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Effects of Some Entomopathogen Fungi on *Apis mellifera* L. and *Bombus terrestris* L.

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Verticillium lecanii,
Honey bee,
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Toxic effect

Abstract: Bees are essential elements of agricultural production because of their role in pollination. However, some practices during production, especially pesticide applications have negative effects on bee life and behaviours. In order to decrease the side effects of pesticides, alternative methods, especially biological control, gained importance. In this study, effects of preparations containing; *Beaveria bassiana*, *Verticillium lecanii* and *Metarhizium anisopliae* var. *anisopliae*, and also Chlorpyrifos-ethyl for comparison, on *Apis mellifera* and *Bombus terrestris*, were investigated. Preparations were applied with two different methods; directly as food and by spraying, with concentrations recommended by the producer companies. In the first method, bees were fed with 5 ml solutions of the preparations mixed with sucrose solution and antenna, wing, leg and abdomen movements of the bees were controlled and scored 4 hours after applications. In the second method, bees were sprayed with the preparations and mortality rates were found. As a result of feeding method, entomopathogen preparations slightly affected the movement of the honey bees, while Chlorpyrifos-ethyl, almost totally inhibited their movement. Similarly, entomopathogens had no harmful effect on the movement of bombus bees, but the insecticide totally inhibited their movement. In the spraying method, the insecticide killed all the individuals of both bee species, while entomopathogens caused the death of only a few individuals.

Bazı Entomopatojen Fungusların *Apis mellifera* L. ve *Bombus terrestris* L.'e Etkileri

Anahtar Kelimeler
Beauveria bassiana,
Metarhizium anisopliae var. *anisopliae*,
Verticillium lecanii,
Bal arısı,
Bombus arısı,
Toksik etki

Özet: Arılar tozlaşmadaki önemli rolleri nedeniyle bitkisel üretimin vazgeçilmez unsurlarıdır. Ancak üretim sırasındaki bazı işlemlerin, özellikle de pestisit uygulamalarının arıların canlılığı ve davranışları üzerinde olumsuz etkileri bulunmaktadır. Pestisitlerin istenmeyen etkilerini azaltmak amacıyla alternatif yöntemler, bunlar arasında da biyolojik mücadele önem kazanmıştır. Bu çalışmada *Beaveria bassiana*, *Verticillium lecanii* ve *Metarhizium anisopliae* var. *anisopliae* ile karşılaştırma ilacı olarak Chlorpyrifos-ethyl etken maddeli preparatların, *Apis mellifera* ve *Bombus terrestris*'e etkisi incelenmiştir. Preparatlar yedirme ve püskürtme şeklinde iki yöntemle, önerilen dozlarda uygulanmıştır. İlk yöntemde, sukroz çözeltisi ile karıştırılarak hazırlanmış preparatlar arılara 5'er ml yedirilmiş ve 4 saat sonra anten, kanat, bacak ve abdomen hareketleri kontrol edilerek puanlanmıştır. İkinci yöntemde ise preparatlar arılara püskürtülmüştür. Çalışma sonucunda, entomopatojenlere ait preparatlar ağız yoluyla uygulandığında bal arılarının hareketleri üzerinde çok düşük seviyede etkili olurken, Chlorpyrifos-ethyl arı hareketlerini neredeyse tamamen engellemiştir. Aynı şekilde, bombus arılarının hareketleri üzerinde entomopatojenlerin hiçbir olumsuz etkisi olmazken insektisit bunların hareketini de tamamen engellemiştir. Püskürtme yönteminde ise insektisit her iki arı türüne ait bireylerin tamamını öldürürken, entomopatojenler çok az sayıda bireyin ölümüne neden olmuştur.

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1. Introduction

Honey bees and bombus bees have important role in the pollination of plants [1,2]. Albert Einstein mentioned that human can live only 4 years if bees disappear. Actually he meant that human and animal life will be distressed by the absence of pollination without the activity of bees [3]. Recently bombus bees gained importance in greenhouse production to obtain high and qualified yield [4,5]. Bees have been negatively affected by the human activities, especially from pesticide applications in agriculture. There are many studies on the harmful effects of agricultural chemicals both on honey bees and bombus bees [6-13]. In U.S.A. 121 different pesticides and their metabolites were determined in bee products such as pollen, beewax and honey [14]. Pesticides have various effects on bees. Besides direct lethal effect, they shorten bee life [15,16], cause disorientation [17], disrupt memory and brain metabolism, decrease learning performance [18,19], and disrupt motor functions [20,21].

