, 2020). The biological cycle of fungi such as *Metarhizium anisopliae* and *Beauveria bassiana* begins when their conidia adhere to the surface of the host insect. After the conidia adhere to the insect, they initiate germination by forming a germ tube and hyphae. Then, with the activation of extracellular cuticle-degrading enzymes, the fungi penetrate the host insect (Goettel & Lacey, 1995). After penetration, the hyphae reach the blood. At this point, the insect activates its internal defense mechanisms against the development of the fungus. The pathogenic fungus escapes the insect's defense mechanisms and forms hyphae bodies. The death of the insect occurs due to anomalies in the blood, the effect of toxins associated with cessation of feeding and depletion of nutritional reserves, growth of hyphae bodies and mycelium (Khachatourians, 1998). Due to all these stages, the duration of action of entomopathogenic fungi is long and therefore they could not compete with traditional pesticides.

Although it has been proven that effective against cockroaches (Cochran, 1995; Ebeling, 1995; Zurek et al., 2003; Gore & Schal, 2004) and low toxicity to mammals (Murray, 1998; Cox, 2004) the use of inorganic insecticide boric acid (H_3BO_3) has been limited due to its long-lasting effect against cockroaches, as is the case with entomopathogenic fungi (Yang et al., 2021).

Therefore, it was aimed to determine the microbial control potential of some entomopathogenic fungi on American cockroach *P. americana* (L.) adults and the effect of their use together with boric acid on the virulence of fungi in this study.

MATERIAL and METHOD

Rearing of Cockroaches

The *P. americana* culture used in the tests was obtained from the samples caught from Kahramanmaraş province in Türkiye in 2020. Insects were grown in plastic boxes (86×40×29 cm) in a climate room with controlled conditions (30±1 °C, 65±10% relative humidity). Eight cardboard chicken egg trays (10×10×10 cm) were placed in the plastic boxes for insects to hide and lay eggs. In order to ensure air circulation, air was added to the boxes with an aquarium pump. To meet the water need, 500 ml containers filled with water containing tissue paper were used in plastic boxes. Commercial dryed dog food (Bonnie®) was given to the insects to meet their nutritional needs. During the breeding process, the plastic boxes were kept closed in order to provide dark conditions.

Boric Acid

The boric acid used in the study was produced by Sigma-Aldrich Laborchemikalien and is 99.5% - 100% purity. It was stored at room temperature (25 ºC) until used in the tests.

Origin of Entomopathogen Fungi

The entomopathogenic fungi, which was used in the study, were obtained from the fungus collection of Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Plant Protection. *B. bassiana* 5-4 isolate was obtained from a single spore culture of isolate 151138 originally obtained from *Rhyzopertha dominica* (Er et al., 2016b). *Metarhizium robertsii* S3 isolate was obtained from a single spore culture of isolate F17-2-1, which was originally obtained from *Sitophilus oryzae* (Er et al., 2016a).

Table 1. The location where the entomopathogenic fungi used in the tests were obtained, the year of isolation and the host

Çizelge 1. Testlerde kullanılan entomopatojen fungusların elde edildiği yer, izolasyon yılı ve konukçusu

Fungus	İsolate	City	Year	Host
Beauveria bassiana	5-4	Şanlıurfa	2013	*Rhyzopertha dominica*
Purpureocillium lilacinum	31304	Kahramanmaraş	2013	*Sitophilus oryzae*
Beauveria varroae	35727	Konya	2013	*Rhyzopertha dominica*
Beauveria varroae	120601	Adana	2013	*Cryptolestes ferrugineus*
Metarhizium robertsii	S3	Gaziantep	2013	*Sitophilus oryzae*

Mass Production of Fungi

The entomopathogenic fungi used in the study were grown using rice substrate (Barış & Er, 2021). Firstly, rice was kept in water for 12 hours for production, then filtered and placed in 200 gram bags. Calcium sulfate ($CaSO_4$) and calcium carbonate ($CaCO_3$) salts were added into the bags and mixed for homogeneous dispersion (Nirmala et al., 2005). The bags were sterilized at 121 °C using an autoclave and then left to cool down to 25 °C. For each 100 g of rice in the bags, 2×10^8 conidia were inoculated and mixed. The bags were sealed and incubated for 14 days. (25±2 °C, 12 s photoperiod). The bags opened after 14 days were left to dry for 10 days. The fungi were sieved from dried rice, placed in glass jars and kept ready for tests at +4 °C. The conidia density of the mass-produced fungi was calculated.

