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Efficiency of the Iraqi Isolates of Beauveria Bassiana & Metarhizium Anisopliae in Controlling the Greater Wax Moth Galleria Mellonella L.

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Abstract. The current study was conducted in the laboratories of the Plant Protection Department/College of Agriculture and Forestry/University of Mosul during the year 2019-2020. The aim of this study was to evaluate the efficiency of the Iraqi isolate of the biological control fungi *Beauveria bassian* and *Metarhizium anisopliae*as chemical pesticides alternatives in controlling different stages of the greater wax moth *Galleria mellonella*. The results of the biological effect of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* on the greater wax moth life stages showed that the cumulative mortality of eggs increased gradually with increasing concentration used. However, *Beauveria bassiana* was more efficient in achieving higher mortality rates than *M. anisopliae* in general. The highest cumulative mortality rate for eggs was at the highest concentration (7.5g/l) for both fungi. On the other hand, it was found that the highest mortality rate in wax moth larvae was in*B. bassiana*combined treatmentwhere spraying the larvae and the feed. The third and fourth larval instars were more sensitive than the sixth and seventh larval instars, which were not affected by the lower concentrations of the pesticide (2.5 and 5 g/L), regardless of pesticide type. The results also showed that the mortality rate in all phases increases with the increase in the post-treatment period. The results of the study indicated the superiority of *B. bassiana* in all treatments, concentrations and periods after treatment and led to the highest mortality rate compared with using *M. anisopliae*. The cumulative mortality rate in the greater wax moth was directly proportional to the increase in treatment methods and the increase in concentration and the time period after treatment.

Keywords: Biological control, insects, *Metarhizium anisopliaeas*, honeybee , *Beauveria bassian*.

Introduction

The greater wax moth, *Galleria mellonella* L., is a permanent and important economic pest with a worldwide spread that attacks honeycombs and pollen inside honey bee hives and in the storeroom. The risk of infection with waxmoth increases at the end of the honey season in the period of storing wax frames in preparation for the next season, causing great economic losses as a result of infecting stored honeycombs and weak bee colonies (Warhust and Goebel, 1995). One of the most widely used methods in controlling this pest is chemical control using pesticides such as methyl bromide, aluminum phosphate and calcium cyanide,; nevertheless, it equires special care to prevent it from affecting honey (Goodman etal., 1990).

In general, chemical pesticides are often associated with toxic effects on honey bees and other beneficial insects. The emergence of resistance in more than 600 types of insect pests against various chemical pesticides increases the importance of finding suitable alternatives to chemical pesticides or at least adopting chemical control methods that leads to rationalizing the consumption of chemical pesticides and reducing their negative effects (Hasan et al., 2013). Accor dingly, biological control agents have become more attractive to researchers in the field of integrated pest control, and perhaps Entomopathogenic fungi (EPF) is one of the most important of these factors because of its ease of release into nature, ease of braking the large number of pathogenic strains from them and the possibility of being subject to the applications of genetic engineering techniques (Khan etal., 2012).The EPF are promising alternatives to chemical pesticides due to the high specialization of their families and ease of production in large quantities as well as killing their hosts through contact (Shahid et al., 2012). One of the possible control plans for G. mellonella in its various stages is the use of pathogenic fungi. In a study of five isolates of Metarhizium (DLCO-AA14, DLCO-AA5, DLCO-AA109, and IMI330189) and one isolate of Beauveria (ITTA18), Namusana and Emiru (2010) demonstrated the possibility of using these fungi in controlling the Greater Wax Moth with different concentrations. 2 x 410, 2 x 510, 2 x 610 and 2 x

710), which led to more than 90% mortality of the insect after 13 days of treatment. Therefore, the current study aimed at evaluateing the efficiency of the Iraqi isolate of the biological control fungi *Beauveria bassian* and *Metarhizium anisopliae* as chemical pesticides alternatives in controlling different stages of the major wax moth.