In recent years, environmentally friendly methods, especially biological control, have increasingly been used as an alternative to pesticides. Among the beneficial organisms used in the biocontrol of pests, entomopathogenic fungi have important role and their usage have gradually been increasing [22-25]. Now there are 750-1000 described entomopathogenic fungus species. In addition, from 1960's 171 fungus preparations, most of which contains *Beauveria bassiana* (Bals.-Criv.) Vuill., *Metarhizium anisopliae* (Metchnikoff) Sorokin) and *Isaria fumosorosea* Wize, have been used in the biocontrol of agricultural pests [26]. Although there are many studies on the effects of entomopathogens on harmful organisms mainly insects, there are less report on their effects on beneficial insects and bees [27-30]. Conflicting results were obtained in the studies performed to determine the effects of entomopathogens on bees. It was reported that high concentrations (10^6-10^8 spore/bee) of *B. bassiana* shortened the lifespan of honey bees [31]. In another study, *M. anisopliae* was found to be more pathogenic for bees than *B. bassiana* [32]. In contrast, when bees were fed with sucrose solution containing *M. anisopliae*, *B. bassiana* and *V. lecanii*, mortality rates were higher than controls when bees were fed with the latter two entomopathogens, while *M. anisopliae* did not significantly affect the mortality rates of the bees [33]. In another study, it was determined that the infestation of a dust formulation of *B. bassiana* on bees significantly decreased average lifespan of the bees [34]. Similarly, *M. anisopliae* and *B. bassiana* commercial preparations were compared in terms of their effects on vitality of honey bees. It was found that both preparations decreased the rates of alive individuals and the latter entomopathogen was more effective [35]. In a study performed both under laboratory conditions and on beehives, it was found that *B. bassiana* and *M. anisopliae* caused slight infection on the bees but they didn't cause any change in bee behaviour, larval development and colony features [36]. Furthermore, some researchers mentioned that bombus bees could be used as a vector for the dissemination of entomopathogen fungus *B. bassiana* in greenhouses [37].

In this study, effects of three entomopathogen fungus preparations and an insecticide with active ingredient Chlorpyrifos-ethyl for comparison, on vitality and motor functions of honey bees and bombus bees, were investigated..

2. Material and Method

The main materials of the study were *Apis mellifera* L. and *Bombus terrestris* L. Honey bees were obtained from a beekeeper (Ümit Ferahzade, Beyşehir, Konya) and bombus bees from Koppert® company in Turkey. Two entomopathogens used in the study; *Beauveria bassiana* (Bals.-Criv.) Vuill. and *Verticillium lecanii* (Zimm.) Viegas (Syn.: *Lecanicillium lecanii* R. Zare & W. Gams) were obtained from Agrobest company. The third entomopathogen isolate used in the study was *Metarhizium anisopliae* (Metsch.) Sorokin var. *anisopliae* Strain F52 (Syn.: *M. brunneum* Petch) isolated from *Cydia pomonella* and was obtained from Swansea University (UK). Insecticide Chlorpyrifos-ethyl produced by Dow AgroSciences company was also used for comparison. Commercial names, rates of active ingredients, formulation types and doses of the preparations were given in Table 1.

Tablo 1. Preparations used in the experiments

Active Ingredient	Commercial Name	Rate and Formulation	Doses
Beauveria bassiana strain Bb-1	Nostalgist BL®	1.5% SL	0.25 ml/l
Verticillium lecani strain V1-1	Nibortem®	1.5% SL	0.25 ml/l
Metarhizium anisopliae var. *anisopliae*	Strain F52	9X10^{11} cfu/kg G	0.10 gr/l
Chlorpyrifos-ethyl	Dursban4®	480 g/l EC	1.50 ml/l