Table 2. The number of conidia contained in 1 milligram of entomopathogenic fungi

Çizelge 2. Entomopatojen fungusların her 1 miligramında ihtiva ettikleri spor sayıları

Fungus	İsolate	Number of conidia (conidia mg^{-1})
Beauveria bassiana	5-4	0.1×10^9
Purpureocillium lilacinum	31304	0.08×10^9
Beauveria varroae	35727	0.1×10^9
Beauveria varroae	120601	0.1×10^9
Metarhizium robertsii	S3	0.032×10^9

Germination Tests

The fungi, which was used in the study were subjected to germination tests before being used in pathogenicity tests (Barış & Er, 2021). Potato Dextrose Agar (PDA) medium was used to determine germination rates. A sample of 1 mg was taken from each of the fungi and vortexed for 2 minutes in 10 ml of 0.01% Tween80. The resulting suspension was diluted to a concentration of 10^6 conidia ml^{-1} and then transferred to petri dishes containing PDA and spread with the help of a spatula. Petri dishes were kept in incubators at 30±2 °C for 24 hours. Thereafter, the conidia were examined under a light microscope at 40x magnification and those with germ tubes equal or longer than the conidia were considered germinated. The germination ratios were calculated after examining a minimum of 100 conidia from each of the three replicate plates. As a result of the germination test, the germination rates of the conidia of *B. bassiana* 5-4, *Purpureocillium lilacinum* 31304, *Beauveria varroae* 35727, *B. varroae* 120601 and *M. robertsii* S3 isolates were approximately 94%, 68%, 94%, 92% and 84%, respectively.

Experimental Design

Glass jars (1 lt) were preferred as the application area for mortality tests. The jars were sterilized using 96% ethyl alcohol. Powdery fungi and boric acid were weighed on a precision scale (0.00001 g). Weighed fungi and boric acid poured into the bottom of the jars (38.5 cm^2) and spread homogeneously with the using of a brush. Five *Periplaneta americana* adults, four up to five days after emergence, were acommodated in the each jar. The jars were covered with sterile cheesecloth to prevent insects from escaping. The tests were carried out in a climate chamber, at 30±1 °C, 65±10% relative humidity, in a randomized parcel design with

3 replications for each dose. Dead-alive counts were made and recorded every 24 hours. In order to prevent the insects from dying due to natural causes such as hunger, one piece of dog food was placed in each jar.

Pathogenicity and Toxicity Tests

In the first test with pure form of entomopathogenic fungi, each fungus was applied at a dose of 25 mg cm^{-2} and lower dose applications were started in line with the results obtained from this test. *B. bassiana* 5-4, *B. varroae* 35727, *B. varroae* 120601 isolates were applied at 0.1×10^9, 0.5×10^9, 1.0×10^9 and 1.5×10^9 conidia cm^{-2} doses. *P. lilacinum* 31304 isolate was applied at 0.08×10^9, 0.4×10^9, 0.8×10^9 and 1.2×10^9 conidia cm^{-2} doses. *M. robertsii* S3 isolate was applied at 0.08×10^9, 0.4×10^9, 0.8×10^9 and 1.2×10^9 conidia cm^{-2} doses. Mortality at 48, 96, 120, 144, 168 and 192 hours was recorded by counting the dead-alive.

In the toxicity tests with boric acid, doses of 0.1, 0.5, 1, 2.5, 5, 10 and 20 mg cm^{-2} were applied and the effects were tested after 48, 96, 120, 144, 168 and 192 hours by counting dead-alive. In the first test to determine the effect of combined use, 0.1×10^9 conidia cm^{-2} *B. bassiana* 5-4 and 1, 0.5 and 0.1 mg cm^{-2} boric acid were applied together. In the second test, 1 mg cm^{-2} boric acid and 0.05×10^9 and 0.01×10^9 conidia cm^{-2} *B. bassiana* 5-4 were applied together. The effects of the applied doses after 48, 96, 120, 144, 168 and 192 hours were tested by counting dead-alive.

Statistical Analyzes

After the data obtained as a result of the tests were subjected to arcsine transformation, they were subjected to one way and two way ANOVA using the Minitab 17 statistics program. Differences between means were determined by the Tukey test at the 5% significance level ($P<0.05$).

RESULTS and DISCUSSION

Pathogenicity Tests

Mortality rates caused by pure form of entomopathogenic fungi on *P. americana* adults are presented in Table 3, 4, 5, 6, 7 and 8. In general, mortality rates increased proportionally with the dose and exposure time. As seen in Table 3, in the first test performed with a fixed dose (25 mg cm^{-2}) of entomopathogenic fungi, at least 50% mortality was observed in all isolates except *P. lilacinum* within the first 48 hours, and 100% mortality was occured in all isolates at the end of 96 hours. Statistically, in the first 48 hours, *P. lilacinum* isolate caused a significantly lower mortality rate compared to other fungi (6.6%). It was determined that *B. varroae* 35727 isolate caused the highest mortality rate (80%) in 48 hours. When the exposure time reached 96 hours, 100% mortality occurred in all isolates.

Table 3. Mortality rates (%) (+ s.e.) of *Periplaneta americana* adults exposed to fixed dose (25 mg cm^{-2}) entomopathogenic fungi

Çizelge 3. Sabit dozda (25 mg cm^{-2}*) entomopatojen funguslara maruz burakılan Periplaneta americana erginlerinin ölüm oranları* (%) (+ s.h.)

