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Original article (Özgün makale)

Effects of the entomopathogenic fungus, *Beauveria bassiana*, with adipokinetic hormone, on *Myzus persicae* and *Trialeurodes vaporariorum*

İsmail KARACA[1], Özlem GÜVEN[2]*, Umesh Kumar GAUTAM[3], Tuğçe ÖZEK[4]

Entomopatojen fungus, *Beauveria bassiana*'nın *Myzus persicae* ve *Trialeurodes vaporariorum* üzerindeki etkisinin adipokinetik hormon ile arttırılması

Öz: Bu çalışmanın amacı, adipokinetik hormon (AKH) ile birlikte uygulanan entomopatojen fungusların etkilerini araştırmaktır. Adipokinetik hormon ile tek ve kombine uygulanan *Beauveria bassiana* izolatları yeşil şeftali yaprak biti, *Myzus persicae* ve sera beyazsineği *Trialeurodes vaporariorum* üzerinde test edilmiştir. *M. persicae*'nin *T. vaporariorum*'a göre uygulamaya daha duyarlı olduğu bulunmuştur. *M. persicae* üzerinde BMAUM M6-4 ve BMAUM LD 2016'nın tek ve hormon ile birlikte uygulamasında sırasıyla %58, %67, %95 ve %95 ölüm oranı görülmüştür. Aynı şekilde *T. vaporariorum*'a bu fungusların tek ve hormon ile birlikte uygulanması sonucu sırasıyla %44, %68 ve %45, %78 oranında ölüm gözlenmiştir. *M. persicae* ve *T. vaporariorum*'da Bovine Serum Albumin (BSA) standardı ile 595 nm'de ölçülen protein seviyesi, BMAUM M6-4+AKH uygulamasında en yüksek besin mobilizasyonunu ortaya çıkarmıştır. Adipokinetik hormon tedavisinde karbonhidrat (glikoz) düzeyinin kontrol grubuna göre biraz arttığı bulunmuştur En düşük glukoz düzeyi entomopatojen fungus uygulamasında ölçülmekle birlikte, en yüksek düzey adipokinetik hormon tedavisi ile birlikte *B. bassiana* izolatlarında saptanmıştır. Entomopatojen funguslarla uygulanan AKH'nin etki mekanizması tam olarak anlaşılamamıştır. Bu nedenle, böceklere karşı gelecekteki entegre yönetim stratejileri için bu alanda yapılacak daha ayrıntılı çalışmalara ihtiyaç vardır.

Anahtar sözcükler: Adipokinetik hormon, *Beauveria bassiana*, Entomopatojen fungus, *Myzus persicae, Trialeurodes vaporariorum*

[1] Department of Plant Protection, Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Türkiye
[2] Department of Biology, Faculty of Science and Letters, Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Türkiye
[3] Biology Centre, CAS, and Faculty of Science, Institute of Entomology, University of South Bohemia, Branišovská, České Budějovice, Czech Republic
[4] Department of Plant Protection, Faculty of Agriculture, Isparta University of Applied Sciences, Isparta, Türkiye (This Author is a 100/2000 YÖK PhD Scholar)
* Corresponding author (Sorumlu yazar): ozlemk@ksu.edu.tr
ORCID ID (Yazar sırasıyla) : 0000-0002-0975-789X; 0000-0002-1775-8323; 0000-0001-6322-9839; 0000-0002-9787-442X
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Abstract: The aim of this study was to investigate the effects of the co-application of entomopathogenic fungi (EMFs) and adipokinetic hormone (AKH) on the green peach aphid, *Myzus persicae,* and the greenhouse whitefly, *Trialeurodes vaporariorum*. Single and combined applications of two *Beauveria bassiana* isolates with AKH were tested. The green peach aphid was more susceptible to treatments than the greenhouse whitefly. Mortality rates of 58%, 67%, %95, and 95% were observed for the single and combined applications of BMAUM M6-4 and BMAUM LD 2016, respectively, against *M. persicae*. The application of these fungi singly and in combination with AKH against *T. vaporariorum* caused mortality rates of 44%, 68% and 45% and78%, respectively. The protein level measured at 595 nm with the standard, Bovine Serum Albumin (BSA), for *M. persicae* and *T. vaporariorum* revealed that the highest level of nutrient mobilization was for the BMAUM M6-4+AKH treatment. The level of carbohydrate (glucose) was slightly higher in the AKH treatments when compared to the control group. The lowest glucose level was measured for the entomopathogenic fungi application, and highest level was determined for the isolates combined with AKH. The mechanism of action of AKH applied with *B. bassiana* isolates is not fully understood. Therefore, more detailed studies are needed in this area to determine the potential of this promising approach for inclusion in IPM programs for the control of these insect pests.

