Evaluating Effectiveness of Pseudomonas Fluorescens and Serratia Marcescens against Galleria Mellonella

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Abstract

During this study, some species of bacteria were isolated and diagnosed from Musca domestica on the outer surface and gut of flies, which were collected from different areas (vegetable and butcher shops) in Baghdad province. The most common isolates were found to be Pseudomonas fluorescens bacteria with a rate of 40%, followed by Staphylococcus lentus bacteria by 32%, and Serratia marcescens bacteria by 28%, thus confirming that Musca domestic is an important mechanical transporter that helps spread pathogens. The results showed that the best effective dilution in the first larvae of Galleria mellonella is 10-4 for Pseudomonas fluorescens bacteria with a mortality and smut rate of 15.62%, followed by a concentration of 10-6 with 12.5%, and then a concentration of 10-8 with a rate of 9.73% dead larva. As for the pupa, the 10-6 concentration gave the eclosion failure rate, followed by a 10-4 concentration, then a 10-8 concentration of 6.25% and 3.12%, respectively.

Serratia marcescens bacteria recorded the highest rate of larvae mortality and smut in concentration 10-6 at 12.5%, followed by a concentration of 10-8 with 9.73%, while the concentration of 10-4 gave the lowest rate of mortality and smut reached 3.12% dead larva, 10-4 concentration recorded the highest pupa emergence failure rate of 12.5%, followed by a concentration of 10-8, 10-6 with 6.25% and 3.12%.

Keywords: Galleria mellonella, Effectiveness, Pseudomonas fluorescens, Serratia marcescens, Musca domestica.

1. Introduction

Home flies Musca domestica belonging to the Muscidae family play the role of mechanical transporter for transportation of many pathogens. The species belonging to Diptera families are one of the most important insect pests carrying these pathogens, including species from the Culicidae family, which transmits the pathogens of malaria, dengue fever and yellow fever and also meningitis.

Psychodidae is a family that transport the pathogen of Leishmania and the Muscidae family of carrying cholera pathogens, trachoma, Shigella dysenteriae and Salmonella typhi, while the Calliphoridae family causes myiasis to humans and animals. This provides an opportunity for epidemics to spread and to kill millions of people and animals. Musca domestica is one of the most dangerous pests and poses a financial threat to the livestock industry, particularly poultry [1-4].

Because of this, human beings have to fight them, reduce their spread and reduce their damage, as in recent years they have been used to use bio-control pests for their ability to reduce the harm of these pests through the use of pathogens such as virus, bacteria and fungi, especially since they are specialized and of a non-destructive nature in the environment, so many of these organisms have been used as alternatives to chemical pesticides [5]. The current study aimed at using Pseudomonas fluorescens and Cerratia marcescens in the bio-control of Galleria mellonella insect and the proof of bacterial pathogenesis.

Eguchi et al. [6] explained the ability of the large waxworm to resist various microorganisms, and these insects have an effective defense system against microorganisms for survival, as the cellular defense factor in this cellular and mechanical defense system responds to pathogens and for parasites through phagocytosis and node formation [7], because they produce specific proteins and induced lymph and are effective against various protein toxins induced by the pathogen [8].

2. Materials and Methods

Collecting samples: The current study included the collection of (50) samples of domestic flies, Musca domestica from different environmental places from Baghdad province (vegetable and butcher shops) and collected during October 2021 as flies were caught by a special network and placed inside sterile glass containers and after a period of collection the insects were transported to the laboratory and all were killed by putting them inside the freezer at zero centigrade temperature for five minutes.

Isolation and diagnosis of bacteria

Bacterial isolates were diagnosed based on the morphological and microscopic characteristics of growth colonies and was diagnosed using the Vitek-2 system as instructed by the manufacturer.

Insect breeding

Wax frames infected with Galleria mellonella were collected from different beehives in Baghdad province. The wax was cut and placed in a wooden breeding box with dimensions (30x 30x 30 cm) whose base is wood and its three sides of the metal wire, but its front is tulle cloth and put the box in the laboratory and the insects were

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continuously fed with dark wax after being frozen for (3) days, for the purpose of sterilization. For the purpose of getting insect stages on an ongoing basis.

Pathology of Galleria mellonella larvae

Preparation of bacterial suspension

The bacterial suspense of potato's dextrose broth is prepared according to [9], after that four glass containers capacity 100 ml are prepared and potato's dextrose broth is placed inside. Several colonies of bacteria were taken by a sterile transporter (loop) and transported into the container containing the broth as they were incubated at a temperature of 37°C for 7 days and then filtrated the suspensor by millipore filter paper (0.45µm). Several of its concentrations were prepared for the purpose of conducting the experiment.

Preparing series of bacterial suspension

The method is based on the preparation of a successive decimal series of bacterial suspension, using nine sterile test tubes capacity 10 ml with nine sterile pipet, each test tube contains 9 ml of sterile distilled water and then 1 ml of bacterial suspension (Stock) is added by the first pipet to the first test tube, which is 10 ml in size, representing the first dilution 1/10 or 10-1.

1 ml is then pulled from the first test tube by the second pipet and placed in the second test tube to a size of 10 ml, which represents the second dilution (1/100) or (10-2) and so until the eighth dilution (1/1000000000) or (10-8) according to [10, 11].