Isolates *İzolatlar*	**Post-treatment time (hour)** *Uygulama sonrası süre (saat)* 48	72	96	$F_{6.14}$	P
Beauveria bassiana 5-4	53.3±17.6 ABb	93.3±6.6 Aa	100±0 Aa	9.25	0.000
Purpureocillium lilacinum 31304	6.6±6.6 Bb	80±11.5 Aa	100±0 Aa	30.52	0.000
Beauveria varroae 35727	80±11.5 Ab	100±0 Aa	100±0 Aa	3.60	0.023
Beauveria varroae 120601	73.3±6.6 Ab	100±0 Aa	100±0 Aa	53.17	0.000
Metarhizium anisopliae S3	66.6±17.6 Ab	100±0 Aa	100±0 Aa	3.81	0.018
Control *Kontrol*	0±0	0±0	0±0		
$F_{4.10}$	4.73	2.18	-		
P	0.021	0.145	-		

- Two-way analysis of variance (ANOVA) was applied to the data and the differences between the means were determined according to the Tukey test at the 5% significance level ($P<0.05$), different capital letters in the same column and different lowercase letters in the same row are statistically different from each other (dose: $F_{4,70}= 6.84$, $P<0.0001$; exposure time: $F_{6.70}= 52.39$, $P<0.0001$; dose × exposure time: $F_{24,70}= 3.58$, $P<0.0001$).

- Verilere çift yönlü varyans analizi (ANOVA) uygulanmış ve ortalamalar arasındaki farklılıklar %5 önem seviyesinde Tukey testine göre belirlenmiştir ($P<0.05$), aynı sütundaki farklı büyük harfler ve aynı satırdaki farklı küçük harfler istatistiksel olarak birbirinden farklıdır (doz: $F_{3,56}= 34.84$, $P<0.0001$; maruz kalma süresi: $F_{6.56}= 18.98$, $P<0.0001$; doz × maruz kalma süresi: $F_{18.56}= 1.27$, $P= 0.243$).

Table 4. Mortality rates (%) (+ s.e.) of *Periplaneta americana* adults exposed to *Purpureocillium lilacinum* 31304
Çizelge 4. Purpureocillium lilacinum 31304 izolatına maruz burakılan Periplaneta americana erginlerinin ölüm oranları (%) (+ s.h.)

Purpureocillium lilacinum-31304	**Post-treatment time (hour)** *Uygulama sonrası süre (saat)*								
	48	72	96	120	144	168	192	$F_{6.14}$	P
0.08×10^9 con. cm^{-2}	0±0Aa	0±0Aa	0±0Ba	6.6±6.6Ba	6.6±6.6Ba	6.6±6.6Ba	13.3±6.6Ba	1.00	0.463
0.4×10^9 con. cm^{-2}	0±0Aa	6.6±6.6Aa	20±11.5ABa	20±11.5ABa	26.6±6.6ABa	33.3±13.3ABa	40±11.5ABa	2.87	0.049
0.8×10^9 con. cm^{-2}	0±0Ac	13.3±6.6Abc	26.6±6.6Aab	40±11.5ABab	46.6±6.6Aab	60±11.5Aa	60±11.5Aa	9.61	0.000
1.2×10^9 con. cm^{-2}	0±0Ac	26.6±13.3Abc	46.6±6.6Aab	60±0Aab	66.6±6.6Aa	66.6±6.6Aa	73.3±6.6Aa	13.28	0.000
Control *Kontrol*	0±0	0±0	0±0	0±0	0±0	0±0	6.6±6.6		
$F_{3.8}$	-	1.55	7.94	5.22	12.05	8.38	6.73		
P	-	0.275	0.009	0.027	0.002	0.008	0.014		

- Two-way analysis of variance (ANOVA) was applied to the data and the differences between the means were determined according to the Tukey test at the 5% significance level (P<0.05), different capital letters in the same column and different lowercase letters in the same row are statistically different from each other (dose: $F_{3,56}$= 34.84, P<0.0001; exposure time: $F_{6.56}$= 18.98, P<0.0001; dose × exposure time: $F_{18.56}$= 1.27, P= 0.243).

- Verilere çift yönlü varyans analizi (ANOVA) uygulanmış ve ortalamalar arasındaki farklılıklar %5 önem seviyesinde Tukey testine göre belirlenmiştir (P<0.05), aynı sütundaki farklı büyük harfler ve aynı satırdaki farklı küçük harfler istatistiksel olarak birbirinden farklıdır (doz: $F_{3,56}$= 34.84, P<0.0001; maruz kalma süresi: $F_{6.56}$= 18.98, P<0.0001; doz × maruz kalma süresi: $F_{18.56}$= 1.27, P= 0.243).

Table 5. Mortality rates (%) (+ s.e.) of *Periplaneta americana* adults exposed to *Metarhizium robertsii* S3
Çizelge 5. Metarhizium robertsii S3 izolatına maruz burakılan Periplaneta americana erginlerinin ölüm oranları (%) (+ s.h.)