Preparations were applied to the bees by two methods; feeding and spraying. At first 2 M sucrose solution in 5 cm Petri dishes were placed 10 m away from the beehives in the field and bees visiting the dishes were picked in small plastic boxes. Bees were brought to the laboratory and kept at -18°C for a few minutes [38]. When they become inactive, they were fixed to plastic syringe from their thorax, without preventing the movement of their head, legs and abdomen. After a few minutes, they were checked if they were healthy or not, by touching a small cotton with sucrose solution to their antenna and inspecting their response. Each bee was fed by sucrose solution until they become full and checked again after 24 hours and healthy bees were selected. Then 5 µl solutions of the entomopathogen and insecticide formulations were prepared, mixed with 5 µl sucrose solutions and bees were fed with the solutions dropped in petri dishes. After the bees finished all the solutions, they were kept in the laboratory for 4 hours and then they were scored according to the movement of their proboscis, antenna, legs and abdomen. Bees which couldn't move any of their body parts were given 0 point, bees moving their proboscis, antenna, legs or abdomen slowly and irregularly were given 1 point, and bees moving their body parts normally were given 2 points for each of their body parts. Thus, bees normally moving all of their body parts were given 8 points, while those totally paralysed were given 0 point [39]. Experiments were performed with 5 replicates and 5 bees were used for each replicate. Bees in the control group were fed only with 2M sucrose solution.

Applications were changed a little bit for bombus bees, since they have different behaviour and response. Beehives with about 60-70 bombus bees were kept under red light and bees were transferred to falcon tubes in groups of 5 bees. Tubes were kept at -18°C for 3-4 minutes and after the bees became inactive, they were fixed onto plastic syringe from their thorax. Bees were then fed with preparations mixed with sucrose solution and scored for their motor functions 4 hours after feeding, similar with honey bees experiment.

For the spraying method, honey bees were put into plastic boxes (10x10x10 cm) and preparations with recommended doses were applied on them by using a simple hand sprayer. Bees in the control group were sprayed with distilled water and 5 replications, and 5 bees in each replicate, were used in the experiment. Dead and alive honey bees were recorded 4, 12 and 24 hours after spraying. Preparations were applied to bombus bees in beehives and bees were checked one week after the applications. Three beehives were used each with 60-70 bees.

JMP (Ver. 8) program was used to evaluate the results. Data were subjected to analyses of variance and Tukey's multiple comparison test was used to compare the means. Efficacy of the preparations were calculated by Abbott's formula [40].

3. Results

As a result of feeding method, there was no significant difference among the movements of the body parts of honey bees fed with entomopathogen preparations and controls. This result showed that the entomopathogens had no negative effect on motor functions of honey bees. In contrast, movement scores of the bees fed with Chlorpyrifos-ethyl were statistically different and formed another group. This meant that the insecticide significantly inhibited the motor functions of honey bees. Similar results were obtained with the bombus bees. All the individuals fed with the entomopathogen preparations were scored with 8 point as in control group showing that entomopathogens had no inhibitory effect on bombus bee's motor functions. Bombus bees fed with Chlorpyrifos-ethyl became motionless and it was observed that all were dead 4 hours after the application (Table 2).

Table 2. Effects of feeding with the entomopathogens and Chlorpyrifos-ethyl on motor functions of the bees (Mean ± SE)

Applications	Body Movement Scores	
	Honey bee	Bombus bee
Control	7.96±0.04 a*	8.0±0.00 a*
Beauveria bassiana	7.36±0.39 a	8.0±0.00 a
Verticillium lecani	7,04±0.39 a	8.0±0.00 a
Metarhizium anisopliae var. *anisopliae*	7.36±0.39 a	8.0±0.00 a
Chlorpyrifos-ethyl	0.36±0.22 b	0.0±0.00 b

* Means in the same column shown by the same letter were not significantly different from each other according to Tukey's test ($p \leq 0.05$)

As a result of spraying method, it was determined in the first observation made 4 hours after the application, Chlorpyrifos-ethyl caused death of all the bees, while in the control and entomopathogen applications, all bees were alive. In the second observation made 12 hours after the application, it was observed that all the bees in the control group and those in *B. bassiana* and *V. lecanii* applications were still alive, while a few bees were dead in the group sprayed with *Metarhizium anisopliae* var. *anisopliae*. In the third observation made 24 hours after the application, all entomopathogens caused death of a few bees, but means were not significantly different from the control group where all the bees were alive (Table 3).