Key words: Hormone, *Beauveria bassiana*, Entomopathogenic fungi, *Myzus persicae, Trialeurodes vaporariorum*

Introduction

The rapid growth of the global population has increased the demand for agricultural products and the importance of pest control has grown in response. Chemicals applied to control insect pests have brought many problems in terms of environmental and human health. Sustainable agriculture and ecological agriculture have become more popular and reconizeded by the public to restore ecological balance and to minimize the use of chemicals. The demand for products that do not contain pesticide residues and the new laws and regulations introduced by the European Union have been effective as a driving force in the application of biological control methods to reduce the amount of pesticide applications (EFSA 2021).

Biological control, which is an important part of integrated pest management (IPM) programs, utilizes predation, parasitism, herbivory, and pathogenicity against insects, mites, other pests andplant diseases. Among the pathogens, myco-biocontrol is the use of fungi to lower the pest density before crop damage the economic threshold. Many insect groups may be infected by over 700 species of fungi recorded as pathogenic. Entomopathogenic fungi (EMFs) such as *Metharizium anisopliae* (Metschn.) Sorokin, and *Beauveria bassiana* (Bals.-Criv.) Vuill. are well characterized with respect to pathogenicity against several insects, and have been used for the control of agricultural pests worldwide (Lacey et al. 2001).

Many studies have shown that the insect endocrine system, particularly various insect neuropeptides, can be used as potential and specific physiological targets for pest control, predominantly adipokinetic hormone (AKH) (Gäde & Goldsworthy 2003; Hoffmann & Lorenz 1998; Rayne & O'Shea 1997; Roeder 1999). The mechanisms responsible for neuropeptides synthesis; their transport, secretion, binding; and various physiological aspects offer numerous opportunities for a new neuropeptide-based insect control strategy. Insect stress neurohormones appear to be particularly suitable for this purpose due to their roles in physiological processes. This is because stress conditions, such as starvation, injury, poisoning, infectionn and intense activity, cause severe metabolic stress and the requirements of animals under these stress conditions are basically similar, i. e,they must activate their energy stores to eliminate or minimize the effects of the stressor or stresses (Gäde & Goldsworthy 2003; Kodrík et al. 2015).

Insect metabolism is predominantly controlled by the adipokinetic hormones (AKHs) belonging to the AKH/RPCH peptide family. This hormone is synthesized, stored and released by neurosecretory cells in the corpora cardiaca (CC), a neuroendocrine gland associated with the brain. One of the most important functions of AKHs is to control the energy part of insect metabolism. In general, they act as typical stress hormones by activating the metabolism of lipids, carbohydrates and/or certain amino acids to store more energy. Among other functions, the AKHs stimulate the heartrate (Scarborough et al. 1984), regulate general body movements (Kodrík et al. 2002) and starvation-induced foraging behavior (Lee & Park 2004); participate in the activation of antioxidant mechanisms (Kodrík et al. 2007), enhance food intake and digestive processes in the insect gut (Kodrík et al. 2012; Bodláková et al. 2017), and also interact with the humoral and cellular immune systems (Goldsworthy et al. 2002). In terms of usage, AKHs show their effects after penetrating the insects cuticle (Kodrík et al. 2002; Lorenz 2003).

Interestingly, recent studies have shown that AKHs have an adverse effect on the defence reactions against insecticidal stress (Plavšin et al. 2015). The co-application of insecticides with AKH increased the insect mortality in comparison to that induced by the insecticides singly. Co-application of AKH with permethrin (Kodrík et al. 2010) and endosulfan and malathion (Velki et al. 2011) against *Pyrrhocoris apterus* (Linnaeus, 1758) (Heteroptera: Pyrrhocoridae), and pirimiphos-methyl and deltamethrin (Plavšin et al. 2015) against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), caused significant mortality. The mechanism underlying the interaction of AKH with insecticides to increase insect mortality is not curremtly understood; however, it has been suggested that an increase in insect metabolism after AKH treatment might play a role (Kodrík et al. 2015). Increasing metabolism may cause easier and faster penetration of insecticides to the tissues and cells. This hypothesis was supported by the determination of metabolic density. Higher carbon dioxide production was measured after insecticide application with AKH than after insecticide application singly (Kodrík et al. 2015).