Metarhizium robertsii S3	**Post-treatment time (hour)** *Uygulama sonrası süre (saat)*								
	48	72	96	120	144	168	192	$F_{6.14}$	P
0.032×10^9 con. cm^{-2}	0±0Aa	0±0Aa	6.6±6.6Ba	6.6±6.6Ca	6.6±6.6Ba	20±11.5Ba	20±11.5Ba	1.13	0.392
0.16×10^9 con. cm^{-2}	0±0Aa	13.3±6.6Aa	26.6±13.3Ba	26.6±13.3BCa	46.6±17.6ABa	53.3±17.6ABa	60±20ABa	2.88	0.049
0.32×10^9 con. cm^{-2}	0±0Ac	33.3±6.6Abc	46.6±6.6ABb	66.6±13.3ABab	80±11.5ABab	93.3±6.6Aa	93.3±6.6Aa	15.15	0.000
1.2×10^9 con. cm^{-2}	13.3±6.6Ab	33.3±17.6Ab	80±0Aab	93.3±6.6Aa	93.3±6.6Aa	93.3±6.6Aa	93.3±6.6Aa	8.27	0.001
Control *Kontrol*	0±0	0±0	0±0	0±0	0±0	0±0	0±0		
$F_{3.8}$	4.00	2.92	8.25	10.38	9.94	8.16	7.30		
P	0.052	0.000	0.008	0.004	0.004	0.008	0.011		

- Two-way analysis of variance (ANOVA) was applied to the data and the differences between the means were determined according to the Tukey test at the 5% significance level (P<0.05), different capital letters in the same column and different lowercase letters in the same row are statistically different from each other (dose: $F_{3,56}$= 45.64, P<0.0001; exposure time: $F_{6.56}$= 19.53, P<0.0001; dose × exposure time: $F_{18.56}$= 1.50, P<0.125).

- Verilere çift yönlü varyans analizi (ANOVA) uygulanmış ve ortalamalar arasındaki farklılıklar %5 önem seviyesinde Tukey testine göre belirlenmiştir (P<0.05), aynı sütundaki farklı büyük harfler ve aynı satırdaki farklı küçük harfler istatistiksel olarak birbirinden farklıdır (doz uygulaması: $F_{3,56}$= 45.64, P<0.0001; maruz kalma süresi: $F_{6.56}$= 19.53, P<0.0001; doz uygulaması × maruz kalma süresi: $F_{18.56}$= 1.50, P=0.125).

P. lilacinum 31304 isolate was the isolate with the lowest pathogenicity among all isolates, so that even at the highest dose (1.2×10^9 conidia cm^{-2}) after 192 hours, this isolate could not cause 100% mortality in the population (Table 4). In another study with this fungus, Toledo-Hernández et al. (2019), tested the pathogenicity of different *P. lilacinum* isolates against the adults of Mexican fruit fly *Anastrepha ludens* (Loew) (Diptera: Tephritidae). With their results, they found that the mortality rates ranged from 28.8% to 52.4%, and the LT_{50} value was 18 days or more.

Although the *M. robertsii* S3 isolate was able to kill at least half of the population at high doses (0.32×10^9 and 1.2×10^9 conidia cm^{-2}) and after 96-120 hours and caused nearly 100% mortality after 192 hours, at low doses (0.032×10^9 and 0.16×10^9 conidia cm^{-2}) could not cause a high mortality rate in the population even after 192 hours (Table 5).

Table 6. Mortality rates (%) (+ s.e.) of *Periplaneta americana* adults exposed to *Beauveria bassiana* 5-4
Çizelge 6. Beauveria bassiana 5-4 izolatına maruz burakılan Periplaneta americana erginlerinin ölüm oranları (%) (+ s.h.)

Beauveria bassiana 5-4	**Post-treatment time (hour)** *Uygulama sonrası süre (saat)*							
	48	72	96	120	144	168	$F_{6.14}$	P
0.1×10^9 con. cm^{-2}	0±0Ac	0±0Bc	66.6±6.6Bb	93.3±6.6Ba	93.3±6.6Ba	100±0Aa	65.77	0.000
0.5×10^9 con. cm^{-2}	26.3±13.3Ab	73.3±13.3Aa	80±11.5Aa	100±0Aa	100±0Aa	100±0Aa	13.43	0.000
0.10×10^9 con. cm^{-2}	26.6±17.6Ac	73.3±6.6Ab	100±0Aa	100±0Aa	100±0Aa	100±0Aa	18.96	0.000
0.15×10^9 con. cm^{-2}	26.6±6.6Ab	93.3±6.6Aa	100±0Aa	100±0Aa	100±0Aa	100±0Aa	35.43	0.000
Control *Kontrol*	0±0	0±0	0±0	0±0	0±0	0±0		
$F_{3.8}$	1.93	22.41	68.75	1.00	1.00	-		
P	0.203	0.000	0.000	0.441	0.441	-		

- Two-way analysis of variance (ANOVA) was applied to the data and the differences between the means were determined according to the Tukey test at the 5% significance level (P<0.05), different capital letters in the same column and different lowercase letters in the same row are statistically different from each other (dose: $F_{3,56}$= 21.65, P<0.0001; exposure time: $F_{6.56}$= 80.69, P<0.0001; dose × exposure time: $F_{18.56}$= 4.88, P<0.0001).
- Verilere çift yönlü varyans analizi (ANOVA) uygulanmış ve ortalamalar arasındaki farklılıklar %5 önem seviyesinde Tukey testine göre belirlenmiştir (P<0.05), aynı sütundaki farklı büyük harfler ve aynı satırdaki farklı küçük harfler istatistiksel olarak birbirinden farklıdır (doz: $F_{3,56}$= 21.65, P<0.0001; maruz kalma süresi: $F_{6.56}$= 80.69, P<0.0001; doz × maruz kalma süresi: $F_{18.56}$= 4.88, P<0.0001).