Materials and Methods

Different larval ages and adults of the Greater Wax Moth were collected from breeding farms in the Entomology Laboratory/Department of Plant Protection/College of Agriculture and Forestry/Mosul University. Larvae were collected with a soft fine brush and placed in 500 cm3 plastic breeding containers covered with muslin cloth and provided with freezing sterile beeswax pieces for feeding the larvae (Taqi, 2007; Karim, 2011). For the purpose of obtaining a pure permanent culture, the young pupae were transferred to a sterile 10 x 10 x 30 cm plastic containers which were covered with muslin cloth and tightly closed using a rubber band. The pupae were monitored until the adults emergence. Cotton pieces saturated with 10% sugar solution were placed in the boxes to feed the adults and motivate them to lay eggs. Besides, pieces of black cardboard were placed inside the boxes for the purpose of laying eggs and eggs were collected every 24 hours. Sufficient numbers of eggs were isolated to obtain the young larval ages (third and fourth instar). As for the sixth and seventh larval instars, they were obtained by isolating sufficient numbers of larvae of the former age and transferred to breeding boxes with continuous monitoring until they molt and reached the required stage according to the treatment. The pupal stage was obtained from the seventh instar larvae in breeding boxes and was monitored until they entered the pupal stage, while the others were left to complete the pupal stage and to be young adults which were collected for the purpose of conducting subsequent experiments.

Fungal Isolates Used in the study

In this study, the Iraqi isolates of *B.bassiana* and *M.anisopliae* classified by the British Commonwealth Institute of Fungi (CMI) were used. The fungal isolates were obtained from the College of Agriculture, University of Baghdad. The vitality of the fungi B. bassiana and M.anisopliae was confirmed and grown in 9 cm Petri dishes on PDA sterilized medium with streptomycin antibiotic to prevent bacterial growth. The dishes were placed in the incubator at 25°C and 85% humidity, and after 7-10 days the dishes were examined and the growth and viability of the fungi under study were confirmed.

The treatments included the use of fungi at four levels of concentration 0, 2.5, 5, and 7.5 g / l, and the ratio of the active substance in the preparation was 1 x 710 reproductive units / g. The treatments were carried out according to the stage of the insect, as eggs and pupae were sprayed with the preparation. While the treatments of larvae and adults included three methods, which is the direct spray treatment. Treating food (wax) were both methods (direct spraying + food treatment) (Al-Mallah and Al-Jubouri, 2012). All treatments were with 5 replicates in the experiment which was designed according to the fully randomized design of factorial trials. The results were analyzed according to a computer-based analysis of variance table in the SAS program, and the averages were compared with Duncan's polynomial test at a probability level of 5%. (Al-Zubaidy and Al-Falahy ،2016)

Bioassay of *Beauveria Bassiana* and *M.anisopliae* on Different Life Stage of the Great Wax Moth:

Effect on Eggs

Wax moth eggs at 24 hours old were taken with 10 eggs for each replicate with 5 replicates for each treatment. The cardboard pieces containing the eggs were placed in 9 cm Petri dishes, then the eggs were sprayed with 5 ml of the sporotrichosis preparation according to the treatment using a hand sprayer from a height of approximately 20cm. The control treatment was sprayed with distilled water. The eggs were monitored until hatching, and the death rate was calculated (Al-Mallah and Al-Jubouri, 2012).

Effect on larvae

Ten larvae were treated with five replicates of each larval age (3rd, 4th, 6th and 7th) by direct spraying of the larvae, the treatment of food or both methods together. Larvae were transferred to plastic containers with a volume of 500 cm 3 covered with muslin cloth and placed in an incubator at 30 °C and 70% humidity. Transactions were monitored daily, and after seven days of treatment, death data was recorded every 3 days (7, 10, 13, 16 and 19 days) according to the method of Skrobek et al. (2008) and the mortality values were corrected according to the Orell and Shneider equation (Al-Mallah and Al-Jubouri, 2012).

Effect on pupa

The pupae were isolated after molting of the seventh instar larvae at a rate of 10 pupae for each treatment with 5 replicates representing each treatment. After spraying with 5 ml of each concentration and according to the treatment, pupae were placed in 500 cm 3 plastic containers top covered with muslin cloth in case of the emergence of adults. Incubated at 30 C° and 70% humidity. The pupae were monitored until they became adults and the corrected mortality rate was calculated as formerly mentioned.

