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Biological Control of Fungi *Beauveria bassiana* and *Metarhizium anisopliae* in the Bluefly *Lucilia sericata* (Diptera: Calliphoridae)

Rabah H. Saady
Ministry of Education/ Salahedin/Iraq
Abdulrahman J. Mansoor
Department of Biology/College of Medicine/University of Tikrit/Iraq
Husham N. Hameed
Department of Physiology/College of Education/ University of Samarra/Iraq

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corresponding author:
Rabah H. Saady
rabah.h.saady.bio@tu.edu.iq
Abdulrahman J. Mansoor
ajmansoor@tu.edu.iq
Husham N. Hameed
Dr.hushamnaji@gmail.com

ABSTRACT

The aims of this study are to find out the biological efficiency of the fungi *Beauveria bassiana* and *Metarhizium anisopliae* in some instar of the bluefly *Lucilia sericata* (Diptera: Calliphoridae). The study was done Samarra University in controlled conditions of 28 ± 3°C and a relative humidity of 65 ± 2%. with a lighting period of 16 hours per day. The droplet suspension is prepared by growing it on Sabouraud dextrose agar (SDB) medium in a flask of 250 ml by 150 ml of the used medium. *M. anisopliae* caused 89.29% for the first instar at 1×10^5 spore/ml after 72 h. *M. anisopliae* caused lowest mortality 67.43% at 1×10^3 spore/ ml after 24 h, *B. bassiana* caused 85.76% for the first instar at 1×10^5 spore/ml after 72h, while B. *bassiana* caused 68.40% at 1×10^3 spore/ ml after 24 h. *M. anisopliae* caused 78.03% of females and 72.35% of male at 1×10^5 spore / ml after 72h. *B. bassiana* caused 74. 35% for female and 62.63 % for male at 1 x 10^5 spore / ml after 72 h. of treatment.

Keywords: Bluefly, fungal suspension, *Beauveria bassiana*, *Metarhizium anisopliae*.

INTRODUCTION

Recent studies focus on using safer materials to combat harmful organisms (Canassa *et al*., 2019). The use of some types of fungi to control harmful insects has become one of the modern methods (Elbanhawy *et al*., 2020). *M. anisopliae* is a soil fungus that causes diseases to various insects because of its parasitism on them and the spores it produces have a green color on them (Canassa *et al*., 2020). This fungus *M. anisopliae* produces many toxic secondary metabolites that play an important role in combat harmful organisms (Dara, 2019). The fungus *M. anisopliae* is a facultative parasite with a wide range that includes more than 200 species of different orders of insects (Gulzar *et al*., 2021). The fungus spores of *B. bassiana* in the form of a white powder and the cells are oval in shape, the fungus *B. bassiana* can parasitize on many insect larvae and thus can be considered biocides (Mantzoukas *et al*., 2019). Biopesticides are widely used nowadays (Atallah and Mekhlif *et al*., 2017). Combat harmful insects have achieved positive results (Yeseen, 2020). Biological control has become one of the modern methods (Zahra *et al*.,2017). Due to the scarcity of research on isolating the pathogens of the blue fly *L. sericata* and isolating the fungus *B. bassiana* and *M. anisopliae* from naturally infected blue fly *L. sericata* larvae were not previously conducted in Iraq, this study wase suggested.

MATERIALS AND METHODES

Sample collection

The green fly *L. sericata* was collected by different traps, then kept in boxes measuring 30 x 30 x 50 cm, controlled through a hole in one side. They were fed with pieces of meat, and after obtaining the larvae, they are raised in other boxes with the use of the mentioned food at 28 ± 2 °C and a relative humidity of 65 ± 2% to reach the pupae, then the temperature is reduced to 25 °C to obtain the adults. The study was conducted from the beginning of March until the end of October 2023.