Table 7. Mortality rates (%) (+ s.e.) of *Periplaneta americana* adults exposed to *Beauveria varroae* 35727
Çizelge 7. Beauveria varroae 35727 izolatına maruz burakılan Periplaneta americana erginlerinin ölüm oranları (%) (+ s.h.)

Beauveria varroae 35727	**Post-treatment time (hour)** *Uygulama sonrası süre (saat)*								
	48	72	96	120	144	168	192	$F_{6.14}$	P
0.1×10^9 con. cm^{-2}	0±0Ab	0±0Bb	6.6±6.6Bab	6.6±6.6Bab	6.6±6.6Cab	20±0Bab	26.6±6.6Ba	4.07	0.014
0.5×10^9 con. cm^{-2}	0±0Ac	20±11.5Bbc	20±11.5Bbc	33.3±17.6Babc	60±11.5Babc	80±11.5Aab	86.6±13.3Aa	6.28	0.002
1×10^9 con. cm^{-2}	33.3±17.6Ab	93.3±6.6Aa	100±0Aa	100±0Aa	100±0Aa	100±0Aa	100±0Aa	11.13	0.000
1.5×10^9 con. cm^{-2}	33.3±13.3Ab	86.6±6.6Aa	100±0Aa	100±0Aa	100±0Aa	100±0Aa	100±0Aa	21.27	0.000
Control *Kontrol*	0±0	0±0	0±0	0±0	0±0	6.6±6.6	6.6±6.6		
$F_{3.8}$	4.67	21.13	35.54	22.06	47.09	26.77	16.74		
P	0.036	0.000	0.000	0.000	0.000	0.000	0.001		

- Two-way analysis of variance (ANOVA) was applied to the data and the differences between the means were determined according to the Tukey test at the 5% significance level (P<0.05), different capital letters in the same column and different lowercase letters in the same row are statistically different from each other (dose: $F_{3,56}$= 138.87, P<0.0001; exposure time: $F_{6.56}$= 24.95, P<0.0001; dose × exposure time: $F_{18.56}$= 2.71, P<0.003).
- Verilere çift yönlü varyans analizi (ANOVA) uygulanmış ve ortalamalar arasındaki farklılıklar %5 önem seviyesinde Tukey testine göre belirlenmiştir (P<0.05), aynı sütundaki farklı büyük harfler ve aynı satırdaki farklı küçük harfler istatistiksel olarak birbirinden farklıdır (doz: $F_{3,56}$= 138.87, P<0.0001; maruz kalma süresi: $F_{6.56}$= 24.95, P<0.0001; doz × maruz kalma süresi: $F_{18.56}$= 2.71, P<0.003).

Beauveria isolates, on the other hand, were observed to cause nearly 100% mortality in the population after 192 hours (except 0.1×10^9 conidia cm^{-2} of isolate 35727), even at low doses (0.1×10^9 and 0.5×10^9 conidia cm^{-2}) (Table 6, 7, 8). *B. bassiana* 5-4 isolate was the most effective isolate, with the lowest dose (0.1×10^9 conidia cm^{-2}) able to kill at least half of the population after a short exposure time (96 hours) (Table 6). In order to see the effectiveness of *B. bassiana* 5-4 isolate at lower doses (0.05 and 0.01 conidia cm^{-2}), effective results could not be obtained from the second test conducted with this isolate, so this test did not make a significant contribution to the study (Table 9).

In tests conducted with boric acid, mortality rates increased proportionally with the dose and exposure time. Doses of 20, 10, 5, 2.5 and 1 mg cm^{-2} caused 100% mortality in insects at 120, 144, 168 and 192 hours. At the end of the test, 0.1 and 0.5 mg cm^{-2} doses caused 40% and 86.6% mortality (Table 10).

Combined Treatments

The results of the tests conducted using the entomopathogenic fungus and boric acid together are

presented in Table 11-12. Fixed dose of *B. bassiana* 5-4 (0.1×10^9 conidia cm^{-2}) and different doses (0.1, 0.5 and 1 mg cm^{-2}) of boric acid were applied to insects and there were no statistically significant differences between the different doses during the test period. Mortality rates increased significantly as the exposure time increased, and after 168 hours, 100% mortality occurred in all tests (Table 11). In the second test, fixed dose of boric acid (1 mg cm^{-2}) and different doses of *B. bassiana* 5-4 (0.01 and 0.05×10^9 conidia cm^{-2}) was applied to insects and there was no statistically significant difference between the two doses during the test, except at 144 and 168 hours. Mortality rates increased significantly as exposure time increased (Table 12). Tables 11 and 12 also show that combined tests can partially increase insect mortality.

Table 8. Mortality rates (%) (+ s.e.) of *Periplaneta americana* adults exposed to *Beauveria varroae* 120601
Çizelge 8. Beauveria varroae 120601 izolatına maruz burakılan Periplaneta americana erginlerinin ölüm oranları (%) (+ s.h.)