Pupae taken from the permanent farm were placed in sterile plastic containers (10 x 10 x 30) cm covered with muslin cloth and left until the adults emergence. To control the adults movement, they were subjected to freezing for a few seconds, then 10 adult males and females were distributed in sterile plastic bottles 950 cm 3 (7 x 16) cm covered with a piece of muslin cloth and the treatments were applied as in case of larvae with 5 replicates. The adults were fed on a peiece of cotton saturated with 10% sugar solution treated with fungal spores or untreated with the fungus in the control adults. The bottles were incubated at 30C° and 70% humidity. The mortality rate was recoded every 3 day from day 7 to day 19 after treatment and the corrected mortality rate was calculated (Al-Mallah and Al-Jubouri, 2012).

Confirmation of the Efficiency of Fungi Under Study

In order to confirm that the wax moth mortality was caused by the fungus, the different dead stages of the wax moth were taken and superficially sterilized using 70% ethanol for 3 seconds (Odindo 1994) and placed in Petri dishes over moist tissues and incubated at 25 °C and 90% humidity for 7 days. Then the samples were taken out and examined to confirm the growth of the mycelium of the fungus externally on the bodies of larvae, pupae and adults (Emiru Syoum, 2001). The pathogenicity was compared between the concentrations of the two fungi depending on the speed of causing mortality (Pr et al., 2012) and the mortality rate was calculated in the insect stages using Abbots (1925) equation according to Al-Mallah and Al-Jubouri (2012).

Results

Effect of*B. Bassiana* and *M. Anisopliae*on the Greater Wax Moth Eggs

The results of Table (1) showed that the highest mean of bee venom in relation to the interference readings was 0.232 mg / colony in the treatment of milking bee population of 10 frames (TMV2) during the period from 1-6/5/2021 compared with the lowest mean of interaction which was 0.061 recorded during the period from 26/6-1/7 for the same treatment. While the highest mean readings of the interaction in the treatment of bee venom milking bee population was 5 frames (TMV1) during the period from 1-6/5/2021 and rised to 0.125 mg/colony compared with the lowest average of 0.022 mg/colony during the period 5/29-3 /6/2021 for the same treatment. As for the general mean of the transactions, the highest mean was recorded by the TMV2 treatment which rised to 0.1136 mg/colony compared with the lowest general mean of 0 mg/colony by the TCM2 treatment. As for the TMV1 treatment, the highest mean recorded rised to 0.0527 mg/colony compared with the lowest mean which was zero mg/colony for the treatment of TCM1. As for the general mean of the effect of readings, the highest general mean was recorded on 1/6/2021 which mounted to 0.1787 mg/colony, compared with the lowest mean at the date of reading on 6/1/7/2021 which rised to 0.0423 mg/colony due to the increase in the amount of the milked venom during the period from 1-6/5 to the significant increase in the number of workers and the abundance of food from pollen and nectar and the appropriate weather conditions of temperature and relative humidity which reached 26.72 ° C and 41.61%, respectively (Figure 1, 2). This means that the amount of venom was clearly affected by the increase in bee population as the amount of milked venom in the treatment of bee population was 10 bee frames twice the amount of the venom of milked in the treatment of bee population 5 bee frames and in the different months of the year. The study found that June was the best month for milking bee venom. These results were consistent with what was found by Omar and Khodairy (2003)& Zhou et al (2003) studies. They found significant differences in quantity of milked venom as an effect of the months of milking. They recorded the highest mean milking of venom in June compared with March, while Mohamed's study (2017) recorded that spring and summer seasons have the highest production of bee venom compared with the rest of the seasons. Spring and summer seasons have the highest production of bee venom compared with the rest of the seasons. The general mean of the amount of venom in both spring and summer seasons was 0.230 and 0.292 mg /colony respectively. Badawy (2022) found that spring showed a significant increase in the The results of Table (1) showed that the cumulative percentage of egg mortality increased gradually with increasing pesticide concentration. The treatment of the biocide*B. bassiana* recorded the highest cumulative mortality rate for eggs at a concentration of 7.5 g/L with a mortality rate of 82%, compared with the treatment of *M.anisopliae* with the same concentration, which led to 64% (Table1). The rates of eggs mortality varied between treatments according to the type of pesticide and the concentration used. And the lowest egg mortality rate was (22%) in the treatment of *M. anisopliae* pesticide at a concentration of 2.5 g / liter, noting that no egg mortality was observed in the comparison treatment (Table1).

