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Compatibility of entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopiliae* with the parasitoid *Bracon hebetor* for controlling of *Ephestia cautella*

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Abstract:

This study was conducted to evaluate the compatibility of the fungi Beauveria bassiana and Metarhizium anisopiliae with the parasitoid Bracon hebetor in controlling of Ephestia cautella. The results revealed that the highest effect of fungi was at concentration of 10^9 spore / ml., the percentages of mortality were 85% and 70% for B. bassiana and M. anisopliae respectively. The LT50 value of B. bassiana was 1.92 days, while it was1.82 days for M. anisopliae. The LC_{50} value was 2.8 x 10^3 spore/ml and 1.1 x 10^5 spore/ml for Beauveria bassiana and Metarhizium ansiopilae respectively. The combination of mentioned agents enhanced their activity in controlling the Ephestia cautella larvae , the mortality rates were 94% and 93% when the parasitoid combined with B. bassiana and M. anisopliae respectively ,while it was 70 , 56.7 , 51.6% for these agents separately. The highest mortality of the parasitoid due to these fungi was recorded after 7 days of treatment with the concentration of

10^9spore/ml of B. bassiana (73.3%) and it was 70% for M. anisopliae, and the LC_{50} value were 2.7 x 10^3 and 1.86 x 10^5 for mentioned fungi respectively.

Key words: entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopiliae*, parasitoid *Bracon hebetor*, *Ephestia cautella*

INTRODUCTION

The date palm tree, *Phoenix dactylifera* is exposed to infestation by various kinds of insects and diseases. One of the most important insect species is the *Ephestia cautella* (Walker), it was the main insect pest in Iraq that infested dates in orchard and continues to stores throughout the months of the year (Abd al-Husain, 1985; Khadir, 1998; Al-Taweel & Al-Jboory, 2007). If this infestation left without any treatment the dates will seriously affected and become unsuitable for human consumption (Hameed, 2002). During storage period methyl bromide (CH3Br) usually applied to control this pest in spite of its dangerous (Ahmed, 1998), moreover *E. cautella* is capable to develop resistance to it (Ahmed, 1998) and this fumigant appeared to be carcinogenic agent to human being in addition to its ability to deplete Ozone layer (Leesch *et al*., 1992; Marcot, 1993; Ross &Vail, 1993). These reasons stimulate researchers to find alternative ways against such pests (Al-Taweel and others , 1990; Talebi et al., 2011). The biological control of this pest is one of the alternative and most effective methods of controlling this pest, which includes the use of natural enemies such as insect parasitoids or entomopathogenic fungi. The insect parasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae) is one effective natural enemy on many insect larvae in particular the Pyralidae family, which including the target insect in this study, as well as other field pests, (Gurbuz, Aksoylar, 2006; Landge et al., 2009). Entomopathogenic fungi

play a major role in the control of many insect pests. There are more than 750 species of Entomopathogenic fungi, the most important are *Beauveria*, *Metarhizium*, *Verticillium* and *Paecilomyces* (Dent, 2002). *Beauveria bassiana* strains are the most virulence on the *Ephestia kuehniella*, followed by the strains of fungus *Metarhizium anisopliae* and *Verticilium lecanii* (Draganova and Markova, 2006).

Sultan (2016) demonstrated the possibility of compatibility between the insect predator *Chrysoperla carnea* (Stephens) , the fungus *Beauveria bassiana* and *Metarhizium anisopliae* against the *Ephestia cautella* (Walker).

According to importance of *Ephestia cautella* on dates due to its significant damage, this study was carried out to evaluate the efficiency and the compatibility of *Beauveria bassiana* , *Metarhizium ansiopliae* and *Bracon hebetor* against this pest.

MATERIALS AND METHODS

Insects

The target insect (*E.cautella*) were reared on artificial diet 81% crushed whole wheat, 1% yeast, 6% syrup(dibis) and 12% glycerol. Stock cultures were maintained at 27±2°C and 60-70% relative humidity (RH), with 16 hours photo phase and 8 hours Scot phase.