Entomopathogens pose a serious threat to insects. Therefore, insects develop several defense mechanisms that help protect them, or at least reduce the impact of this type of stress. A similar insect response was observed between the entomopathogenic nematode *Steinernema carpocapsae* (Ibrahim et al. 2017) and the entomopathogenic fungus, *Isaria fumosorosea* (Gautam et al. 2020a,b), in combination with AKH. The synergy of entomopathogenic fungi with AKH seems to be very promising.

The main goal of this study was to demonstrate whether the application of external AKHs significantly enhances the efficacy of the entomopathogenic fungus, *B. bassiana*, against the green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), and the greenhouse whitefly, *Trialeurodes vaporariorum* (Westw.) (Hemiptera: Aleyrodidae).

Materials and Methods

Insect rearing

Two insect species, the green peach aphid, *M. persicae,* and the greenhouse whitefly, *T. vaporariorum,* were used in this study. *Myzus persicae* and *T. vaporariorum* were reared on pepper plants and eggplants, respectively, at 25 ± 1 °C, and 65 ± 5% RH and 16: 8 hours Light: Dark (L: D) in separate insect rearing rooms. In order to maintain the colonies, the old pepper plants and eggplants were replaced with clean plants from the plant rearing room every week.

Fungal strains and preparation of inoculum

The strain of the entomopathogenic fungus, *B. bassiana* (BMAUM M6-4), used in this study was isolated from soil samples in Isparta Province, Türkiye (Baydar et al. 2016), by using the "*Galleri*a trap method" (Zimmermann 1986). The other *B. bassiana* isolate, BMAUM LD 2016, was isolated from adult *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) collected from the campus of Süleyman Demirel University in, Isparta, Türkiye.

Conidia from 2-3 weeks-old fungal culture were harvested in 30 ml of 0.03% w/v Aqueous Tween® 80 and conidial concentration was determined by using a Thoma haemocytometer. The final concentration was adjusted to 1×10^7 conidia/ml in 100 ml of 0.03% w/v Aqueous Tween® 80. Conidial viability was determined by inoculating a 1 ×10^5 conidia/ml spore suspension on Potato dextrose agar (PDA) and evaluating the germination of 100 spores after 24 h of incubation at 25 ± 2°C. Conidia viability always exceeded 90% for both strains of *B. bassiana*.

Adipokinetic hormone (AKH)

The adipokinetic hormone, Acypi-AKH: pGlu-Val-Asn-Phe-Thr-Pro-Thr-Trp-Gly-Gln-NH2 (Jedlička et al. 2012) used in this study was provided by Prof. Dalibor Kodrík (Institute of Entomology ASCR, Biology Center, České Budějovice, Czech Republic).

Insect treatment with entomopathogenic fungi and adipokinetic hormone

The leaf dipping method was used for application of the entomopathogenic fungal spores for testing against *M. persicae*. Clean pepper leaves were dipped for 5 seconds in the 1 × 10^7 conidia/ml spore suspension and dried on filter paper. Spore treated leaves were placed in Petri dishes containing 12-15 ml of sterile water agar (2%, w/v) to minimize dehydration of the leaves. Ten nymphs were transferred to each leaf and one hour after contact with the fungal spores, 2 µl of Acypi-AKH (80 pmol of Acypi-AKH in 20% methanol of Ringer's solution) was dropped on each nymph with a fine tipped brush.

The insect treatments were entomopathogenic fungus singly, entomopathogenic fungus with AKH, AKH singly, and control. Each trial was conducted withfive replications, and all trials were repeated at least twice on different dates using different fungal cultures and insect generations.

For the control group, sterile distilled water with 0.03% w/v Tween® 80 was used in the application of the *B. beauveria* strain, and 2µl solvent was used in the hormone application. The experiments were carried out in a climate chamber (Termaks®) at 25 ± 1 °C temperature, 60 ± 5% humidity and 16: 8 hours L:D periods. Dead and living individuals of the insects were recorded daily.