The results showed a direct relationship between the concentrations and the percentage of mortality, and the presence of significant differences between the two fungi used in the study, and the reason for this may be due to the moral difference between the two fungi in terms of the nature of the fungus in parasitism and the type and nature of the substances secreted during the parasitism process and the ability of the fungus to penetrate the eggshell. The reason may also be due to the integration of the enzymatic and mechanical activities of the fungus, as the fungus *B. bassiana* is able to secrete protease enzymes, ketones, lipases and toxins as well as a strong mechanical action (Charnley, 2003). The results of the study agreed with Al-Moussawi's results (2015), who indicated that the highest cumulative mortality rate was recorded in the eggs of the greater wax moth when using the sporophyte suspension of *M. anisopliae* at a concentration of $1*7^{10}$ spores/ml compared with the lowest concentrations. The use of mushroom *B. bassiana* with concentration $250*10^{5}$ recorded the highest.

Table 1. Effect of biopesticides *B. bassiana* and *M.anisopliae* on Egg Mortality of the Greater Wax Moth.

Treatments	Eggs mortality at different concentrations (ml/L)				Average
	25 %	5 %	7.5 %	Control (DW)	
B. bassiana	48 C	62 B	82 A	0 F	48 A
M.anisopliae	22 E	40 D	64 B	0 F	31.5 B
Average	35 c	51 B	73 a	0 D	

Values are means of five replications. Means that followed by the same letter are not significantly different according to Duncan's multiple range tests ($P \leq 0.05$)

Cumulative mortality rate in the greater wax moth eggs, and concentration $250*1^{10}$ recorded the lowest mortality rate (Karim, 2011). Vey (2001) indicated that the fungus B.b secretes a number of compounds Beauvericin, Valinomycin and Bassianolide that are toxic to insects and fatal to their embryos. On the other hand, between Genthner and his group (1998), the filtrate of M. anisopliae contains toxic substances such as Methelen chloride, which may cause toxicity to the embryos of aquatic animals. Al-Mihna (2011) also used the spores of *M. anisopliae* to kill the eggs of An.stephensi and Cx mosquitoes. Quinquefasciatus at a concentration of $2×5^{10}$. Ali (2007) also indicated that exposing Cx.pipiens L. eggs to spores of B. bassiana led to the death of them all. Santos and his group (2009) confirmed that the hatching rate of Aedes aegypti mosquito eggs exposed to spores of *M. anisopliae* at a concentration of $2.8×2^{10}$ spores/ml led to a reduction in hatchability to 50% at 98% humidity.

Effect of *Beauveria. Bassiana* and *M. anisopliae*on Greater Wax Moth Larvae

The results of both Tables (2 and 3) showed that food spraying + larval spraying recorded the highest cumulative mortality rate of larvae in all periods and was 100% when using the highest concentration (7.5 ml/L) after 19 days of treatment in the third and fourth larval instars. This percentage slightly decreased in the sixth and seventh larval ages and was 90% and 88%, respectively. The cumulative mortality rates of larvae varied according to the study factors, the lowest percentage was in the food spray treatment at a concentration of 2.5 g/L. No larval mortality was observed in the sixth and seventh larval instars in the two feeding and larval spraying treatments in the first period compared with the mixed method (see Tables 2 and 3). The reason may be that the body tissues of the first three larval instars were delicate. Also, the incomplete defense system of the modern larvae gave the opportunity for the fungus enzymes to cause fatal damage to these ages. The results of the current study agreed with Karim's results (2011) as using the fungus *B. bassiana* by direct spraying the larvae with increased concentration which gave better results than the method of spraying food and using the lower concentration.