Fungus culture:

The fungal pathogen used in present study were cultured on potato dextrose agar medium (PDA) autoclaved at 121○C (15 Psi) for 15-20 minutes and poured into sterilized Petri plates. The Petri plates containing PDA medium were incubated at 27 ± 1○C, 80 ± 5% relative humidity and photoperiod of 12 hours. The conidia were harvested gently by scraping the surface of 15-days old culture with inoculation needle. The conidia were

suspended in distilled water containing 0.1% Tween-80. The mixture was stirred on a magnetic shaker for 10 minutes. The hyphal debris was removed by filtering the mixture through fine mesh sieve. The conidial concentration of final suspension was determined by direct count using haemocytometer. Suspension concentrations (1×10^5 , 1×10^7 and 1×10^9 conidia ml^{-1}) was prepared and used in bioassay.

Bioassay

Immersion method

The fifth larval stage of the *E.cautella* (10 larvae per replicate) was immersed for 10 seconds in different concentrations (1×10^5 , 1×10^7 and 1×10^9 conidia ml^{-1}) of the fungal suspension of *Beauveria bassiana* and *Metarhizium ansiopliae* and in distilled water for the control treatment (Jarrahi and Safavi, 2016).The excess moisture was removed from the treated larvae by placing them on filter paper and then transferred to Petri dish containing 5 grams of diet and incubated for 72hr under controlled laboratory conditions . Mortality rates were calculated and the dead insects were placed moisturized filter paper for 7 days to determine the cause of mortality (the fungal growth) (Jarrahi and Safavi, 2016). The experiment was replicated three times.

Exposure to treated filter papers with fungal suspension

E. cautella larvae were exposure to treated filter papers with different concentrations of fungal suspension (1×10^5 , 1×10^7 and 1×10^9 conidia ml^{-1}) of *Beauveria bassiana* and *Metarhizium ansiopliae* in Petri dishes supplied with 5 grams of diet, and incubated for 72 hours at 27 ± 1 ° C , 60-70% relative humidity and photoperiod 8:16 hours (D: L). Daily mortality was recorded and dead insects were transferred to the moisturized filter paper for 7 days to determine the cause of

mortality depending on the fungal growth (Jarrahi and Safavi, 2016). The experiment was replicated three times.

Efficiency of *B.hebetor*

Couples of newly emerging adults of *B.hebetor* were kept in test tubes 2.5 x 7.5 cm for mating (adults were fed on 10% honey solution) and then were introduced in glass bottles of 7.5 x 12 cm containing 100 larvae of fig moth for 24, 48 and 72 hours., At each time, the percentage of paralyzed larvae were calculated. The experiment was replicated ten times.

Integrated Effect of entomopathogenic fungi and prasitoides

This experiment was carried out by immersing the fifth larval stage of the *E.cautella* in the LC50 concentration of the fungus *B. bassiana* and *M. ansiopliae* for 10 seconds. Each 100 treated larvae were placed in plastic bottles 12 x 11 cm with pair of adult parasitoides and covered with muslin, then incubated under controlled laboratory condition. The results are recorded after 72 hours to determine the mortality rate and reasons. These results compared with result of treated larvae with fungi alone and parasitoid alone.

Effect of fungi on the adult of *Bracon hebetor*

The effect of the fungus on the parasitoides adults as well as the LC_{50} and the LT_{50} was determined by exposure the parasitoide adult (10 individuals) to treated filter papers with fungal suspension. And then placed in a glass container with a piece of cotton saturated with 10% sugar solution and incubated under controlled laboratory condition. Mortality rates and its reasons were determined.

Exposure of parasitoides adult to treated host larvae with fungal suspension.

The larvae of *E.cautella* were dipped in the fungal suspension of *B. bassiana* and *M.ansiopliae* (20 larvae per replicate) for each fungal concentration and in distilled water as control. 10 adult of parasitoides (5 males and 5 females at age of 24-48 hours) were introduced in container of treated larvae and supplied with a piece of saturated cotton by sugar solution (10%) to feed parasitoides and incubated under controlled laboratory condition. Mortality rate of parasitoides was calculated with determining the reason of mortality. The experiment was replicated ten times.