Fungi isolation steps

The different stages of *L. sericata* larvae were collected from different regions of Saladdin governorate, then the samples were examined to investigate the samples infected with the fungus *M. anisopliae* and *B. bassiana* and this was inferred by the change in the color of the larvae to a reddish-orange color, and we could see the filaments of the *M. anisopliae* on the larvae, while the fungus *B. bassiana* appears as a white to yellow powder on the larvae of *L. sericata*. The dead and weak larvae are placed in 70% alcohol (ethanol) for 5 minutes then placed in sodium hypochlorite 0.05 for three minutes after that they are placed in sterile water, then on sterile filter paper, and with sterile forceps they are put in a special dish containing the appropriate medium to isolate *M. anisopliae* then placed in the incubator at 28 ± 2C° for 7 days.

Identification of fungi

M. anisopliae and *B. bassiana* isolated from the larvae of *L. sericata* was taken part of the fungus culture and put on slide with some of sterile water and placing the cover of the slide after that it was examined under 40x magnification in plant protection/ College of Agriculture/ Tikrit University, and the taxonomic key was relied upon to reach the species leve (Ahmed, 2019).

Save *invitro* fungi

In the culture medium of glass tubes with a capacity of 15 ml, a space is made for each tube, then the fungus is transferred by a sterile carrier to each tube. The test tube is transferred to the incubator at 28 ± 2, then after seven days it is transferred to refrigerator.

Preparation of the fungal suspension

The droplet suspension is prepared by growing it on SDB medium in a flask of 250 ml by150ml of the used medium and kept in an incubator at 28C° for 7 days. It is shaken daily for the purpose of distributing the fungal growth, after that Filtration is done by passing the fungal extract through several layers of gauze, we put 1 ml from the filtrate on a cells Haemocytometer, where number spores in each of the squares is calculated by the largest squares on the sides, then the total number is divided by four to obtain the average number of spores in one box, then multiply this result by 1×10^4 to obtain the number of spores in 1 ml of the fungal suspension, we get a concentration of 2×10^6 spores/ ml (Abeer *et al*., 2007). For the purpose of obtaining a concentration lower than the equation (Al-Ghanmi, 2020).

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Vital test

Biological test for different concentrations of *M. anisopliae* and *B. bassian* in the first and third larval instars of the blue fly *L. sericata* after 24, 48 and 72 hours. I took 30 larvae that had been prepared by isolating the larvae of the previous stage in the breeding tubes until moulting and reaching the required stage as was done. Dependence on the size of the head of the larvae, which increases to double with the age of the larvae to the next stage, and for each concentration and for each of the two species separately, and distributed to four containers, three of which contain 100 ml of each concentration of the suspension. The control agent contains sterile water, with a small brush transferred to a 200 ml glass flask containing sterile water, 10 mg/cm sterile larvae food was added. The pots containing the treated larvae were incubated in 28 ±2C°.

Biological testing in adults

Sufficient numbers of pupae of each species were taken from the permanent farm and placed individually in tubes of 10 ml capacity and closed with cotton swabs, until they were transformed into adults. 10 adults, males and females, of both types, were distributed separately in one liter plastic bottles covered with a piece of tulle cloth, and each repeater was sprayed with a hand sprayer from a height of approximately 10 cm after making a hole that allowed spraying the fungal suspension, and then it was closed again after the end of spraying, control treatment was sprayed with distill water. Treated adults were transferred to plastic bottles of 1 liter capacity, inside each of which was a cotton saturated with 10% sugar solution in a petri dish with a diameter of 9 cm. This experiment was repeated three times for each concentration and for both types, and the same as the control treatment. I hugged the bottles treatment in the incubator at 28 ± 2C°.

RESULTS AND DISCUSSION

(Table 1) showed that *M. anisopliae* caused 89.29% for the first instar at 1×10^5 spore/ml after 72 h, *M. anisopliae* caused 52.73% at 1×10^3 spore/ml after 24 h. treatment, while no mortality rate was recorded in the control coefficient. As for the third larval stage, we note that the highest mortality was 82.62% at 1×10^5 spore/ml after 72 hours, while *M. anisopliae* caused 67.43% at 1×10^3 spore/ml after 24 h of treatment.

Table 1: Larval stages of *L. sericata* affected by various concentrations.