In general, wax moth larvae reduced the effect of pathogens with their external defense system. Therefore, the combination of the two methods of direct spraying of the larvae and spraying the food material helped the speed of entry of the fungal pathogen into the body of the larva through food and respiratory openings; thus, increasing the speed and rate of larvae mortality although the main way for the entry of these fungi was to penetrate the body wall through the germination tube. Eguchie et al. (1986) showed that the greater wax moth is highly resistant against various microorganisms including B. bassiana and M. anisopliae. This includes different defensive methods (Lavine & Strand 2002). The infection of most insects was through direct penetration of the body wall by the germ tube, which was formed two days after the adhesion of spores to the insect and the infection developed when the appropriate heat and humidity were available (Steinhause, 1949). Bocuias and Pendland (1998) indicated that the infection of insects with the fungus is not only through the body wall but also through the open bronchioles or through the alimentary canal.

Table 2. Effect of bBio-pesticide *Beauveria. Bassiana* on Larvae Mortality of the Greater Wax Moth.

Larval age	Spray	%Mortality at different day post treatment																				Average	Treat. Average
		7 dpt Concentration ml/L				10dpt Concentration ml/L				13dpt Concentration ml/L				16dpt Concentration ml/L				19dpt Concentration ml/L					
		2.5	5	7.5	Cont.	2.5	5	7.5	Cont.	2.5	5	7.5	Cont.	2.5	5	7.5	Cont.	2.5	5	7.5	Cont.		
3rd instar	Feed	2	4	8	0	6	10	14	0	12	16	24	0	34	36	44	0	54	60	66	0		Spray feed
	Larvae	6	10	14	0	14	22	24	0	26	32	48	0	52	64	78	0	62	72	82	0	30.807	18.25
	Combined	10	14	20	0	18	26	34	0	40	50	62	0	64	72	90	0	76	84	100	0		
4th instar	Feed	0	2	8	0	4	6	16	0	16	16	24	0	40	52	52	0	50	56	66	0		Spray larvae
	Larvae	4	6	10	0	6	10	16	0	20	24	36	0	42	52	68	0	58	66	74	0	27.614	24.05
	Combined	6	10	14	0	10	14	20	0	36	42	64	0	60	62	84	0	72	82	100	0		
6th instar	Feed	0	0	0	0	2	6	8	0	12	18	20	0	34	40	46	0	46	54	60	0		Combined
	Larvae	0	0	0	0	4	10	14	0	14	20	28	0	40	44	54	0	54	60	70	0	22.982	
	Combined	0	2	2	0	6	10	16	0	26	32	46	0	52	60	64	0	70	80	90	0		
7th instar	Feed	0	0	0	0	2	4	6	0	12	16	18	0	30	34	42	0	44	52	56	0		31.01
	Larvae	0	0	0	0	4	8	12	0	12	18	24	0	48	54	56	0	52	56	70	0	21.473	
	Combined	0	0	2	0	6	8	14	0	22	26	38	0	44	50	54	0	66	78	88	0		
L.S.D. ($P \leq 0.05$)		Concentrations=0.673						Days=0.752						Interaction= 5.211									

Values are means of 5 replications. Means are compared according to the least significant difference L.S.D. ($P \leq 0.05$).

Table 3. Effect of bio-pesticide *Metarhizium Anisopliae*on Larvae Mortality of the Greater Wax Moth.