Experimental design and statistical analysis:

The experiments were designed in a completely randomized design CRD, and data were analyzed by using SPSS program, and Duncan multiple range test (DMRT) to compare the means in probability level of 0.05. Standard probit analysis was used to obtain LC50 and LT50 values.

RESULTS AND DISCUSSION

Effectiveness of fungal isolates against the larvae of figs moth *Ephestia cautella.*

Immersion method

The results of immersion last larval stage of *E. cautella* in different concentrations of fungal suspension of local isolates of *B. bassiana* and *M. anisopliae* appeared that the susceptibility depended on the concentrations and the isolates, the mortality have increased with increasing of concentrations (Table 1), highest impact of both isolates was at concentration of 10^9 spore / ml (85% and 70%) after four days of treatment by *B.*

bassiana and *M. anisopliae,* respectively , and the lowest impact (77 and 50% respectively) was at the concentration of 10^5 spore / ml.

The median lethal time (LT_{50}) which determines the virulence of fungal isolation has been associated with the concentration, it was ranged from 1.92 - 2.9 days at concentrations of 10^9 and 10^5 spore \ ml respectively for *B. bassiana* and 1.82 - 3.92 days for the fungus of *M. Anisoplia.*

Comparing the fungal isolates depending on the median lethal concentration LC50 showed that the fungus of *B. bassiana* was more pathogenicity its LC 50 value was 2.8×10^3 spore / ml compared with the fungus *M. anisopliae* , which reached to 1.1×10^5 spore \ ml.

Table (1): Mortality Percentage (after 4 days), the median lethal concentrations (LC_{50}) and the median lethal time (LT_{50}) of *Ephestia cautella* larvae treated by immersion method with different concentrations fungal suspensions of *B.bassiana* and *M. anisopliae.*

Treatment	the focus	% Of mortality	LT50	X^2	P Values	LC50	X^2	P value
B.bassiana	10^5	60.5ab	2.9	14.4	0.044	2.8×10^3	11.5	0.17
	10^7	80 c	2.43	18.7	0.001			
	10^9	85 c	1.92	11.7	0.11			
M. Anisopliae	10^5	50a	3.92	5.3	0.62	1.1×10^5	5.5	0.35
	10^7	63ab	2.66	6.5	0.48			
	10^9	70b	1.82	7.64	0.37			

The rates followed by the same letter in the same column did not differ significantly according to the Duncan test (0.05)

The pathogenicity is the most important indicator when measuring the effectiveness of entomopathogenic fungi against pests and the foundation of biological laboratory tests (Robert and St Leger, 2004), Fungal isolates were elected as successful biological agents according to their pathogenecity,

easy of production and adaptation to the environmental conditions (Reay, et al., 2008).

Interesting of using of pathogenic microorganisms was increased especially entomopathogenic fungi to combat warehouse insects because of low toxicity and risks to mammalian and the environment. Their effect was occurred by contact with the insects and penetrate their bodies through analysis of the integument and then spread inside the body and causing mortality (Cox and Wiking,1996). According to the mentioned reasons, the entomopathogenic fungi was tested against many warehouses insects in the laboratory and field (Kavallieratos et al , 2006; Sabbour and Abd El-Aziz, 2007a, b , 2010). One of the mechanisms that causing insect mortality by *Beauveria bassiana* and *M anisopliae* is produce a number of toxic compounds (Zacharuk, 1971; Vey et al. 2001). In a local study the pathogenicity of three isolates of *B. bassiana* fungus were tested against larvae of *Ephestia cautilla* , the highest pathogencity of three isolates was at the concentration 10^{6} spore / ml, which achieved mortality percentage 92.7 and 91.2 and 90.3% after 18 days and these isolates achieved protection of dates in store for six months, with the percentage of injury , 1.3, 1 , and 3% for the three isolates ,while it ranged between 5 - 16.5% in the control (Jassim and Laith 2012). Using of *Beauveria bassiana* , *Metarhizium anisopliae* and *Isaria fumosorosea* against *Plodia interpunctella*, *Ephestia cautella* and *E. Kuehniella* showed that the highest efficacy was for *Beauveria bassiana* fungus and effectiveness was enhanced by addition of diatomaceous (Sabbour et al. 2013). Dragamora and Markoa (2006) Tested Four fungal isolates of *Beauveria bassiana* and two isolates of *M.anisopiliae* and one isolate *of* the *fungus Verticillium lecanii* against larvae of *Ephestia kuehniella* and the percentage mortality was measured within 8 days, the virulence was determined by LT