	Stage	Concentration spore/ ml	Percentage mortality /hours			Average concentration effect	Average treatment effect
			24	48	72		
M. anisopliae	**First**	1×10^3	52.73	73.36	82.37	C 69.48	A 73.59
		1×10^4	59.31	76.98	84.67	B 73.64	
		1×10^5	62.00	81.67	89.29	A 77.65	
		control	0	0	0	D 0	
	Third	1×10^3	43.67	62.19	72.51	C 59.45	B 65.26
		1×10^4	51.21	67.00	78.18	B 65.46	
		1×10^5	58.86	71.21	82.62	A 70.89	
		control	0	0	0	D 0	

**Similar capital letters in one column vertically means no significant difference.

(Table 2) showed that *B. bassiana* caused 85.76% in relation to the first larval instar at 1×10^5 spore/ml after 72 h. *B. bassiana* caused 49.16% at 1×10^3 spore/ml after 24 h. of treatment. Mortality rate was not recorded in the control coefficient. As for third larval stage, we note that *B. bassiana* caused 75.81% at 1×10^5 spore / ml after 72 h. *B. bassiana* caused the lowest mortality rate of 40.68 % at 1 x 10^3 spore/ ml after 24 h. no mortality recorded in the control laboratories.

Table 2: Larval stages of *L.sericata* affected by various concentrations.

Type of fungi	Stage	Concentration spore/ ml	Percentage mortality/hours			Average concentration effect	Average treatment effect
			24	48	72		
B. bassiana	**First**	1×10^3	49.16	60.86	78.31	C 62.77	A 68.61
		1×10^4	54.00	69.21	81.94	B 68.38	
		1×10^5	64.29	74.00	85.76	A 74.86	
		control	0	0	0	D 0	
	Third	1×10^3	40.68	53.00	67.21	C 59.81	B 63.11
		1×10^4	51.23	62.89	71.27	B 61.79	
		1×10^5	59.50	67.93	75.81	A 67.74	
		control	0	0	0	D 0	

**Similar capital letters in one column vertically means no significant difference.

(Table 3) showed that *M. anisopliae* caused 72.35% for males at 1×10^5 spore / ml after 72 hours, while *M. anisopliae* caused 41.82% at 1×10^3 spore/ml after 24 h, mortality percentage was not recorded in the control coefficient. As for females, we notice the caused 78.03 % at 1×10^5 spore/ml after 72h. *M. anisopliae* caused 42.00% at 1×10^3 spore/ml after 24 hours of treatment, while no mortality rate was recorded in control plants.

Table 3: Male and female of *L. sericata* affected by various concentrations.

Type of fungi	Stage	Concentration spore/ ml	Percentage mortality /hours			Average concentration	Average treatment effect
			24	48	72		
M. anisopliae	**male**	1×10^3	41.82	52.31	59.38	C 51.17	B 59.07
		1×10^4	49.39	61.56	69.34	B 60.09	
		1×10^5	56.19	69.38	72.35	A 65.97	
		control	0	0	0	D 0	
	Female	1×10^3	42.00	57.84	63.97	C 54.60	A 63.88
		1×10^4	54.91	66.93	73.15	B 64.99	
		1×10^5	64.31	73.85	78.03	A 72.06	
		control	0	0	0	D 0	

*Similar capital letters in one column vertically means no significant difference.

(Table 4) shows that *B. bassiana* caused 62.63% for male at a concentration of 1×10^5 spore/ml after 72 h, B. *bassiana* caused 24.37% at 1×10^3 spore/ml after 24h, mortality rate was not recorded in the control coefficient. As for females, we notice the caused 74.65% at 1×10^5 spore / ml after 72 h, B. *bassiana* caused 39.74% at 1×10^3 spore / ml after 24 h, no mortality percentage was recorded in the control coefficient**.**

Table 4: shows male and female of *B. bassiana* affected by various concentrations.