Table 3. Effects of spraying with the entomopathogens and Chlorpyrifos-ethyl on the number of alive *Apis mellifera* individuals (Mean ± SE)

Applications	Mean Number of Alive Bees		
	4. hour	12. hour	24. hour
Control	5.0 ± 0.0 a*	5.0 ± 0.0 a	5.0 ± 0.0 a
Beauveria bassiana	5.0 ± 0.0 a	5.0 ± 0.0 a	4.6 ± 0.2 a
Verticillium lecanii	5.0 ± 0.0 a	5.0 ± 0.0 a	4.6 ± 0.2 a
Metarhizium anisopliae var. *anisopliae*	5.0 ± 0.0 a	4.8 ± 0.2 a	4.6 ± 0.4 a
Chlorpyrifos-ethyl	0.0 ± 0.0 b	0.0 ± 0.0 b	0.0 ± 0.0 b

* Means in the same column shown by the same letter were not significantly different from each other according to Tukey's test ($p \leq 0.05$)

According to the results obtained 24 hours after the applications, the insecticide showed 100% efficacy on both bee species (Figure 1, 2).

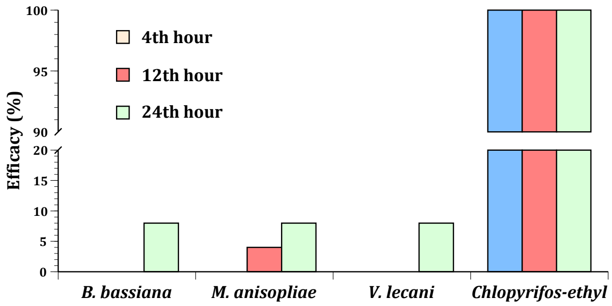

Figure 1. Efficacy of the entomopathogens and Chlorpyrifos-ethyl on *Apis mellifera*, 4, 12 and 24 hours after spraying (%)

Similar results were obtained in the observation made one week after the application of preparations by spraying method, on the beehives containing *B. terrestris* individuals. Chlorpyrifos-ethyl caused death of all the bees when applied by spraying method, while entomopathogen applications caused death of a small number of bees and statistically arranged in the same group with control (Table 4). When the results were evaluated by Abbott's formula, the efficacy of the entomopathogen preparations on bombus bees were less than 2%, while spraying the insecticide had 100% efficacy (Figure 2).

Table 4. Effects of spraying with the entomopathogens and Chlorpyrifos-ethyl on the number of alive *Bombus terrestris* individuals (Mean ± SE)

Applications	Mean Number of Alive Bees
Control	65.00 ± 0.00 a*
Beauveria bassiana	64.00 ± 1.00 a
Verticillium lecani	64.33 ± 0.66 a
Metarhizium anisopliae var. *Anisopliae*	64.67 ± 0.33 a
Chlorpyrifos-ethyl	0.00 ± 0.00 b

* Means shown by the same letter were not significantly different from each other according to Tukey's test (p≤0.05)

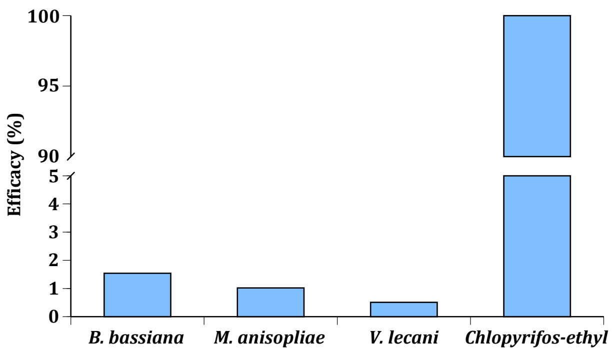

Figure 2. Efficacy of spraying with the entomopathogens and Chlorpyrifos-ethyl on *Bombus terrestris* (%)