$_{50,}$ results appeared that the isolate Bb383 of *B. bassiana* caused the highest lethal effect of larvae (87.88%), followed by Bb399 and Bb382 ,mortality rates was 68.84% and 60%respectively. Lowest lethal effect was by two isolates of *M.anisopiliae* and one isolate of the fungus *V. Lecanii.* Bb383 isolate was the most virulent, according to the value of the median lethal time with limited confidence 5.234- 4.81 days and rate of 5.019 days. Virulence of ten fungal isolates of the fungus *Beauveria bassiana* and *Metarhizium anisopliae* under laboratory conditions against the third larval stage of *E. kuehniella*, by using the method of immersion (10 ml) for 5 seconds, percentage of the cumulative mortality was between of 11-92% for *Metarhizium anisopliae* and the LC_{50} values ranged between 5.4×10^{7} to 3.4×10^{8} and for *Beauveria bassiana* the mortality was ranged between 17-88% and the LC_{50} between 8.3×10^{5} to 6.5×10^{6} (Faraji et al. , 2013). According to Wakefield (2005) the greatest results were obtained within the Mycopest project of definition of the most entomopathogenic fungi efficacy in the control of stores insects, work within the project appeared that the *B bassiana* found in cereals stores in United Kingdom had better control to insect and mites pests. Four of the 12 isolates were tested for *B bassiana* fungus caused 100% mortality of *E.kuehniella* larvae after 10 days of treatment by concentration of 8×1 conidia / ml. According to the Lord in2005 the application of entomopathogenic fungi achieved development when combined with other materials such as *Diatomaceous earth*.

Exposing to treated filter paper with fungal suspension

The results of treatment the last larval stage of *E.cautella* to different concentrations of *B. bassiana* and *M. anisopliae* on filter paper treated with fungal suspension (Table 2)

showed that the mortality rate was 60 and 56% at concentration of 10^5 spore / ml and LT_{50} was 3 and 3.68 days for mentioned fungi respectively, and at 10^9 spore / ml the mortality was 73 and 71% and the LT_{50} was 2.9 and 2.3 days respectively. The LC_{50} value was 1.4×10^2 and 6.4×10^3 respectively.

Table (2): Percentage of mortality (after 4 days) , the median lethal concentrations (LC_{50}) and the median lethal time (LT_{50}) of *Ephestia cautella* larvae treated by filter paper method with different concentrations of *B.bassiana* and *M. anisopliae* Suspensions

Treatment	the focus	% Of mortality	LT50	X^2	P	LC50	X^2	ValuesP
B.bassiana	10^5	60a	3.0	6.26	0.51	1.4×10^2	4.9	0.66
	10^7	66 ab	2.96	9.5	0.22			
	10^9	73 b	2.9	12.6	0.08			
M. Anisopliae	10^5	56 a	3.68	2.45	0.93	6.4×10^3	5.5	0.6
	10^7	60 a	3.75	8.14	0.32			
	10^9	71 b	2.3	4.2	0.76			

The rates followed by the same letter in the same column did not differ significantly according to the Duncan test (0.05)

The main elements of integrated management of the store products is a combination of a number of low - risk control methods, it is not easy and effective control the store pests by one way (Kavallieratos et al. , 2006), in this area the entomopathogenic fungi Provide alternative strategy to chemical control. Many studies documented the effectiveness of entomopathogenic fungi such as *B.bassiana M. anisopliae* against insect pests on store products and some existing commercially (Ekesi, 2001). Other studies has given equal encouragement to various Isolates of *B. bassiana M. anisopliae* for the control of *Plodia interpunctella* and *E. kuehniella (Zeller)* (Bischoff and Reichmuth, 1997)

Rice and Cogburn (1999) recorded 80-100% mortality after 21 days of treatment of *Sitophilus oryzae* and *Rizopertha dominc* and *Tribolium castanium* by *B.bassiana* .