Type of fungi	Stage	Concentration spore/ ml	Percentage mortality /hours			Average concentration		Average treatment effect	
			24	48	72				
B. bassiana	**male**	1×10^3	37.24	45.93	52.26	C	48.14	B	51.00
		1×10^4	43.62	49.84	54.21	B	49.22		
		1×10^5	50.37	54.00	62.63	A	55.64		
		control	0	0	0	0			
	Female	1×10^3	39.74	52.17	61.42	C	51.08	A	60.23
		1×10^4	49.62	63.05	71.43	B	61.36		
		1×10^5	61.02	69.15	74.65	A	68.26		
		control	0	0	0	D	0		

**Similar capital letters in one column vertically means no significant difference.

This study agreed with a study (Velavan in *et al*., 2022) which it was proved that the use of fungi *M. anisopliae* and *B. bassian* against the adults of the banana weevil *ODOIporus longicollis* banana weevil led to effective effects through penetration of the outer cuticle layer, legs and abdominal area, and this was shown after 15 days treatment by using different concentrations of fungi *M. anisopliae* and *B. bassian*. Our current study is similar to a study (Kisaakye *et al*., 2021) in which it was shown that the fungus *B. bassian* caused 50% of the adults of the banana weevil *ODOIporus longicollis*, *M. anisopliae* caused 80% of the adults. The study agrees with (Rabah and Abdulrahman, 2020) the results of the laboratory tests are shown *V. lecanii* fungi cause highest mortality ratio 89.63% for the second larval *of culex pipiens.* This study is consistent with (Ali *et al*., 2022) the fungus *Metarhizium anisopliea* was used in biological control of housefly *Musca domestica and* achieved positive results.

Comparison of the bioefficiency of *M. Anisopliae* against the blue fly *L. Sericata.*

By comparing the general average of the killing rate for all concentrations, it was found that there were significant differences in terms of the effect on the first and second in stars, male and female blue fly, where *M. anisopliae* had more influence on the first instar than the rest of the stages, while *M. anisopliae* had less effect on the male Fig. (1).

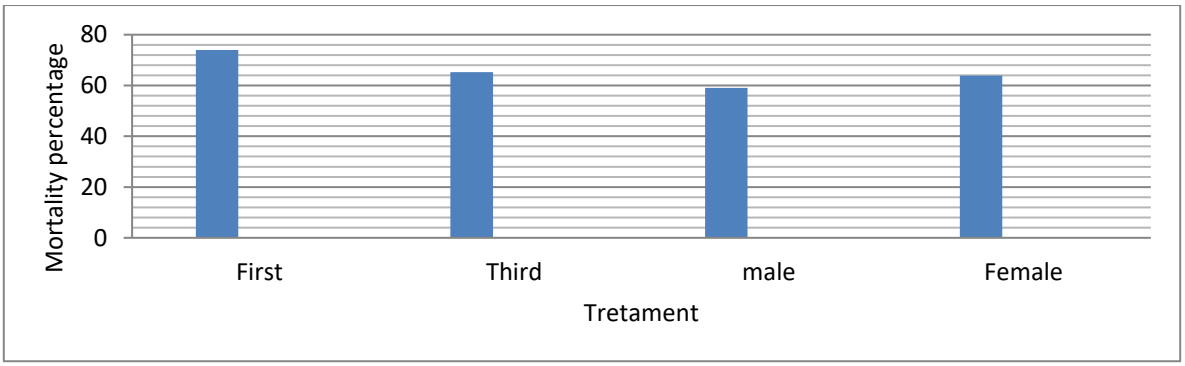

Fig. 1: Comparison of the bioefficiency of *M. anisopliae* against male and Female blue fly fly *L. sericata.*

Comparison of the bioefficiency of *B. bassiana* against the blue fly *L. sericata*

By comparing the general average of the killing rate for all concentrations, it was found that there were significant differences in terms of the effect on the first and second in stars, male and female blue fly, where the fungus *B. bassiana* had more influence on the first instar than the rest of the stages, the form of the fungus *B. bassiana* was less effective on the male. Fig. (2)