Larval age	Spray	%Mortality at different day post treatment																				Average	Treat. Average
		7 dpt Concentration ml/L				10dpt Concentration ml/L				13dpt Concentration ml/L				16dpt Concentration ml/L				19dpt Concentration ml/L					
		2.5	5	7.5	Cont.	2.5	5	7.5	Cont.	2.5	5	7.5	Cont.	2.5	5	7.5	Cont.	2.5	5	7.5	Cont.		
3rd instar	Feed	0	2	6	0	4	8	10	0	10	14	20	0	28	26	34	0	50	52	58	0		Spray feed
	Larvae	2	6	10	0	8	14	18	0	20	26	38	0	34	54	68	0	52	64	78	0	24.526	14.6
	Combined	6	8	10	0	12	16	18	0	32	38	56	0	54	66	84	0	68	74	90	0		
4th instar	Feed	0	0	4	0	2	8	12	0	12	14	24	0	36	42	42	0	40	46	56	0		Spray larvae
	Larvae	2	4	6	0	4	8	12	0	14	20	26	0	34	42	60	0	48	56	64	0	22.701	19.05
	Combined	4	6	8	0	8	10	16	0	24	30	46	0	54	52	72	0	64	72	84	0		
6th instar	Feed	0	0	0	0	2	8	6	0	4	16	20	0	22	30	36	0	36	44	52	0		Combined
	Larvae	0	0	0	0	2	8	10	0	8	16	14	0	30	34	42	0	44	50	60	0	18.456	
	Combined	0	0	0	0	6	10	10	0	20	26	36	0	40	48	52	0	60	70	80	0		
7th instar	Feed	0	0	0	0	2	2	6	0	4	8	10	0	20	24	32	0	36	42	46	0		25.225
	Larvae	0	0	0	0	4	6	10	0	4	10	16	0	28	44	44	0	42	46	60	0	16.947	
	Combined	0	0	0	0	4	6	12	0	20	24	30	0	36	44	42	0	56	70	76	0		
L.S.D. ($P \leq 0.05$)		Concentrations=0.806						Days=0.901						Interaction= 6.245									

Values are means of 5 replications. Means are compared according to the least significant difference L.S.D. ($P \leq 0.05$).

In general, the highest cumulative mortality rate in the larvae of the major wax moth was in the early larval ages, and the mortality rate decreased with the age of the larva. This is mostly due to the small size of the larval ages and thus the lack of defense bodies and the weak thickness of the chitin layer in the body wall which made it more sensitive to the penetration of fungi. This is consistent with Al-Haidari (2009) when the fungus was used to control the larvae of the corn stalk borer where the mortality rate was 46.0% for the first larval ages, compared with 20% for the large larval ages at the same concentration. These results agreed with the results of Al-Obaidi and Samir (2011) when using the fungus B. bassiana in controlling cotton leaf worm larvae, explaining that the effect increased with increasing exposure time.

Effect of *B. bassiana* and *M. anisopliae* on Greater Wax Moth Pupae

The results of Table (4) showed that there were significant differences between treatments in the cumulative mortality rates of the Great Wax Moth virgins according to the type of mushroom and the study factors. The mortality rates gradually increased with the increase in the concentration used. The treatment of B. bassiana was superior by recording the highest cumulative mortality rate of virgins (46%) at the concentration of 7.5 g/L, compared with the treatment of *M. anisopliae* with the same concentration which led to a mortality rate of 28%. The lowest mortality rate (9%) was recorded in the treatment of the fungicide *M. anisopliae* at a concentration of 2.5 g/L.

Table 4. Effect of bio-pesticides *B. bassiana* and *M. anisopliae* on Pupae Mortality of the Greater Wax Moth.

Treatments	Eggs mortality at different concentrations (ml/L)				Average
	25 %	5 %	7.5 %	Control (DW)	
B. bassiana	12 c	24 bc	46 a	0 e	20.5 A
M.anisopliae	6 d	12 c	28 b	0 e	11.5 B
Average	9 C	18 B	37 A	0 D	

Values are means of five replications. Means that followed by the same letter are not significantly different according to Duncan's multiple range tests ($P \leq 0.05$)

The low effect of the two fungi with low concentration could be due to the presence of cocoons surrounding the insect which acted as a hydrophobic layer and reduced the penetration of spores.. Also, the cuticle of the pupae wss more solid than the larval stages because the structure of chitin reached its highest level in the stage before pupation (Ferkovich et al., 1981). The results of the study agreed with Karim's (2011) that the emergence of differences between treatments and concentrations is due to the efficiency and ability of the fungus *B. bassiana* to penetrate the silken tissue of the cocoon and affect the pupae inside it. Al-Moussawi (2015) also mentioned that there is a direct relationship between the concentrations used for the fungus *M. anisopliae* and the mortality rate of virgins, and that the decrease in the effect of suspended or filtered fungi in the pupae is due to the presence of pupae that act as a hydrophobic layer.