Before the application of treatments, ten adult greenhouse whiteflies, were released into mesh tent cages with clean tomato plants to stimulate egg laying. After ten days, the tomato leaves containing the same age nymphs were used in the treatments. Entomopathogenic fungi were applied to *T. vaporariorum* by using the leaf dipping method, as previously mentioned (Gautam et al. 2020a). However, leaves with nymphs were used instead of clean ones. The excess insects were removed to leave ten nymphs on each leaf. The same treatments and conditions mentioned above for *M. persicae* were applied to *T. vaporariorum*.

Nutrient Levels

Nutrient availability plays an important role in regulating insect growth and controlling insect homeostasis. The analysis of nutrient levels in the bodies of insects infected with entomopathogenic fungi was performed with the following kit and the protocol determined by the manufacturer; the level of protein was measured with The Bicinchoninic Acid Protein Test Kit (Sigma Aldrich) (Stoscheck 1990) and the Bovine Serum Albumin (BSA) was used as the standard. Protein readings were performed at 595 nm (T80 UV / VIS Spectrometer, PG Instruments Ltd).

In the experiments, 5 aphids and 10 whitefly individuals were used for each replication. One hour after treatment with the EMF, 2 µl of Acypi-AKH (80 pmol of Acypi-AKH hormone) was applied on the top of each insect. These insects were then homogenized in potassium phosphate solution (PBS) (pH 7.0). Ten µl of this solution was transferred to an Eppendorf tube for each individual; the insects were crushed with a plastic crusher and centrifuged at +4 °C 10000 g for 10 minutes. The supernatants were used for the assays. Samples were stored at -20 °C until they were processed.

Digestive enzyme activity

For the activity of digestive enzymes, the glucose level was determined, and for this determination only the *B. bassiana* (BMAUM LD 2016) isolate and AKH were applied, as described earlier. Thirty aphids and thirty whiteflies were removed one hour after application and placed in 1.5 ml Eppendorf tubes containing 80% methanol and 1% HCL. The glucose level was analyzed with a SHIMADZU HPLC (RID 10A detector, Aminex HPX-87C carbohydrate column), and the results were obtained in mg/g.

Data Analyses

The statistical analyses were performed with use of the SPSS (ver. 17) and JMP (ver. 8) package programs. The data were subjected to one-way analysis of variance (ANOVA) and subsequently the Tukey test to compare each treatment. Insect mortalities were corrected according to Abbott’s formula (Abbott 1925), and percentage mortalities were calculated. Probit analysis on the median lethal time (LT_{50}) of the *B. bassiana* isolate’s application singly and combined with AKH, were calculated. Statistically significant difference was established at the level of $P < 0.05$.

Results

The effect of entomopathogenic fungal isolates and AKH hormone

The effects of two isolates of the EMF, *B.bassiana*, on the green peach aphid (*M. persicae*) and the greenhouse whitefly (*T. vaporariorum*), were observed from the second day after application (Figure 1). The treatment effects of the isolates and a the hormone on the two insects are presented in Tables 1 and 2.

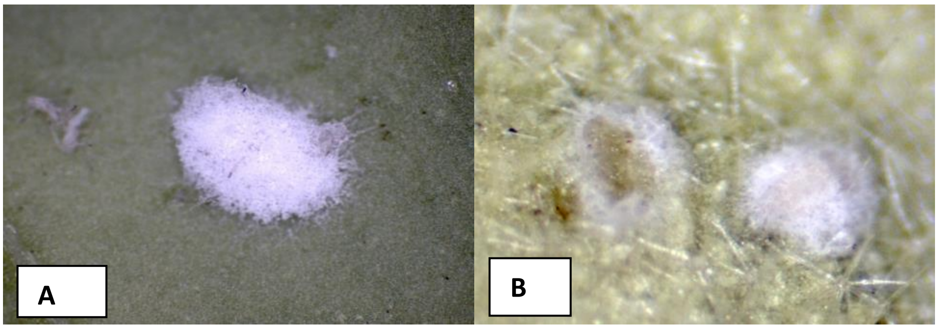

Figure 1. The effects of the entomopathogenic fungus, *Beauveria bassiana,* on the green peach aphid (*Myzus persicae*) (A) and the greenhouse whitefly (*Trialeurodes vaporariorum*) (B).