Beauveria varroae 120601	**Post-treatment time (hour)** *Uygulama sonrası süre (saat)*								
	48	72	96	120	144	168	192	$F_{6.14}$	P
0.1×10^9 con. cm^{-2}	0±0Ad	0±0Bd	0±0Bd	13.3±6.6Bcd	53.3±6.6Bbc	86.6±13.3Aab	93.3±6.6Aa	27.01	0.000
0.5×10^9 con. cm^{-2}	20±11.5Aa	53.3±29ABa	73.3±17.6Aa	93.3±6.6Aa	93.3±6.6Aa	93.3±6.6Aa	93.3±6.6Aa	2.80	0.052
1×10^9 con. cm^{-2}	6.6±6.6Ac	60±11.5ABb	93.3±6.6Aa	100±0Aa	100±0Aa	100±0Aa	100±0Aa	32.90	0.000
1.5×10^9 con. cm^{-2}	26.6±17.6Ab	80±0Aa	100±0Aa	100±0Aa	100±0Aa	100±0Aa	100±0Aa	19.83	0.000
Control *Kontrol*	0±0	0±0	0±0	0±0	0±0	0±0	0±0		
$F_{3.8}$	1.32	4.25	22.54	31.10	18.02	0.69	0.67		
P	0.333	0.045	0.000	0.000	0.001	0.583	0.596		

- Two-way analysis of variance (ANOVA) was applied to the data and the differences between the means were determined according to the Tukey test at the 5% significance level (P<0.05), different capital letters in the same column and different lowercase letters in the same row are statistically different from each other (dose: $F_{3,56}$= 38.48, P<0.0001; exposure time: $F_{6.56}$= 36.49, P<0.0001; dose × exposure time: $F_{18.56}$= 3.07, P<0.002).

- Verilere çift yönlü varyans analizi (ANOVA) uygulanmış ve ortalamalar arasındaki farklılıklar %5 önem seviyesinde Tukey testine göre belirlenmiştir (P<0.05), aynı sütundaki farklı büyük harfler ve aynı satırdaki farklı küçük harfler istatistiksel olarak birbirinden farklıdır (doz: $F_{3,56}$= 38.48, P<0.0001; maruz kalma süresi: $F_{6.56}$= 36.49, P<0.0001; doz × maruz kalma süresi: $F_{18.56}$= 3.07, P<0.002).

Table 9. Mortality rates (%) (+ s.e.) of *Periplaneta americana* adults exposed to lower application doses of *Beauveria bassiana* 5-4
Çizelge 9. Beauveria bassiana 5-4 izolatının alt uygulama dozlarına maruz burakılan Periplaneta americana erginlerinin ölüm oranları (%) (+ s.h.)

Beauveria bassiana 5-4	**Post-treatment time (hour)** *Uygulama sonrası süre (saat)*					
	48	96	144	192	$F_{6.14}$	P
0.05×10^9 con. cm^{-2}	0±0Ab	20±0Aa	26.6±6.6Aa	26.6±6.6Aa	26.85	0.000
0.01×10^9 con. cm^{-2}	0±0Aa	0±0Ba	0±0Ba	0±0Ba	-	-
Control (*Kontrol)*	0±0	0±0	0±0	0±0		
$F_{1.4}$	-	-	53.17	53.17		
P	-	-	0.002	0.002		

- Two-way analysis of variance (ANOVA) was applied to the data and the differences between the means were determined according to the Tukey test at the 5% significance level (P<0.05), different capital letters in the same column and different lowercase letters in the same row are statistically different from each other (dose: $F_{1.28}$= 395.81, P<0.0001; exposure time: $F_{6.28}$= 26.85, P<0.0001; dose × exposure time: $F_{6.28}$= 26.85, P<0.0001).

- Verilere çift yönlü varyans analizi (ANOVA) uygulanmış ve ortalamalar arasındaki farklılıklar %5 önem seviyesinde Tukey testine göre belirlenmiştir (P<0.05), aynı sütundaki farklı büyük harfler ve aynı satırdaki farklı küçük harfler istatistiksel olarak birbirinden farklıdır (doz: $F_{1.28}$= 395.81, P<0.0001; maruz kalma süresi: $F_{6.28}$= 26.85, P<0.0001; doz × maruz kalma süresi: $F_{6.28}$= 26.85, P<0.0001).

In this study, 5 different isolates of entomopathogenic fungi and boric acid were tested on *P. americana* adults and the combined effect of the fungi isolates showing the highest pathogenicity with boric acid was investigated. Although pure entomopathogenic fungi and boric acid applications can cause high mortality in cockroaches, it can be clearly seen that the effect duration period are long. With the combined application of boric acid and entomopathogen fungus, this period can be shortened by creating a combined effect. Considering the parallel studies on this subject, Zurek et al. (2002), investigated the toxicity of the combined use of the entomopathogenic fungus *M. anisopliae* (Metschnikoff) and boric acid to the German cockroach *Blattella germanica* in a similar study. When they used *M. anisopliae* alone, they achieved a mortality rate of more than 92% after 28 days at a dose of 8.96×10^9 conidia m^{-2}. When they used powdered *M. anisopliae* conidia together with a powder formulation containing 12.5% boric acid or drinking water

containing 0.1% boric acid, they achieved 100% mortality at the end of 8 and 10 days, respectively. In another study, Dayer & Karvandian (2016), found that when they applied 8 gr kg^{-1} boric acid and 5.3×10^8 conidia 10 gr^{-1} feed formulation to 30 *B. germanica* adults, boric acid did not show any side effects on *M. anisopliae*, but it increased its virulence in adult German cockroaches. They found that it increased the mortality rate of insects. They measured the LT_{50} value as 35 days in pure *M. anisopliae*, 47 days in pure boric acid, and 21 days in combined use. In the focus of these data, and similarly, the combined use of *B. bassiana* 5-4 and boric acid increased toxicity in some cases in this study.