4. Discussion and Conclusion

In this study, entomopathogen *B. bassiana*, *V. lecani* and *M. anisopliae* var. *anisopliae* preparations were applied to honey bees and bombus bees by feeding and spraying methods. As a result, both on honey bees and bombus bees, no harmful effect of the entomopathogens were determined and mean numbers of alive bees were not significantly different from the control group. Conversely, a commonly used insecticide Chlorpyrifos-ethyl which was used in the study as a comparison pesticide, was found to be very effective and caused death of all the individuals of both bee species. It was previously shown that Chlorpyrifos-ethyl had toxic effect on bees. In a study made in South Italy, it was mentioned that this chemical was one of the most commonly used insecticides and was responsible from the bee losses. Residues of this insecticide were found on 32% of the dead bee samples taken from the areas where bee losses were observed [41]. In a risk evaluation report on pesticide residues and bees, it was indicated that Chlorpyrifos was one of the most risky chemicals for bees and that besides its direct effect, it caused harm by its residues in pollen and beewax [42]. Results of this study were in harmony with the previous records on the toxic effects of this chemical on bees.

There are conflicting reports on the effects of entomopathogens on bees. Some researchers had found them harmless for bees. Furthermore, some of them declared that entomopathogen fungi could be used against bee parasites [43,44], while some others stated that bees could be used to spread entomopathogens in greenhouses [37]. However, there are also some reports on the pathogenic effects of entomopathogens on bees. It was found that high concentrations (10^6-10^8 spores/bee) of *Beauveria bassiana* shortened life span of bees [31]. In another study, *M. anisopliae* were found to be more pathogenic for bees than *B. bassiana* [32]. Similarly, it was determined that *B. bassiana* and *M. anisopliae* caused infection and death of bees even in lower rates under laboratory conditions. But it was reported that the fungi caused no change in the behaviour, larval development and colony features [36]. Effects of commercial preparations of *M. anisopliae*, *B. bassiana* and *Isaria fumosorosea* on three bee species were investigated and two latter fungi caused less than 30% death on all three bee species, while the effect of *M. anisopliae* was higher and changed among 38.9-94.2% depending on the species [45]. In a research, different isolates of *M. anisopliae* were used against bees and it was found that some isolates had high toxic effect while some were less effective [46]. These reports showed that there were virulence differencies among species and also among the isolates belonging to same species and indicated the cause of conflicting results obtained in similar studies.

In a recent study, effects of *M. anisopliae*, *B. bassiana* ve *V. lecani* on honey bees were investigated by three methods. When the spore suspensions of the fungi were sprayed on paper strips coated with starch, placed between the frames in front of the beehives, mortality rate was higher than others in the beehive where *V. lecani* was applied. When the fungi were sprayed on marked areas of the beehive, *M. anisopliae* and *V. lecani* caused higher rates of death, but there were no statistically significant difference among the mortality rates. When bees were fed with sucrose solutions mixed with the fungi, *M. anisopliae* had no significant effect on bee mortality, while mortality rates of bees fed with *B. bassiana* and *V. lecani* were higher than controls [33]. In a similar research, four methods were used to apply *M. anisopliae* and *B. bassiana* commercial preparations on honey bees. *B. bassiana* decreased the rates of alive bees in all methods, while *M. anisopliae* were less effective [35]. These results indicated that the application methods could also change the efficacy of entomopathogens.

It was determined in the present study that honey bees were more susceptible to entomopathogens than bombus bees. Efficacy of the entomopathogens on honey bees reached 8% in the observation made 24 hours after the applications, while on bombus bees it was less than 2% one week after application. In a research made in Finland, it was found that *M. anisopliae* could infect bombus bees, but it would cause no risk if the fungus was applied in soil or on plants not attractive for bombus bees [47]. Conversely, application of a dust formulation of *B. bassiana* on bombus bees decreased the longevity of bees [34]. These reports indicated that the effects of entomopathogens on bombus bees changed depending on the isolates and application methods.

In the previous studies, effects of entomopathogens on bees were only evaluated in terms of bee mortality. While the present study investigated the effects of entomopathogens both on mortality and also on motor functions of honey bees and bombus bees. The results of the study showed that the effects of entomopathogens on bees were relatively low. However, detailed field and greenhouse studies using different application methods will be better to understand the possible long-term effects of entomopathogens on bees. Then entomopathogens can safely be used against insect pests causing harm on agricultural crops, as part of integrated pest management strategies.

