

**Compatibility of the entomopathogenic fungi
Beauveria bassiana and *Metarhizium anisopliae*
with *Chrysoperla carnea***

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Abstract:

*The current study was conducted to evaluate the compatibility of the entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* with *Chrysoperla carnea*. The result revealed that the direct exposure of larval instars of this predator to different concentrations of spores suspension of mentioned fungi have effected on the survival rate and the larval duration. Third instar larvae witnessed a significant increasing in their duration (17.6 days and 15 days) at concentration of 10^9 / ml of *B. bassiana* and *M.anisopliae* respectively comparing with the control (4 days),and this may be resulted from the influence of secondary metabolites and enzymes on hormone secretion . The effect of both fungi at all concentrations on the survival rate of the first and second instar larvae was non-occurred may be due to the molting process , While it was 43.3% and 67% for third instar at concentration of 10^9 spores \ml of *M. anisopliae* and *B. bassiana* respectively. The act of fungi continued on subsequent stages ,so the duration of pupae lasted 19 and 16 days for above treatment*

respectively comparing with the control (8.3 days). The percentage of adults emergence also decreased to 80.2% and 85.2% for previous treatment comparing with 100% for control. One of the important life features of predators is the predation efficiency, this feature for the first and second larval instar reduced to 13 and 37 eggs for each instar respectively at concentration of 10^9 spores / ml of *B. bassiana* ,and 25.7 and 66 eggs by exposing to *M.anisopliae* compared with 34 and 86.3eggs in control. Predation efficiency of third larval stage was affected by *M. anisopliae* and *B.bassiana* at the above concentration, it was 554 eggs and 777.7 eggs respectively ,while it was 1040.3 eggs in control. There was no significant effect on adults longevity after exposing to different concentrations of the fungal suspension , greater reduction was at concentration of 10^9 spores / ml for *M. anisopliae* (16 days for males and 33days for females), and *B.bassiana* (16.7 days for males and 38 days for females),while it was 19.7 and 44.3 days for control. This results encourage the use of both agents in the application of integrated pest management of insect pests.

Key words: entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, *Chrysoperla carnea*

INTRODUCTION

The green lacewings *Chrysoperla carnea* (Stephens), is one of the most important general insect predator as it feeds voraciously on a number of horticultural and agricultural cropping systems including vegetables, fruits, nuts, fibres and forage crops, and forests (Tauber et al. 2000), it has been invested successfully in bio-control programs against agricultural pests (Duelli et al., 2001 Romeis et al., 2014; Meissle et al., 2012).

Modern strategies of pest control depends on using of Entomopathogenic fungi as a successful alternatives methods to chemical pesticides in agricultural pest control (Fan et al., 2007). At least 90 genera and more than 700 species of fungus

is almost a entomopathogenic (Khachatourians and Sohail, 2008)) Most of these belong to Deuteromycota and the most important species of which , that widely used in various countries around the world are *B. bassiana* and *M. anisopliae* (Toledo et al., 2008., 2002,). These species were used locally and internationally as bio-control agents with high efficiency against many agricultural pests (Inglis et al., 2001and safty, 2005). It was reported that among 41 isolates of entomopathogenic fungi from Thailand, *Beauveria bassiana* Bb.5335 and *Metarhizium anisopliae* Ma.7965 were virulent against *Myzus persicae* Sulzer, *Macrosiphum euphobia* (Thomas) (Hom., Aphididae), *Thrips tabaci* Lindeman and *Frakliniella occidentalis* Pergande (Thys., Thripidae)(Thungrabeab, and Aemprapa, 2002; Sengonca,et al.2006; Thungrabeab, et al.2006 a,b). The success of fungal entomopathogens as biological control agents depends not only on high efficacy against insect pests, but also on low virulence against non target insects. In addition, most guidelines for the registration of biopesticides require laboratory testing for infectivity to non target organism. Thus, before considering fungi isolates as biological control agents, it is necessary to investigate their effects on non target insects prior to their release(Thungrabeab and Tongma 2007). Many studies about IPM proved possibility of combining the compatible agents to control pests without any harm to human health and the environment. For the purpose of combining the fungi and predators or parasites we need knowledge , deep study and high coordinate among them to achieve the best results in the control of insect pests and to avoid their damages (Lacey et al., 2015).Integration of the *Chrysoperla carnea* with the fungus *Verticillium lecanii* at concentration of 10^8 spores / ml against beans aphids *Aphis craccivora* was effected in reducing aphids population density and increasing the bean crop in Egypt and these results were better than using of each alone (Abd El -