Table 10. Mortality rates (%) (+ s.e.) of *Periplaneta americana* adults exposed to boric acid
Çizelge 10. Borik asite maruz burakılan Periplaneta americana erginlerinin ölüm oranları (%) (+ s.h.)

Boric acid	Post-treatment time (hour) *Uygulama sonrası süre (saat)*							$F_{6.14}$	P
	48	72	96	120	144	168	192		
0.1 mg cm^{-2}	0±0Bb	0±0Bb	6.6±6.6Cab	13.3±6.6Cab	13.3±6.6Bab	13.3±6.6Bab	40±0Ca	4.07	0.014
0.5 mg cm^{-2}	0±0Bc	0±0Bc	26.6±6.6BCbc	46.6±6.6BCab	80±11.5Aa	86.6±6.6Aa	86.6±6.6Ba	22.25	0.000
1 mg cm^{-2}	0±0Be	6.6±6.6Bde	40±11.5ABCcd	66.6±13.3ABCbc	66.6±13.3ABbc	93.3±6.6Aab	100±0Aa	23.92	0.000
2.5 mg cm^{-2}	0±0Bc	20±11.5ABbc	66.6±6.6ABab	86.6±6.6ABa	86.6±6.6Aa	86.6±6.6Aa	100±0Aa	18.83	0.000
5 mg cm^{-2}	0±0Bd	33.3±6.6ABcd	53.3±6.6ABbc	86.6±6.6ABab	86.6±6.6Aab	100±0Aa	100±0Aa	21.07	0.000
10 mg cm^{-2}	6.6±6.6Bc	26.6±17.6ABbc	80±11.5Aab	86.6±13.3ABa	100±0Aa	100±0Aa	100±0Aa	13.10	0.000
20 mg cm^{-2}	26.6±6.6Ac	60±11.5Ab	73.3±6.6ABb	100±0Aa	100±0Aa	100±0Aa	100±0Aa	49.59	0.000
Control *Kontrol*	0±0	0±0	0±0	0±0	0±0	0±0	0±0		
$F_{6.14}$	1.32	4.25	22.54	31.10	18.02	0.69	0.67		
P	0.000	0.006	0.001	0.001	0.001	0.001	0.000		

- Two-way analysis of variance (ANOVA) was applied to the data and the differences between the means were determined according to the Tukey test at the 5% significance level (P<0.05), different capital letters in the same column and different lowercase letters in the same row are statistically different from each other (dose: $F_{6.98}$= 50.47, P<0.0001; exposure time: $F_{6.98}$= 112.85, P<0.0001; dose × exposure time: $F_{36.98}$= 2.25, P<0.002).

- Verilere çift yönlü varyans analizi (ANOVA) uygulanmış ve ortalamalar arasındaki farklılıklar %5 önem seviyesinde Tukey testine göre belirlenmiştir (P<0.05), aynı sütundaki farklı büyük harfler ve aynı satırdaki farklı küçük harfler istatistiksel olarak birbirinden farklıdır (doz: $F_{6.98}$= 50.47, P<0.0001; maruz kalma süresi: $F_{6.98}$= 112.85, P<0.0001; doz × maruz kalma süresi: $F_{36.98}$= 2.25, P<0.002).

Table 11. Mortality rates (%) (+ s.e.) of *Periplaneta americana* adults exposed to the combined use of fixed dose (0.1×10^9 conidia cm^{-2}) *Beauveria bassiana* 5-4 and different doses of boric acid
Çizelge 11. Sabit dozda (0.1×10^9 konidi cm^{-2}) Beauveria bassiana 5-4 ve farklı dozlardaki borik asidin kombine kullanımına maruz burakılan Periplaneta americana erginlerinin ölüm oranları (%) (+ s.h.)

Beauveria bassiana (5-4) + boric acid	Post-treatment time (hour) *Uygulama sonrası süre (saat)*						$F_{6.14}$	P
	48	72	96	120	144	168		
0.1×10^9 con. + 1 mg cm^{-2}	6.6±6.6Ab	33.3±17.6Ab	60±20Aab	93.3±6.6Aa	100±0Aa	100±0Aa	14.32	0.000
0.1×10^9 con. + 0.5 mg cm^{-2}	0±0Ac	33.3±13.3Ab	80±11.5Aab	86.6±6.6Aa	86.6±6.6Aa	100±0Aa	21.01	0.000
0.1×10^9 con. + 0.1 mg cm^{-2}	6.6±6.6Ab	26.6±13.3Ab	80±11.5Aa	93.3±6.6Aa	100±0Aa	100±0Aa	17.21	0.000
Control *Kontrol*	0±0	0±0	0±0	0±0	0±0	0±0		
B. bassiana (5-4)(0.1×10^9 conidia cm^{-2})	0±0	0±0	66.6±6.6	93.3±6.6	93.3±6.6	100±0		
$F_{2.6}$	0.50	0.11	0.68	0.33	4.00	-		
P	0.630	0.894	0.540	0.729	0.079	-		