Application of the two *B. bassiana* isolates singly and together with AKH were very effective against the green peach aphid (Table 1). On the 1st day after treatments, mortality was observed to increasedover time. After 48 hrs, all applications, including the control, were included in the same statistical group. After 72 hrs, the EMF singly and entomopathogenic fungus with AKH application had more effect on the insect pests, and mean mortality was significantly different between the hormone and control treatment. After 192 hrs, the highest mortalities were observed for the *B. bassiana* isolate, BMAUM LD 2016, and for BMAUM LD 2016 with AKH. There was no significant difference between theAKH and control treatments (Table 1).

Table 1. The mean number of dead nymphs (± SE) of the green peach aphid, *Myzus persicae*, after the application of two *Beauveria bassiana* (isolates singly and with the hormone, AKH singly).

Treatments [a]	Mortality of Nymphs							
	24 hrs*	**48 hrs**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**
BMAUM M6-4	0.2±0.2 a	2.5±1.0 a	3.2±1.2 ab	3.9±1.3 abc	4.0±1.3 bc	5.6±1.3 b	5.8±1.3 b	6.5±1.2 b
BMAUM M6-4 + AKH	0.2±0.2 a	2.7±0.9 a	4.4±1.4 a	5.0±1.4 ab	5.1±1.3 ab	6.3±1.5 ab	6.7±1.6 ab	7.2±1.0 ab
BMAUM LD 2016	0.2±0.2 a	2.2±0.7 a	4.5±1.2 a	6.4±0.9 a	7.3±0.6 ab	8.5±0.3 a	9.5±0.2 a	9.6±0.2 a
BMAUM LD 2016 + AKH	0.2±0.2 a	3.0±1.2 a	4.7±1.0 a	7.6±0.7 a	8.6±0.4 a	8.7±0.3 a	9.5±0.3 a	9.6±0.2 a
AKH	0.0±0.0 a	0.0±0.0 a	0.0±0.0 b	0.3±0.2 c	0.3±0.3 d	1.0±0.3 c	1.0±0.3 c	1.3±0.3 c
Control	0.0±0.0 a	0.0±0.0 a	0.0±0.0 b	0.3±0.3 c	0.3±0.4 d	0.6±0.4 c	0.8±0.4 c	0.9±0.3 c

*Means within a column followed by the same lower case letter are not significantly different (P≥ 05).

[a]BMAUM: Biological Control Research and Application Center, AKH: Adipokinetik Hormone

The two Turkish isolates of *B. bassiana* (EMPs) used in this study werevery effective against the greenhouse whitefly and the green peach aphid. Applications of the entomopathogenic fungi with AKH caused higher mortality that was significantly different from the other treatments (Table 2).

Table 2. Mean number of dead nymphs (± SE) of the greenhouse whitefly, *Trialeurodes vaporariorum* after the application of two *Beauveria bassiana* isolates singly or with thehormone AKH singlyon different days.

Treatments [a]	Mean Mortality of Nymphs						
	24hrs*	**48hrs**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
BMAUM M6-4	0.0±0.0 a	0.0±0.0 a	0.6±0.2 abc	1.2 ±0.3 abc	3.0±1.03 ab	3.8±1.6 ab	4.4±1.2 a
BMAUM M6-4 + AKH	0.0±0.0 a	0.1±0.1 a	1.4±0.4 ab	3.8±1.1 ab	4.6±0.9 a	5.6±1.3 a	6.8±1.2 a
BMAUM LD 2016	0.0±0.0 a	0.0±0.0 a	0.9±0.2 abc	1.5±0.7 abc	3.5±1.5 ab	3.9±0.5 ab	4.5±0.7 a
BMAUM LD 2016 + AKH	0.0±0.0 a	0.6±0.9 a	1.7±0.6 a	4.6±1.5 a	5.02±1.7 a	5.8±1.8 a	7.8±1.4 a
AKH	0.±0.00 a	0.0±0.0 a	0.2±0.1 bc	0.2±0.1 bc	0.2±0.1 b	0.3±0.2 b	0.3±0.2 b
Control	0.0±0.0 a	0.0±0.0 a	0.04±0.03 c	0.07±0.07 c	0.1±0.1 b	0.1±0.1 b	0.1±0.1 b

*Means within a column followed by the same lower case letter arenot significantly different (P ≥ .05).