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References

[1] Özbek, H. 1992. Balarısı (*A. mellifera* L.)'nın bitkilerin tozlaşmasında kullanılması. Doğu Anadolu Bölgesi I. Arıcılık Semineri, 3-4 Haziran, Erzurum, 30-47.

[2] Gösterit, A., Gürel, F. 2005. *Bombus terrestris* (Hymenoptera: Apidae) Arılarının Yayılmasının Ekosistem Üzerine Etkileri. Uludağ Arıcılık Dergisi, 5, 115-121.

[3] Kandemir, İ. 2007. Amerika Birleşik Devletleri'nde Toplu Arı Ölümleri ve Koloni Çökme Bozukluğu (KÇB) Üzerine Bir Derleme. Uludağ Arıcılık Dergisi, 7(2), 63-69.

[4] Genç, F. 1995. Bombus Arıları, *Bombus* spp., ve Türk Tarımı İçin Önemi. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 26(4), 557-568.

[5] Tüzün, A. 2013. Türkiye'deki Vespidae Türlerinin (Hymoneptera: Insecta) Önemi. Arıcılık Araştırma Dergisi, 5(10), 2-6.

[6] Özbek, H. 1983. Arıların Zirai Mücadele İlaçlarından Etkilenmeleri ve Alınacak Önlemler. Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü Yayınları, Ankara, 46s.

[7] Özbek, H. 1985. Pestisitlerin Faydalı Böcek Faunasına Olumsuz Etkileri. Ulusal Çevre Sempozyumu Tebliğ Metinleri, 12-15 Kasım, Adana, 766-775.

[8] Iwasa, T., Motoyama, N., Ambrose, J.T., Roe, M.R. 2004. Mechanism for the Differential Toxicity of Neonicotinoid Insecticides in the Honey Bee, *Apis mellifera*. Crop Protection, 23, 371-378.

[9] Johnson, R.M., Ellis, M.D., Mullin, C.A., Frazier, M. 2010. Pesticides and Honey Bee Toxicity. Apidologie, 41, 312-331.

[10] Rasuli, F., Rafie, J.N., Sadeghi, A. 2015. The Acute Oral Toxicity of Commonly Used Pesticides in Iran, to Honey Bees (*Apis mellifera meda*). J. Apic. Sci., 59 (1), 17-26.

[11] Van Engelsdorp, D., Hayes Jr., J., Underwood, R.M., Pettis, J. 2010. A Survey of Honey Bee Colony Losses in the U.S., Fall 2008 to Spring 2009. Journal of Apicultural Research, 49(1), 7-14.

[12] Ünal, H.H., Oruç, H.H., Sezgin, A., Kabil, E. 2010. Türkiye'de 2006-2010 Yılları Arasında, Bal Arılarında Görülen Ölümler Sonrasında Tespit Edilen Pestisitler. Uludağ Arıcılık Dergisi, 10(4), 119-125.

[13] Stoner, K. A., Eitzer, B.D. 2012. Movement of Soil-Applied Imidacloprid and Thiamethoxam into Nectar and Pollen of Squash (*Cucurbita pepo*). Plos One, 7(6), 1-6.

[14] Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., vanEngelsdorp, D., Pettis, J.S. 2010. High Levels of Miticides and Agrochemicals in North American Apiaries: Implications for Honey Bee Health. PlosOne, 5(3), 1-17.

[15] Mackenzie, K.E., Winston, M.L. 1989. The Effects of Sublethal Exposure to Diazinon, Carbaryl and

Resmethrin on Longevity and Foraging in *Apis mellifera* L. Apidologie, 20(1), 29-40.

[16] Karahan, A., Gül, A., Kutlu, M.A., Karaca, İ. 2017. Thiamethoxam'ın Yaban Arısı (*Vespa* sp.) Üzerine Etkisi. Mehmet Akif Ersoy Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 8 (Ek Sayı 1), 221-227.

[17] Schricker, B., Stephen, W.P. 1970. The Effect of Sublethal Doses of Parathion on Honey Bee Behaviour. Journal of Apicultural Research, 9, 141-153.

[18] Decourtye, A., Armengaud, C., Renou, M., Devillers, J., Cluzeau, S., Gauthier, M., Pham-De´le`gue, M.H. 2004. Imidacloprid Impairs Memory and Brain Metabolism in the Honeybee (*Apis mellifera* L.). Pest. Biochem. Physiol., 78, 83-92.