- Two-way analysis of variance (ANOVA) was applied to the data and the differences between the means were determined according to the Tukey test at the 5% significance level (P<0.05), different capital letters in the same column and different lowercase letters in the same row are statistically different from each other (dose: $F_{2,42}$= 0.40, P<0.673; exposure time: $F_{6,42}$= 49.89, P<0.0001; dose × exposure time: $F_{12,42}$= 0.63, P=0.806).

- Verilere çift yönlü varyans analizi (ANOVA) uygulanmış ve ortalamalar arasındaki farklılıklar %5 önem seviyesinde Tukey testine göre belirlenmiştir (P<0.05), aynı sütundaki farklı büyük harfler ve aynı satırdaki farklı küçük harfler istatistiksel olarak birbirinden farklıdır (doz: $F_{2,42}$= 0.40, P<0.673; maruz kalma süresi: $F_{6,42}$= 49.89, P<0.0001; doz × maruz kalma süresi: $F_{12,42}$= 0.63, P=0.806).

Table 12. Mortality rates (%) (+ s.e.) of *Periplaneta americana* adults exposed to the combined use of fixed dose (1 mg cm^{-2}) boric acid and different doses of *Beauveria bassiana* 5-4

Çizelge 12. Sabit dozda (1 mg cm^{-2}) *borik asit farklı dozlardaki Beauveria bassiana 5-4'ün kombine kullanımına maruz burakılan Periplaneta americana erginlerinin ölüm oranları (%) (+ s.h.)*

Boric acid + *Beauveria bassiana* (5-4)	**Post-treatment time (hours)** *Uygulama sonrası süre (saat)*								
	48	72	96	120	144	168	192	$F_{6.14}$	P
1 mg + 0.05×10^9 con. cm^{-2}	0±0Ac	33.3±6.6Abc	60±20Aab	86.6±13.3Aab	100±0Aa	100±0Aa	100±0Aa	12.93	0.000
1 mg + 0.01×10^9 con. cm^{-2}	0±0Ad	6.6±6.6Acd	33.3±6.6Abc	60±11.5Ab	66.6±6.6Bab	73.3±6.6Bab	93.3±6.6Aa	22.47	0.000
Control *Kontrol*	0±0	0±0	0±0	0±0	0±0	0±0	0±0		
Boric acid (1 mg cm^{-2})	0±0	6.6±6.6	40±11.5	66.6±13.3	66.6±13.3	93.3±6.6	100±0		
$F_{1.4}$	1.00	7.11	1.47	3.02	68.75	53.17	1.00		
P	0.374	0.056	0.292	0.157	0.001	0.002	0.374		

- Two-way analysis of variance (ANOVA) was applied to the data and the differences between the means were determined according to the Tukey test at the 5% significance level (P<0.05), different capital letters in the same column and different lowercase letters in the same row are statistically different from each other (dose: $F_{1,28}$= 30.27, P<0.0001; exposure time: $F_{6,28}$= 31.05, P<0.0001; dose × exposure time: $F_{6,28}$= 0.89, P>0.05).

- Verilere çift yönlü varyans analizi (ANOVA) uygulanmış ve ortalamalar arasındaki farklılıklar %5 önem seviyesinde Tukey testine göre belirlenmiştir (P<0.05), aynı sütundaki farklı büyük harfler ve aynı satırdaki farklı küçük harfler istatistiksel olarak birbirinden farklıdır (doz: $F_{1,28}$= 30.27, P<0.0001; maruz kalma süresi: $F_{6,28}$= 31.05, P<0.0001; doz × maruz kalma süresi: $F_{6,28}$= 0.89, P>0.05).

CONCLUSION

As a result, the lethal effect of entomopathogenic fungi and boric acid against *P. americana* can be accelerated by combination. Both have been specified in the literature to be safe for humans and other mammals. The principle of synergism between boric acid and entomopathogenic fungus is not known exactly, but Gwokyalya & Altuntaş (2019), found in their study that the cytotoxic effect of boric acid significantly suppressed the hemocyte immune response mechanisms such as melanization, encapsulation and nodule formation of *Galleria mellonella* (Lepidoptera: Pyralidae). They revealed that *M. anisopliae* facilitates evasion of these mechanisms, thereby increasing the virulence of the pathogen. Similarly, it is clear that American cockroaches exposed to lethal subdoses of boric acid become more susceptible to *B. bassiana* infection. The advantage of this combination is, it can accelerate the mechanism of action of the entomopathogenic fungus without compromising its viability. However, more research is needed to optimize this formulation, determine its effectiveness under field conditions, and expand its use on other pest species.

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Author Contributions

The authors declare that they have contributed equally to the article.

Conflicts of Interest

The authors declare no conflict of interest.

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