Gawad and Atef, 2008). Salih and Diwan (2006) pointed out the success of the integration of two fungi *Verticillium lecanii* and *Beauveria bassiana* to control whitefly *Bemisia tabaci* in agriculture experiment.

According to the importance and efficiency of the *Chrysoperla carnea* (Stephens) and the entomopathogenic fungi and their role as successful bio-control agents within the integrated management programs throughout the world, this study was conducted to determine the compatibility of *Beauveria bassiana* and *Metarhizium anisopliae* with *Chrysoperla carnea*.

MATERIALS AND METHODS

Insects

The adults of *C.carnea* that originally collected from fields were confined in transparent plastic cups (11 cm in diameter and 7.5 cm high) and supplied with the standard artificial diet consisting of yeast, sugars, and distilled water in the ratio of 4 g: 7 g: 10 mL, respectively [Hagen,&Tassan, 1970]. The top of the plastic cup was covered with black muslin cloth tightened with a rubber band. The eggs laid by females on the walls of the cups and muslin cloth were harvested daily, using forceps to break the stalk beneath the egg. The eggs were placed, with the help of a camel's hair brush, singly in plastic Petri dishes (10 cm in diameter and 1.5 cm high).Newly larvae were fed on eggs of *Ephestia cautella* that was reared such as described by Hamad *et al.* (2008).

Fungus:

The fungal isolates *Metarhizium anisopliae* and *Beauveria bassiana* were grown on 9 cm Petri dishes containing Potato dextrose Agar PDA (39 g l⁻¹) and incubation at 25°C in darkness until colonies fully occupied the dishes then stored at 4 °C for

further use. The fungal suspension was prepared by adding 5 ml sterile distilled water SDW mixed with 500 μ l of tween 80 and gently scraped with sterile scalpel. The suspensions were stirred vigorously for 5 min to break up the spores from the conidiophores and the hypha debris was removed by passing the suspension through fabric cloths. Three concentrations of each fungal isolates 1×10^5 , 1×10^7 and 1×10^9 were determined by the aid of hemocytometer. The viability of spore was determined as in Lacey (1997).

Assay methods

All tests were conducted under the same laboratory conditions ($25 \pm 2^\circ$ C, 60-70% relative humidity and photoperiod 16: 8 h (L:D)).

Effect of fungal isolates on the larval stages

the effectiveness of fungal isolates on the larval stages of the predator has been testing, single larva are used for each replicate at rate of five replicates per treatment that were treated with different concentrations (10^5 , 10^7 , 10^9 spores / ml) of both fungal isolates *B. bassiana* and *M. anisopliae*. and supplied with eggs of *Ephesia* as **diet**, control group was treated with 2 ml of distilled water only (Hossam El Din et al., 2010). Rate of larval age, survival and pupation for each treatment were recorded.

Effect of fungal isolates on pupae

one day old pupae were treated by direct spraying with different concentrations (10^5 , 10^7 , 10^9 spores / ml) of both fungal isolates *B. bassiana* and *M. anisopliae*. and distilled water only as control group. Five pupae are used for each replicate at rate of three replicates per treatment. adult emergence were recorded daily.

Effect of fungal isolates on the survival and longevity of adults

This experiment was conducted on 24 hours adults aged at rate of 10 adults (5 males \times 5 females) for each replicate (three replicates per treatment). They were placed in open sides bottle (diameter of 13 cm and a height of 17.5 cm), its down hole was settled in a glass vase containing a glass container with small piece of cotton saturated with diet of adults, the upper hole was blocked with a piece of muslin for ventilation and to prevent of adults escape. Each replicate were treated by 2 ml of spores suspension of fungal isolates at concentrations (10^5 , 10^7 and 10^9) spore / ml and the control group were treated with 2 ml of distilled water, the survival rate and longevity of adults were calculated .