Efficiency of the parasitoid *B. hebetor* and its interact with fungal isolates against the larvae of moth figs.

The results (fig. 1) appeared that the ability of *B. hebetor* on paralyse the fig moth larvae was 24 ± 3 larvae in the first day and the cumulative rate was 48 ± 9 and 63 ± 19 larvae in the second and third days respectively. Comparing the effectiveness of parasitoid alone and with the Microbial control agents (*B. bassiana* and *M. anisopliae*) showed The combination of biological control agents gave an enhancing to the effectiveness(fig. 2) , the percentage of larvae mortality was 94 and 93% as the impact of parasitois with *B. bassiana* and *M. anisopliae,* respectively , while it was 70% , 56.7% and 51.6% for the parasitoid alone and the mentioned fungi respectively , with significant different between fungi.

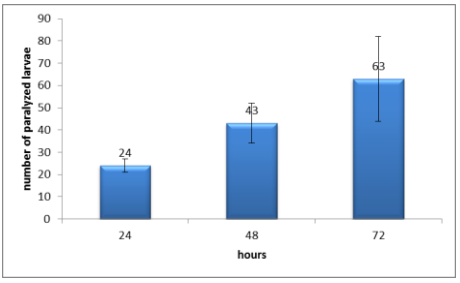
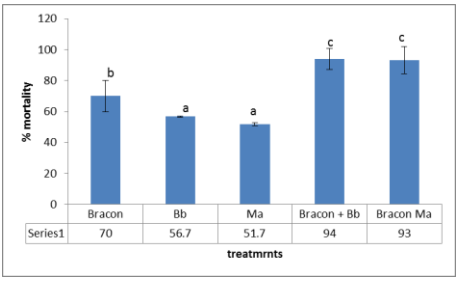


Figure (1) Cumulative parasitism efficiency of parasitoid *Bracon hebetor* on larvae of *Ephestia cautella*



Figure(2) the effectiveness of the combination of biological control agents (parasitoid *Bracon hebetor* , *B. bassiana* and *M. anisopliae)* against the larvae of *Ephestia cautella*

The organization of host population by external parasitoids mainly through injecting of females secretions toxicity in larval host that caused rapid permanently paralysis, which allows for females laying eggs and then feed of parasitoid larvae that caused inhibiting of the defensive response of host larvae (Pennacchio et al., 2014).

This mechanical adopted as a measure of the effectiveness of B. *hebetor* especially toxic secretions that cause complete and permanent paralysis at the level of one part of toxin to 200 million liquid part of the blood host (Weaver et al., 2001). The increase of efficiency is due increasing the sensitivity of paralyzed larvae to fungal infection, speed and increase fungal growth as containing toxic secretions which disable the endocrine and immune system of the host (Kryukova et al. , 2011). Kryukov et al. (2013) confirmed increase the effectiveness of entomopathogenic fungi in Paralyzed individuals compared with non – paralyzed ones and the LC50 value has decreased by 5,000 times after entering the toxicity secretions to the body of the larva and low dose (114 conidia per larva) was sufficient for the development of Fungal growth, this phenomenon is widespread in nature and that individuals that fall down on the surface of the soil and thus more vulnerable to infection. It was indicated to increase of Lepidoptera larvae

sensitivity after injected of parasitoid, such as internal parasitoids *Micropliti scroceiper (*King and Bell, 1978) and *Oomyzuss okolowskii (Dos santos* et al. , 2002) and *Pimplahy pochondriaca (Dani* et al. , 2004).