[a]BMAUM: Biological Control Research and Application Center, AKH: Adipokinetik Hormone.

After calculating the effects of *B. bassiana* isolate (BMAUM LD 2016) singly and together with adipokinetic hormone as percentages, it was determined that mortality in the green peach aphid was significantly higher than in the greenhouse whitefly (Table 3). Also, the BMAUM M6-4 treatment involving green peach aphid nymphs showed significantly lower effects than theBMAUM LD 2016 treatments, singly and with AKH. For the nymphs of both insect species, the BMAUM M6-4 isolate combined with AKH showed higher and statistically significant effects ($F = 59.98$; 5.29, $P \leq 0.05$, df = 3) than the single applications (Table 3).

Table 3. Percentage mortality of *Myzus persicae* and *Trialeurodes vaporariorum* 168 hrs after the application of entomopathogenic fungi singly and combined with the hormone, AKH.

Insect species	Percentage Mortality [a]					
	BMAUM M6-4	**BMAUM M6-4 + AKH**	**BMAUM LD 2016**	**BMAUM LD 2016 + AKH**	**AKH**	**Control**
Myzus persicae	58 ± 3 b*	67 ± 11 ab	95 ± 2 a	95 ± 11 a	10 ± 4 c	8 ± 3 c
Trialeurodes vaporariorum	44 ± 13 a	68 ± 8 ab	45 ± 16 a	78 ± 8 a	3 ± 0.3 b	1 ± 0.2 b

*Mean mortalities within a row followed by the same lower-case letter are not significantly different (P≤ 05).

[a]BMAUM: Biological Control Research and Application Center, AKH: Adipokinetik Hormone.

The effects of *B. bassiana* isolates singly or combined with AKH, depending on the elapsed time, were calculated by using regression curves and equations. For *M. persicae*, the LT_{50} values were 5.8, 4.8, 3.8 and 3.4 days for BMAUM M6-4, BMAUM M6-4 + AKH, BMAUM LD 2016 and BMAUM LD 2016 + AKH, respectively. For *T. vaporariorum,* the LT_{50} values were 7.8, 5.8, 7.5 and 2.5 days for BMAUM M6-4, BMAUM M6-4 + AKH, BMAUM LD 2016 and BMAUM LD 2016 + AKH, respectively.

Protein levels

The total protein levels measured at 595 nm in *M. persicae* nymphs after the application of the *B. bassiana* isolates singly or combined with AKH are given in Figure 2. The lowest protein level was measured in the control group and that was followed by the AKH and BMAUM LD 2016 treatments. The protein level increased slightly in BMAUM LD 2016 + AKH, and the highest level was obtained in the BMAUM M6-4 + AKH treatment (Figure 2).

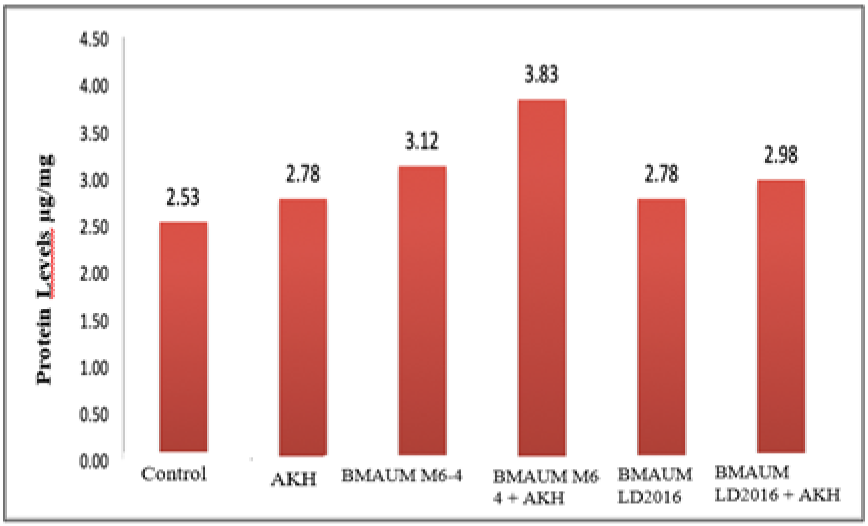

Figure 2. Total protein levels in *Myzus persicae* nymphs after the application of *B. bassiana* isolates singly orcombined with AKH.