[19] Williamson, S.M., Wright, G.A. 2013. Exposure to Multiple Cholinergic Pesticides Impairs Olfactory Learning and Memory in Honeybees. Journal of Experimental Biology, 216 (10), 1799-1807.

[20] Bovi, T.S., Zaluski, R., Orsi, R.O. 2018. Toxicity and Motor Changes in Africanized Honey Bees (*Apis mellifera* L.) Exposed to Fipronil and Imidacloprid. Annals of the Brazilian Academy of Sciences, 90(1), 239-245.

[21] Karahan, A., Gül, A., Kutlu, M.A., Karaca, İ. 2018. Imidacloprid'in Bal Arılarının (*Apis mellifera anatoliaca* ve *Apis mellifera caucasica*) Vücut Fonksiyonları Üzerine Etkisinin Araştırılması. Tr. Doğa ve Fen Derg., 7(1), 24-28.

[22] Öncüer, C. 1984. Zararlı Böceklere Karşı Biyolojik Savaşta Entomopatojen Funguslar. Türkiye Bitki Koruma Dergisi, 8, 177-189.

[23] Groden, E. 1999. Using *Beauveria bassiana* for Insect Management. Proceedings. New England Vegetable and Berry Growers Conference and Trade Show, Sturbridge, MA, 313-315.

[24] Goettel, M.S., Koike, M., Kim, J.J., Aiuchi, D., Shinya, R., Brodeur, J. 2008. Potential of *Lecanicillium* spp. for Management of Insects, Nematodes and Plant Diseases. J. Invertebr. Pathol., 98, 256-261.

[25] Demirci, F., Mustu, M., Kaydan, M.B., Ülgentürk, S. 2011. Laboratory Evaluation of the Effectiveness of the Entomopathogen; *Isaria farinosa*, on Citrus Mealybug, *Planococcus citri*. J. Pest. Sci., 84, 337-342.

[26] Mora, M.A.E., Castilho, A.M.C., Fraga, M.E. 2017. Classification and Infection Mechanism of Entomopathogenic Fungi. Arq. Inst. Biol., 84, 1-10, e0552015.

[27] Ginsberg, H.S., Lebrun, R.A., Heyer, K., Zhioua, E. 2002. Potential Nontarget Effects of *Metarhizium anisopliae* (Deuteromycetes) Used for Biological Control of Ticks (Acari: Ixodidae). Environmental Entomology, 31(6), 1191-1196.

[28] Thungrabeab, M., Tongma, S. 2007. Effect of Entomopathogenic Fungi, *Beauveria bassiana* and *Metarhizium anisopliae* on Non Target Insect. KMITL SCI. Tech. J., 7, 8-12.

[29] Roy, H.E., Brown, M.J., Rothery, P., Remy, L., Michael, W., Majerus, E.N. 2008. Interactions Between the Fungal Pathogen *Beauveria bassiana* and Three Species of Coccinelid: *Harmonia axyridis*, *Coccinella septempunctata* and *Adalia bipunctata*. Bio Control, 53, 265-276.

[30] Roy, H.E., Cottrell T.E. 2008. Forgotten Natural Enemies: Interactions Between Coccinellids and Insect-Parasitic Fungi Eur. J. Entomol., 105, 391-398.

[31] Vandenberg, J.D. 1990. Safety of Four Entomopathogens for Caged Adult Honey Bees (Hymenoptera: Apidae). Econ. Entomol., 83(3), 755-759.

[32] Butt ,T.M., Ibrahim, L., Ball, B.V., Clark, S.J. 1994. Pathogenicity of Entomogenous Fungi *Metarhizium anisopliae* and *Beauveria bassiana* Against Crucifer Pests and the Honey Bee. Biocontrol Science and Technology, 4, 207-214.

[33] Soni, J., Thakur, M. 2011. Effect of Biopathogens on Honey Bees. Pest Technology, 5(1), 86-90.

[34] Karise, R., Muljar, R., Smagghe, G., Kaart, T., Kuusik, A., Dreyersdorff, G., Williams, I.H., Mand, M. 2015. Sublethal Effects of Kaolin and the Biopesticides Prestop-Mix and BotaniGard on Metabolic Rate, Water Loss and Longevity in Bumble Bees (*Bombus terrestris*). Journal of Pest Science, DOI 10.1007/s10340-015-0649-z.