Effect of *B. bassiana* and *M. anisopliae* on the Greater Wax Moth Adults

With regard to the effect of the fungi under study on adult moths, the results showed that the highest mortality rate was recorded in B. bassiana treatment when it was used by both methods (insect spray + food spray) at a concentration of 7.5 g / liter after 19 days of treatment with a cumulative mortality rate of 84 % compared with the M. anisopliae treatment which, in turn, scored 70% under the same conditions and treatments. The lowest cumulative mortality rate for adults was 2% when *M. anisopliae* was treated using insect spray method at a concentration of 2.5 g/L after 7 days of treatment, while the mortality rate was 4% when B. bassiana was used in the same conditions and concentration. The results of the study indicated the superiority of *B. bassiana* in the mortality rate in all treatments compared with its percentage in the case of using *M. anisopliae*.

The higher efficacy of B. bassianamay was due to its production of many enzymes such as protease, chitinase and lipase according to the type and strain of the fungus. These enzymes acted as a catalyst for the binding and according to the components of the insect cuticle, which varry among species (Bocuias and Pendland, 1998).

Using a combination of spraying the food and the insect increased the chances of the fungus entering the insect's body. In the case of food spray treatment, the cumulative mortality rate was higher than the insect spray treatment as the entry of the fungus was faster through food. It may also be due to the effect of fungus secretions of toxins after entering the insect's body with food. The low efficacy in the case of spraying the insect was because the greater wax moth was of the order Lepidoptera. So the presence of scales may hinder the speed of fungal penetration into adults body. Goettel and Inglis (2001) indicated the ability and efficiency of *B. bassiana* in reducing the population density of grasshoppers and showed that the fungus is characterized by rapid killing and a great effect on the insect when provided with the food. Al-Salihi (2013) mentioned that the Iraqi isolates of *B. bassiana* was effective against the adults of the small grain piercing insect due to toxins secreted by the fungus to the food.

The results of this study agreed with what Karim (2011) and Al-Mousawi (2015) stated that the increase in the concentration and duration of exposure to the fungi *B. bassiana* and *M.anisopliae* was directly proportional to the cumulative mortality rate of the greater Wax moth adults.

Table 4. Effect of bio-pesticides *B. bassiana* and *M. anisopliae* on Pupae Mortality of the Greater Wax Moth.

Pesticide	Spray	%Mortality at different day post treatment																				Average	Treat. Average
		7dpt				10dpt				13dpt				16dpt				19dpt					
		Concentration ml/L				Concentration ml/L				Concentration ml/L				Concentration ml/L				Concentration ml/L					
		2.5	5	7.5	Cont.	2.5	5	7.5	Cont.	2.5	5	7.5	Cont.	2.5	5	7.5	Cont.	2.5	5	7.5	Cont.		
Beauveria bassiana	Adult	4	8	10	0	8	12	20	0	12	14	26	0	20	26	40	0	34	46	56	0		Spray adult
	Feed	10	12	14	0	14	18	26	0	16	22	30	0	26	34	42	0	42	50	66	0	23.51	16.44 Spray feed
	combined	14	16	28	0	20	24	42	0	24	30	46	0	36	44	56	0	52	66	84	0		20.88
Metarhizium anisopliae	Adult	2	4	8	0	6	10	18	0	8	14	24	0	20	24	36	0	30	42	46	0		Combined
	Feed	6	10	14	0	12	16	20	0	14	20	26	0	24	30	36	0	36	46	62	0	20.28	27.9
	combined	10	14	20	0	16	20	36	0	20	26	40	0	32	40	46	0	44	58	70	0		
	LSD	Concentrations=0.938								Days=1.048								Interaction= 5.136					

Values are means of 5 replications. Means are compared according to the least significant difference L.S.D. ($P \leq 0.05$).

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