Effect of fungal isolates on the predation efficiency.

the predation efficiency of newly hatched larvae, second and third instar larvae that treated with concentrations of 10^5 , 10^7 and 10^9 spores/ml of both fungal isolates were tested , single larva/replicate(5 replicates/treatment) was placed in Petri dishes 9 cm and was provided with counted eggs of *Ephestia cautella* to feed ,control group was treated with 2 ml of distilled water. the percentage of predation was calculated depending on number of eggs that was supplied daily and number of eggs that were consumed (Hamad al-Rawi, 2008).

Statistical analysis

statistical analysis was conducted using completely randomized design CRD and Duncan test . Probit analysis was used to obtain the median lethal concentrations (LC50) in addition to the time taken to kill 50% (LT50),within SPSS system, version 20.

RESULTS AND DISCUSSION

Effect of fungal isolates on the larval stages

The Influence of different concentrations of spores suspension of *B.bassiana* and *M. anisopliae* on the survival rate and larval duration of *C.carnea* was explained in table(1) .The survival rate of the first and second instar larvae was non-occurred may be due to the molting process that got rid the fungal spores before starting of disease steps, , While the survival rate of third instar was 43.3% when exposed to concentration of 10^9 spores/ml of the fungus *M. anisopliae* and 67% of the concentration of 10^7 spores / ml of the same fungus and the concentration of 10^9 spores / ml of the fungus *B .bassiana*. These results may be due to the effect of fungi on the hormonal system that induce failure of pupation and then death . Developmental duration of the first larval instar was 3 days at concentrations of 10^7 and 10^9 spores / ml of *Baeuvaria bassiana* without significant difference from the same concentrations of *M. anisopliae*, in which the developmental duration was 2.9 days for both concentrations. Second instar larvae **lasts for** 4.3 days when treated with concentration of 10^9 / ml of *M. anisopliae* with significant difference from the same concentration of the fungus *B. bassiana* (3.7 days). Third instar witnessed a significant increasing in the developmental duration that reached to 17.6 days with concentration of 10^9 / ml for the fungus *B. bassiana* in comparison with the control (4 days). The failure in the development of last instar may be due to the influence on hormonal secretion system that may be resulted from secondary metabolites and enzymes,.. In addition to the effects of fungi on the last instar, the influence was continued on subsequent stages, least pupation ratio (43.3%) was occurred at concentration of 10^9 spores / ml of *M.anisopliae* then increasing of pupa duration to 19 days compared with the control (8.3 days). The emergence percentage of adult also

decreased to 80.2% at concentration of 10^9 spores / ml of *M.anisopliae* which was not significantly different from the treatment of fungus *B.bassiana* at same concentration (85.2%) ,while it was 100% in control. the success of fungal entomopathogens as biological control agents depends not only on high efficacy against insect pests, but also on low virulence against non target insects. In addition, most guidelines for the registration of biopesticides require laboratory testing for infectivity to non target organism. Thus, before considering fungi isolates as biological control agents, it is necessary to investigate their effects on non target insects prior to their release(Thungrabeab and Tongma 2007) ,so there was many studies to confirm this aspect. 11 isolates of the fungus *M.anisopliae* and one isolate of the fungus *B.bassiana* were developed to combat Hoppers without side effects on associated natural enemies (Danfa and Vaderlak, 1999). Tounou et al. (2003) revealed that the two isolates of fungi *M. anisopliae* Ma.43 and *P. fumosoroseus* Pfr.12 had caused 98% and 100% mortality of the pest *Empoasca decipiens* (Homoptera) respectively, without impact on eggs parasitoid *Anagrus atomus* (L)(Hymenoptera). It was pointed out no fungal pathogenicity of the isolate *Baeuvaria bassiana* 5335 on the natural enemies such as *Coccinella septempunctata* and *Chrysoperla carnea* and few pathogenicity of isolate *M.anisopliae*7965 (4%)(Thungrabeab and Tongma 2007). Similar results were recorded in the study of Gustavo *et al.* (2005) that third instar is affected and the first and second instars was not affected by immersing them in spores suspension of *Baeuvaria bassiana*. Sewify and El- Arnaouty (1998) studied the effect of two isolates of the fungus *Verticillium lecanii* (Zimm.)on larvae of *Chrysoperla carnea* referring to the high pathogenicity of one isolates on the third larval insrtar and weaken of the predatory capacity and feed rate as well as the emergence of adult and fecundity. On the other hand, Zhu and Kim (2012) found the larvae of