[35] Potrich, M., da Silva, R.T.L., Maia, F.M.C., Lozano, E.R., Rossi, R.M., Colombo, F.C., Tedesco, F.G., de Gouvea, A. 2018. Effects of Entomopathogens on Africanized *Apis mellifera* L. (Hymenoptera: Apidae). Revista Brasileira de Entomologia, 62(1), 23-28.

[36] Alves, S.B., Marchini, L.C., Pereira, R.M., Baumgratz, L.L. 1996. Effects of Some Insect Pathogens on the Africanized Honey Bee, *Apis mellifera* L. (Hym., Apidae). Journal of Applied Entomology, 120(9), 559-564.

[37] Al-mazra'awi, M.S., Shipp, L., Broadbent, B., Kevan P. 2006. Biological Control of *Lygus lineolaris* (Hemiptera: Miridae) and *Frankliniella occidentalis* (Thysanoptera: Thripidae) by *Bombus impatiens* (Hymenoptera: Apidae) vectored *Beauveria bassiana* in Greenhouse Sweet Pepper. Biological Control 37, 89-97.

[38] Hranitz, J.M., Abramson, C.I., Carter, R.P. 2010. Ethanol Increases HSP70 Concentrations in Honeybee (*Apis mellifera* L.) Brain Tissue. Alcohol, 44(3), 275-282.

[39] Duell, E. M. 2012. Honeybee Stress: Behavioral & Physiological Effects of Orally Administered Flumethrin. Bloomsburg University of Pennsylvania, Honors Program, Thesis, 46p, Pennsylvania.

[40] Abbott, W.S. 1925. A Method of Computing the Effectiveness of an Insecticide. Journal of Economic Entomology, 18, 265-267.

[41] Porrini, C., Caprio, E., Tesoriero D., Prisco, G.D. 2014. Using Honey Bee as Bioindicator of Chemicals in Campanian Agroecosystems (South Italy). Bulletin of Insectology, 67 (1), 137-146.

[42] Sanchez-Bayo, F., Goka, K. 2014. Pesticide Residues and Bees - A Risk Assessment. PLoS ONE 9(4), e94482.

[43] Kanga, L.H.B., Jones, W.A., James, R.R. 2003. Field Trials Using the Fungal Pathogen, *Metarhizium anisopliae* (Deuteromycetes: Hyphomycetes) to Control the Ectoparasitic Mite, *Varroa destructor* (Acari: Varroidae) in Honey Bees, *Apis mellifera* (Hymenoptera: Apidae) Colonies. Journal of Economic Entomology, 96, 1091-1099.

[44] Muerrle, T.M., Neumann, P., Dames, J.F., Hepburn, H.R., Hill, M.P. 2006. Susceptibility of Adult *Aethina tumida* (Coleoptera: Nitulidae) to Entomopathogenic Fungi. Journal of Economic Entomology, 99, 1-6.

[45] Toledo-Hernández, R.A., Ruíz-Toledo, J., Toledo, J., Sánchez, D. 2016. Effect of Three Entomopathogenic Fungi on Three Species of Stingless Bees (Hymenoptera: Apidae) Under Laboratory Conditions. Journal of Economic Entomology, 109(3), 1015-1019.

[46] Shaw, K.E., Davidson, G., Clark, S.J., Ball, B.V., Pell, J.K., Chandler, D., Sunderland, K. 2002. Laboratory Bioassays to Assess the Pathogenicity of Mitosporic Fungi to *Varroa destructor* (Acari: Mesostigmata), an Ectoparasitic Mite of the Honey Bee *Apis mellifera* L. Biological Control, 24, 266-276.

[47] Hokkanen, H.M.T., Zeng, Q.Q., Menzler-Hokkanen, I. 2003. Assessing the Impacts of *Metarhizium* and *Beauveria* on Bumblebees. ss 63-71. Hokkanen, H.M.T., Hajek, A.E., ed. 2003. Environmental Impacts of Microbial Insecticides. Dordrecht, Kluwer Academic Publishers. 269p.