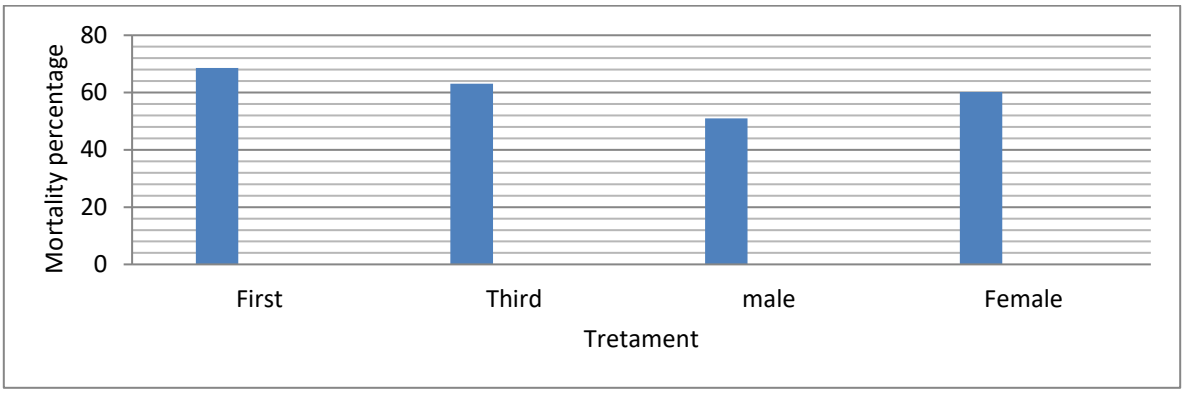

Fig. 2: Comparison of the Bioefficiency of *B. bassiana* against male and female blue fly *L. sericata*.

CONCLUSION

Through the results reached in the current research, we can say that the pathogenic fungi *M. anisopliae* and *B. bassian* can be used as biopesticides in controlling the different stages of the blue fly *L. sericata* (Diptera: Calliphoridae) without leaving negative results affecting the environment or living organisms.

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امكانية استخدام بعض الفطريات في السيطرة على الذبابة الزرقاء *Lucilia sericata* (Diptera: Calliphoridae)

رباح حسن سعدي

وزارة التربية/ صلاح الدين/ العراق

عبد الرحمن جهاد منصور

قسم علوم الحياة/ كلية الطب/ جامعة تكريت/ العراق

هشام ناجي حميد

قسم الفسلجة/ كلية التربية/ جامعة سامراء/ العراق

الملخص

الهدف من هذه الدراسة معرفة الكفاءة الحيوية للفطريات *Beauveria bassiana and Metarhizium anisopliae* في السيطرة البايولوجية على الاطوار اليرقية الأول والثالث وبالغات ذكر وانثى الذبابة الزرقاء *Lucilia sericata* (Diptera:Calliphoridae) اجريت الدراسة في جامعة سامراء في ظروف مختبرية مسيطر عليها 28± 3 درجة مئوية ورطوبة نسبية 65± 3٪. تم تحضير المعلق القطري بتنميتها على وسط Sabouraud dextrose agar (SDB) بدورق 250 مل بمقدار 150 مل من الوسط المستعمل، الفطر *M. anisopliae* سبب اعلى نسبة هلاك 89.29 % للعمر اليرقي الأول عند التركيز 1×10^{5} بوغ / مل بعد 72 ساعة من المعاملة بينما الفطر *M. anisopliae* سبب اقل نسبة هلاك 67 .43 % عند التركيز 1×10^{3} بوغ/ مل بعد 24 ساعة من المعاملة، الفطر *B. bassiana* سبب اعلى نسبة هلاك 85.76 % بالنسبة للعمر اليرقي الأول عند التركيز 1×10^{5} بوغ / مل بعد 72 ساعة بينما الفطر *B. bassiana* سبب اقل نسبة هلاك 68. 40 % عند التركيز 1×10^{3} بوغ/ مل بعد 24 ساعة من المعاملة، وسبب الفطر *M. anisopliae* اعلى نسبة هلاك للإناث .0378 % عند التركيز 1×10^{5} بوغ/ مل بعد 72 ساعة اما بالنسبة للفطر *B. bassiana* سبب اعلى نسبة هلاك للإناث 65 .74 % عند التركيز 1×10^{5} بوغ/ مل بعد 72 ساعة.

الكلمات الدالة: الذبابة الزرقاء، المعلق القطري، الفطريات *Beauveria bassiana, Metarhizium anisopliae*