Shonouda And Nasr (1998) stated that The parasitism efficiency of the *B. hebetor* against *Ephestia Kuehniella* larvae *ranged from* 90 - 100 *% For all instars* except the first instar (0 *%)* For its activity and smallness size. Reinert and King (1971) noted that the release of 250 females of parasitoid leads to 97% mortality of the Indian flour moth larvae (1800 larvae). Nickle and Hagstrum (1981) has indicated that the *B. hebetor* was high efficiency against almond moth *E. cautella* in the stores. The parastoid is more efficient when it is ready for release at an early stage of infection. The paralyzed hosts by parastoids female are ready source for their food and thus increase their survival and fertility and is a suitable place to lay eggs. According to Cline *et al.* (1984) that the release of 50 p airs of *B. hebetor* twice a week in the corn flour store containing an infected packs by *E. cautella:* first packed and tightly closed, and the second packed but without integrated closing , and the third is open, these led to a variation infection rates in the three packages as follows: infections was reached in the first pack to 7.5% compared with 42.5% in the control treatment group , while it was 10% compared to 75% in the control treatment for the second pack, and 50% in third pack compared to 100% in the control treatment .

Keever et al. (1985 and 1986) pointed out the possibility of reducing the infection of *E. cautella Plodia interpunctella* using *B. hebetor* and the predator *X. flavipes* in the store of Pistachio. While Brower and the Press (1990) pointed out the importance of biological control using *B. hebetor* and *Trichogramma pretiosum* against *E. cautella* and *Plodia*

interpunctella, the first achieved a mortality ratio of stages of *Plodia interpunc*tella 66.1%, while the second has achieved 37.3% , release both parasitoids together they achieved 84.3% mortality. Mortality rate of *E. cautella* was 96.7% in the case of the first parasitoid, while it was 97.3% when using the second parasitods while using both parasitoid the mortality was 98% .

Hagstrum and Smittle (1978) stated that when paralyse the larvae of the host by a parasitoids female, the paralyzed larvae remain moist and suitable for feeding, or to lay eggs and the growth of their offspring last enough period, the rate of deterioration of bodies does not exceed 15% per week. Female choose newly and soft paralyzed larvae usually for the purpose of eggs laying, which represents 68.7% of the total laid eggs. The researchers also found that the number of eggs laid by single female on the single larva does not doubling with increase the number of female to 1, 2, 5 and 10 to a single larva of the host , the number of eggs was 13.6, 14.4, 10.5 and 6.7respectively. Some researchers have pointed to the importance of Kairomones that released by some moths that inferred them by *B. hebetor* . Scholler et al. (1997) explain that Kairomones are material if it released the benefit the recipient alone , which includes smells or tastes attractive and exciting to attack the host or prey whether an insect or vegetable host. To reduce the crop field losses and a few risks on the environment and human is recommended to use microbial control of (Hull and Beer, 1985), but the use with parasitoids and predators carries negative effects on the life and effectiveness of those parasitodes and predators (Hajek and St Leger, 1994; Thungrabeab and Tongma, 2007) .

The effect of fungi on parasitoid

Direct effect of fungi on the parsitoid's life showed results (table3) relating to the exposure of the parsitoid

to the fungal suspension in the filter paper that the highest mortality rate after 7 days of treatment was 73.3% by exposure to the concentration of 10^9 spores / ml of fungal suspension of *B. bassiana* and 70% by the same concentration of *M. anisopliae*. The shortest LT50 was 2.3 days of *B. bassiana* and 2.86 days of *M. anisopliae,* the LC50 was 2.7×10^3 and 1.86×10^5 spore / ml for mentioned fungi respectively.

Table 3: Cumulative rates of mortality and median lethal concentrations LC_{50} and median lethal time LT_{50} for *Bracon hebetor* adults exposure to fungal suspension of *B.bassiana* And *M.anisopliae* in filter paper.