The protein levels in *T. vaporariorum* nymphs are presented in Figure 3. Quite similar levels were observed in *T. vaporariorum* nymphs, as were the protein levels in *M. persicae* nymphs, and the highest level was obtained for the BMAUM M6-4 treatment (Figure 3).

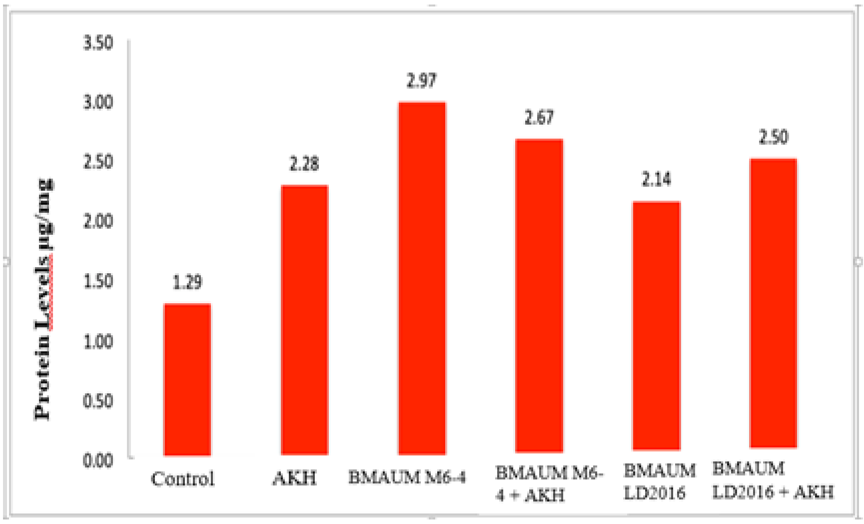

Figure 3. Total protein levels in *Trialeurodes vaporariorum* nymphs after the application of two *B. bassiana* isolates singly or combined with AKH.

Digestive enzyme activity

The activity levels of glucose enzymes across the treatments are presented in Figure 4. The glucose level was slightly higher in the AKH treatments than in the control. The lowest glucose level was measured for the entomopathogenic fungal applications against the nymphs of both insect species, and the highest level was determined for the *B. bassiana* isolate combined with AKH, against *M. persicae* (Figure 4).

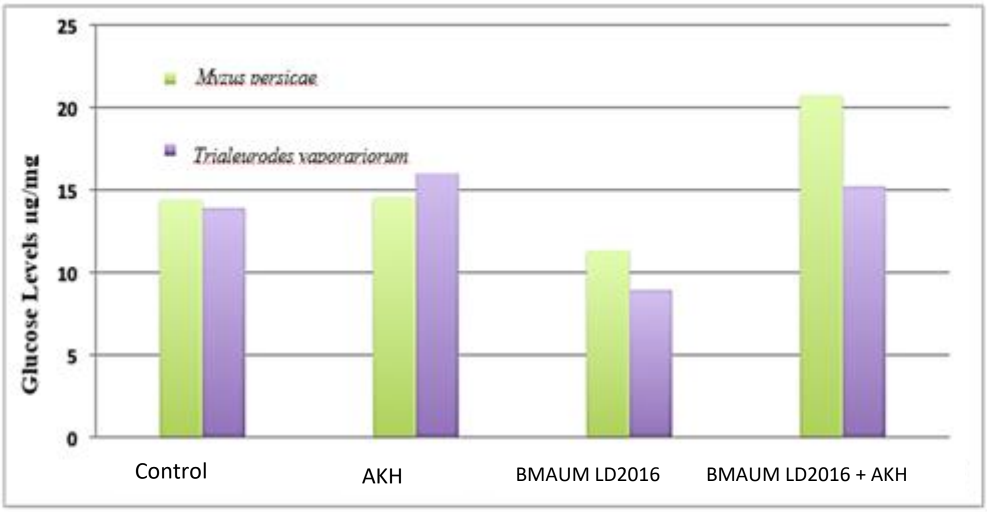

Figure 4. Total glucose levels measured in *Myzus persicae* and *Trialeurodes vaporariorum* treated with the *B. bassiana* isolate (BMAUM LD.2016) applied singly and combined with adipokinetic hormone (AKH).