Chrysoperla carnea contributed to the spread of 89% of spores suspension to a distance of 2.4 meters from the release site, and achieved a 88% mortality in the host (*Myzus persicae*) without affecting the predatory capacity. In applied study .Mensah *et al.* (2015) found the use of isolate *Aspergillus* sp. BC 639 were active against *Helicoverpa* spp. (Lepidoptera) in commercial cotton fields and ineffective on predators, including the larvae of *Chrysopa* spp. In a laboratory study, Leyva *et al.* (2011) revealed that any age of predator *Chrysopa exterior* is not affected by exposed to *Beauveria bassiana* (Bals.) and *Vuillemin* Strain LBB-1 and this encourages the use of both in the application of integrated pest management of insect pests.

Table (1) Effect of different concentrations of *Baeuvaria bassiana* and *Metarhizium anisopliae* on larval instars of the predator *Chrysoperla carnea*

treatment	concentrations	Larval duration (days)			Survival rate of larval instars		
		first	second	third	first	second	third
control	0.0	2.2a	3.3	4	100	100	100a
<i>Baeuvaria bassiana</i>	10 ⁵	2.6b	3.6	b13.3	100	100	b96.6
	10 ⁷	3c	3.6	b16.6	100	100	77 c
	10 ⁹	3c	3.7	b17.6	100	100	67 d
<i>Metarhizium anisopliae</i>	10 ⁵	2.5b	3.6	b14.6	100	100	a100
	10 ⁷	2.9c	3.7	14 b	100	100	67 d
	10 ⁹	2.9c	4.3	15 b	100	100	43.3 e

Table (2) Effect of treating the third larval instar of *Chrysoperla carnea* by different concentrations of *Baeuvaria bassiana* and *Metarhizium anisopliae* on subsequent stages.

treatment	concentration	Pupation percentage	Pupae age	percentage of adult emergence
control	0.0	a100	a8.3	a100
<i>Baeuvaria bassiana</i>	10 ⁵	b 96.6	b15	a100
	10 ⁷	77 c	14.3 b	89.5 b
	10 ⁹	67 d	16 bc	85.2.4 c
<i>Metarhizium anisopliae</i>	10 ⁵	a100	16 bc	a100
	10 ⁷	67 d	b14.5	92.3 b
	10 ⁹	43.3e	c9 1	80.2c

Impact of fungal isolates on the predation efficiency

One of the most important biological parameters for insect predators is searching and Predation capacity which are strongly influenced by environmental factors include entomopathogenic fungi. Treatment of larval instars by different concentrations of *Baeuvaria bassiana* and *Metarhizium anisopliae* was effected on the consumption capacity according to the concentration, the highest decline in predation efficiency was at the concentration of 10^9 spores/ml of *B. bassiana*, it was 13 and 37 eggs for the first and the second instar respectively, with significant differences from the rest concentrations and .The predation capacity of both larval instars at the same concentration of *M. anisopliae* were 25.7 and 66 eggs respectively. The third larval instar has been influenced by the fungi at mentioned concentration, it was reached to 554 eggs, and 777.7 eggs when they exposed to *M. anisoplia* and *B. bassiana* respectively compared with the control that reached to 1040.3 eggs.