Treatment	the focus	% Of death	LT50	X^2	ValuesP	LC50	X^2	ValuesP
B.bassiana	10^5	56.7b	2.82	5.2	0.64	2.7×10^3	12	0.09
	10^7	66.7bc	2.33	9.32	0.23			
	10^9	73.3c	2.3	23	0.03			
M. Anisopliae	10^5	46.7a	3.45	3.63	0.82	1.86×10^5	11.02	0.35
	10^7	63.3bc	3.2	6.79	0.45			
	10^9	70c	2.86	19.2	0.008			

The rates followed by the same letter in the same column did not differ significantly according to the Duncan test (0.05)

The direct effect of fungi on the life of the parasitoid that has been exposure to fugal suspension on the host larvae bodies, the results (table 4) showed that the highest rate of mortality after 7 days of treatment was 83% by the concentration of 10 9 spores / ml of the fungus *B. bassiana* with a significant difference from the fungus *M. anisopliae* at the same concentration (67.5%) and reached the shortest LT50 was 2.1 days for *B. bassiana* and 2.33 days by treatment of the fungus *M. anisopliae*, the LC50 reached $4.2 \times 10\ 5$ and $6.2 \times 10\ 6$ spore / ml for mentioned fungi respectively.

Table (4): Cumulative mortality rates and LT50 and LC50 of adults of *Bracon hebetor* exposed to different Suspensions *B. bassiana* and *M. anisopliae* through the bodies of host larvae .

Treatment	the focus	% Of mortality	LT50	X 2	Values P	LC50	X 2	ValuesP
B.bassiana	10^{5}	47a	3.7	17	0.04	4.2×10^{5}	17.6	0.01
	10^{7}	57b	3.33	19	0.03			
	10^{9}	83 d	2.1	32	0.01			
M. Anisopliae	10^{5}	42.5 a	3.3	53	0.009	6.2×10^{6}	6.7	0.35
	10^{7}	50ab	2.8	69	0.02			
	10^{9}	67.5 c	2.33	46	0.04			

The rates followed by the same letter in the same column did not differ significantly according to the Duncan test (0.05)

Man studies have indicated that the pathogenic fungi effect on natural enemies under the optimum environmental conditions of fungus, for example , Act of *Aphidius nigripes* was obstructed by infection of aphids by *Lecanicillium lecanii* , but the successful biology evolution in a high percentage of parasitoid occurs when the exposure to the fungus was after four days of parasitism (Askary and Brodeur, 1999).

In a study of the impact of two Iranian isolates of *B. bassiana* and *M.anisopliae* using the method of immersion of a n immature individuals of parasitoid, the LC 50 value of IRAN187C *B. bassiana* was 4×10^{9} spore / ml and the mortality rate for other isolates are very low, so the LC50 value didn't account. It confirmed the lack of impact of all isolates on the pupal stages of the parasitoids and recommended using the Isolates in the field of integrated control (Mahdavi et al., 2013). Exposing the two species of *B. hebetor* and *Apoangyrus lopezi* to *B. bassiana* and *M. anisopliae*. Under the development of locust and leaves hoper control program, Danfa and Vandervald in 1999 found that the laboratory application of the fungus Metarhizium spp. and one isolate of *B. bassiana* at a rate of 5× 1012 spore / ml in 280 litres of

water per hectare (the field rate recommendation) gave 100% mortality in *Bracon hebetor* and *Apoanagyrus lopezi*.

Some parasitoids show a decrease in attempts of egging in the fungal infected hosts compared to non - infected, some parasitoids can distinguish the infected hosts. Some lay slightly eggs on the infected hosts compared to non - infected (Brobyn, 1988; Fransen and Van Lenteren, 1993).

In a study on the use of *Lariophagus distinguendus* and *Anisoptermalus calandrae* alone and in combination with the *fungus Beauveria bassiana* against *Sitophilus granarius* on grain, the results showed that the highest suppression of the pest population (99.9%) was due to *L. distinguendus,* and then *A. Calandrae* either when combine both of them the suppression has been 83-98% indicative of a negative impact of fungi on parasitoid (Hansen and Steenberg*,* 2006*).* Increase in mortality can occur when the effect of interference between the pathogen and the parasitoid, the fungal pathogen *Hirsutella cryptosclerotium* and parasitoid *Gyranusoi deatebygi,* both natural enemy of *Rastrococcus invadens,* the fungi has decreased the level of parasitism (Akalach *et al*., 1992).

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