Discussion

Two local Turkish isolates (BMAUM M6-4 and BMAUM LD 2016) of the EMF, *B. bassiana*, collected in Isparta Province, Türkiye, were very effective against the important greenhouse pests, *M. persicae* and *T. vaporariorum*. The low LT_{50} values for these isolates raises the prospect of using them as microbial pesticides in IPM programs. Both the mortality rates and LT_{50} values revealed by this study are within the ranges of the values stated in the literature (Akmal et al. 2013; Kim et al. (2013). There are several reports about the effects of EMFs on aphids and whiteflies (Akmal et al. 2013, Kim et al. (2013); Jandricic et al. 2014; Quesada-Moraga et al. 2006). Testing 44 locally isolated entomopathogenic fungal and 4 commercially used isolates on the three aphid species *M. persicae, Aphis gossypii* Glover and *Aulacorthum solani* Kaltenbach (Hemiptera: Aphididae), showed promising results. Moreover, the local isolates of *B. bassiana* and *M. anisopliae* were more effective than commercially available isolates (Jandricic et al. 2014). The use of *B. bassiana* on four aphid species (*Schizaphis graminum, Rhopalosiphum padi, Brevicoryne brassicae* and *Lipaphis erysimi*) produced LT_{50} values between 2.19 days and 3.73 days (Akmal et al. 2013). Also, similar results for the application of *Isaria* spp., *Lecanicillium* spp., *B. bassiana* and *Cordeceps* spp. on *M. persicae* were reported by Kim et al. (2013).

Studies conducted to test the control of *Bemisia tabaci* author and *T. vaporariorum* by 25 isolates of *B. bassiana* produced 3% to 85% mortality (Quesada-Moraga et al. 2006). In another study, *B. bassiana* caused 46.3% mortality of whitefly (Javed et al. 2019). Feng et al. (2004) investigated the effects of *B. bassiana* on *T. vaporariorum* under greenhouse conditions and recorded 72.1% mortality on the 10th day, 86.3% mortality on the 15th day, and 97.9% mortality on the 25th day.

In the present study, the synergistic effects of an entomopathogenic fungus and AKH were investigated. The study found that the hormone application increased to a certain extent the effect of entomopathogenic fungi after the first few days but the increase was not statistically significant. Although today difficulties in hormone supply and high cost limit the application of these **e**ntomopathogenic fungi and AKH together, this basic research can be built on infuture studies, venturing that the AKHs can be produced synthetically in the future.

Recently, some studies have focused on using insecticides, microorganisms and insect toxins in combination with AKHs (Goldsworthy et al 2002; Goldsworthy et al 2005; Kodrik et al. 2015; Shaik et al 2017; Ibrahim et al. 2018; Gautam et al. 2020a), and they found increased mortality in insects. Ibrahim et al. (2017) investigated the co-application of AKH and *Steinernema carpocapsae* against firebug, *Pyrrhocoris apterus* (Heteroptera: Pyrrhocoridae) adults. The applications increased mortality by about 2.5 times within one day, and carbon dioxide production increased about 2.1 and 1.6 times compared to the control and only EPN-treated insects, respectively. The application of AKHs with the entomopathogenic fungus, *Metarhizium anisopliae*, bacterium, *Bacillus megaterium*, and laminarin, against *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae), increased mortality in comparison to the use of the microorganisms singly (Goldsworthy et al. 2005; Mullen & Goldsworthy 2006). Similar results reported by Gautam et al. (2020a) also revealed that AKHs boosted the lethality of infection by the EMF, *Isaria fumosorosea*, of the cockroach, *Periplaneta americana* (Linnaeus, 1758) (Blattodea: Blattidae).

Conclusions

This current study demonstrated that the application of two *B. bassiana* isolates together with AKH and *T. vaporariorum*, compared to the application of the entomopathogenic fungal isolates singly. The mechanisms of AKH activity have not been clearly explained yet but it may play a role in increasing the metabolic rate and nutrient mobility in insects, which in turn increase the effects of entomopathogenic fungi. More studies need to be conducted to understand the physiological and biochemical modes of action of AKH in insects under stress situations. Overall, this study produced a useful body of information that can substantially contribute to the improvement of control strategies for two important pests in IPM programs bring interesting results potentially usable also in pest management strategies.

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