The decline in the food consumption of predators is one of the possible effects of the sub lethal doses of entomopathogenic fungi (Hajek and Goettel, 2000). *C.carnea* larvae that infected by *Lecanicillium longisporum* consumed less number of Aphids than those consumed by non infected individuals (Sewify and El- Arnaouty (1998) as well as the results (Roditakis et al., 2008) appeared the consumption rate of *Myzus persicae* are decreased when *C.carnea* larvae infected by the fungus *Lecanicillium longisporum*. Also it was found decreasing in predation capacity of the predator *Dicyphus hesperus* that infected by the fungus *Paecilomyces fumosoroseus* (Alma, 2005) On the other hand, many of the predator species avoided feeding on fungal infected prey, It was recorded avoid predation of infected whiteflies with fungi from predator *D.hesperus* (Labbe et al ,. 2006) the predator *Anthocoris*

nemorum wasn't fed on infected aphids with the fungus *B. bassiana* (Meyling and Pell, 2006).

Table (3) Effect of different concentrations of spores suspension of *Baeuvaria bassiana* and *Metarhizium anisopliae* on predation efficiency of larval instars of *Chrysoperla carnea* feeding on *Ephestia cautella* eggs.

treatment	concentrations	Predation rate of larval instars		
		first	second	third
control	0.0	34.0 a	86.3 a	1040.3 a
<i>Baeuvaria bassiana</i>	10 ⁵	22.7 cd	52.7 c	959.3 a
	10 ⁷	17.3 d	46.7 c	802 b
	10 ⁹	13 e	37.3 d	777.7 bc
<i>Metarhizium anisopliae</i>	10 ⁵	30.7 a	a 83	672.3 cd
	10 ⁷	27.7 b	ab79	613 d
	10 ⁹	25.7 b	b66	554 d

Impact of fungal isolates on adults longevity.

In addition to direct infection, entomopathogenic fungi may cause sub lethal effects in non target insect, the results (table 4) showed that there was significant reducing in adult females longevity those that exposed at age of 24 hours to various concentrations of fungal suspension. The longevity of males and females exposing to *M.anisopliae* at concentration of 10⁹ spores/ml were 15 and 31.7 days respectively, and they were 16.7 days and 38 days after exposing to *B.bassiana* with the same concentration compared with the control that was 19.7 and 44.3 days respectively.

Results of this study was agreed with Habib ,(2015) that pointed out that the impact of the spores suspension of two isolates of *B. bassiana* (*Bals.*) and two isolates of *M. anisopliae* at concentrations (10⁵ 10⁷ 10⁹) spore / ml on longevity and survival rate of adults of *Dacus ciliates* (males and females) were varied depending on the increasing of concentrations . The results obtained by Hossam El-Din *et.al* (2010) referred to the absence of significant differences between the concentrations of fungus *B. bassiana* in their effect on adults longevity, males longevity that treated at age of 24 hours with

concentrations of 10^5 , 10^3 and 10^1 spore / ml was 11.9 , 11.4 and 12.2 days respectively, compared with the control treatment (17.9 days), longevity of females that treated at age of 24 hours with mentioned concentrations were 11.6, 12.0 and 12.1 days, respectively and at control treatment was 18.1 days. The different among concentrations is explained by several reasons, including the amount of fungal spores reached to the insect body and the failure of some fungi to penetrate of cuticle and the ability to secrete enzymes that analyses the insect integument. (Bekheit and Abo El-Abbas , 2000, Silva and Messias, 1986).

Table (4) Effect of *Baeuvaria bassiana* and *Metarhizium anisopliae* on adults longevity of *Chrysoperla carnea*

treatment	concentrations	Adults longevity (days)		means
		males	females	
control	0.0	19.7	44.3	32
<i>Baeuvaria bassiana</i>	10^5	19.7	40.0	29.85
	10^7	19.3	40.7	30
	10^9	16.7	38.0	27.3
means		18.85	40.75	27.8
	0.0	19.7	44.3	32
<i>Metarhizium anisopliae</i>	10^5	15.3	33.7	24.5
	10^7	15	31.7	24.5
	10^9	16	33	23.35
means		16.5	35.7	26.1
LSD	Fungi=6.38 , concentrations= 7.99 , sex= 5.04 Fungi x concentrations =10.16 , fungi x sex = 6.02 , concentration x sex = 10.22 Fungi x concentration x sex= 13.71			

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