Statistical analysis

Statistical Analysis System -SAS (2018) used data analysis to study the effect of different treatments on characteristics studied in a complete random design (CRD), and compared significant differences between mean with the lowest significant difference-LSD test.

3. Results and Discussion

Identification of samples

The current study included the collection of 50 samples of the domestic fly insect to isolate and diagnose some species of bacteria on the outside surface of the fly (legs and wings) and digestive system, which were collected from different areas of Baghdad province, including (butcher and vegetable shops, houses and waste collection places), diagnostic tests included the detection of (11) genus of gram negative bacteria for each of the following genera: Escherichia coli, Staphylococcus lentus, Pseudomonas fluorescens, Salmonella enteric, Serratia marcescens, Enterobacter cloacae, Enterobacter aerogenes, Raoultella ornithinolytica, Sphingomonas paucimobilis, Shigella sp. and Micrococcus sp. Pseudomonas fluorescens, which were isolated from different regions, recorded the highest prevalence rate of 40%, followed by Staphylococcus lentus bacteria by 32%, followed by Serratia marcescens and Escherichia coli bacteria by 28%, and Raoultella ornithinolytica bacteria by 24%, Salmonella enteric recorded an isolation rate of 20%, Enterobacter cloacae, Enterobacter aerogenes and Sphingomonas paucimobilis recorded an isolation rate of 8%, and Micrococcus sp. bacteria recorded the lowest prevalence rate of 4% as in the table (1).

Table (1): Prevalence of bacterial species isolated from domestic flies and present in different environments.

Bacteria	Cllecting places		Rate
	Vegetable sgop	Butcher shop	
Escherichia coli	3	4	7(%28)
Staphylococcus lentus	3	5	8(%32)
Pseudomonas fluorescens	4	6	10(%40)
Salmonella enteric	3	2	5(%20)
Serratia marcescens	4	3	7(%28)
Enterobacter cloacae	2	0	2(%8)
Enterobacter aerogenes	1	1	2(%8)
Raoultella ornithinolytica	3	3	6(%24)
Sphingomonas paucimobilis	1	1	2(%8)
Micrococcus spp.	1	0	1(%4)
Total	25	25	50
LSD value	--	--	* 7.26
*P≤0.05			

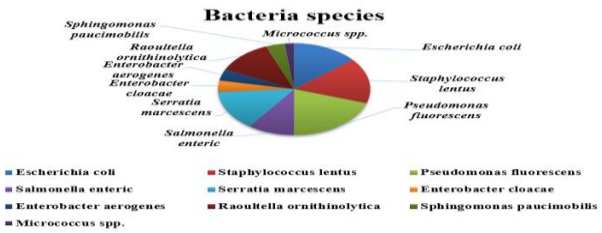

Figure (1): Shows the prevalence of bacterial genera isolated from domestic flies and present in different environments.

A study conducted by Fotedar [12], confirmed that the domestic fly is a mechanical transporter that helps in the spread of pathogens and this is consistent with the current study in which the domestic fly was found to be an important mechanical transporter.

The current results for Enterobacter sp. and Pseudomonas sp. bacteria also agreed with a study carried out by Nazari et al. [13] where the results of the current study showed that the gram-negative bacteria, including Staphylococcus lentus bacteria are one of the most dominant bacteria and repeatedly as the results of the current study are close to a local study conducted by [14], the results were consistent as for Staphylococcus lentus with the findings of [15].

Current results for Escherichia coli, Serratia marcescens, Shigella sp., Salmonella sp. and Staphylococcus sp. also agreed with a study conducted by Baker et al. [16] where other bacterial species similar to a study by Vazirianzadeh et al. [17] isolated as there are many studies indicating the role of domestic flies in the transport of bacteria.

Sulaiman et al. [18] isolated 18 species of intestinal bacteria of multiple species of flies in Malaysia, as well as Nazni et al. [19] isolated Bacillus sp. bacteria, Micrococcus sp. and Staphylococcus sp. and Escherichia sp. from the outer surface of the body of the domestic fly in addition to isolating it from the vomit and waste of the domestic fly.

Banjo et al. [20] also isolated bacterial species of domestic

fly larvae in Nigeria, similar to isolated species from the current study, while in Germany Förster et al. [21] isolated pathological bacteria such as Eshreshia coli, Campylocterjejuni from the intestines and the outer surface of the domestic fly body. Holt et al. [22] referred to Salmonella sp. isolation from the domestic fly as well as Nazni et al. [19] indicated that newly emerging flies are free of pathological bacterial.

Pathology of bacteria on the larvae of Galleria mellonella L.

Two genus were taken from bacteria isolated from Musca domestica and the food of the larvae of G. mellonella was sprayed with three different concentrations 10-4, 10-6 and 10-8, and after a month's follow-up, the results in the table (2) showed the presence of mortality and smut for treated larvae as it was recorded in Pseudomonas fluorescens bacteria the highest mortality rate at 10-4 was 15.62%, followed by a 10-6 concentration was 12.5%, and a concentration of 10-8 was recorded the lowest mortality and smut rate 9.37%.

Serratia marcescens recorded the highest mortality rate of 12.5% at 10-6, followed by a 10-8 concentration of 9.37%, while a 10-4 concentration of 3.12%, indicating a positive relationship between exposure duration and killing rate in high and low concentrations. The bacteria caused the treatment larvae to turn brown as showed in picture (1).

Table (2): Pathology of bacteria on G. mellonella.

No.	Bacteria	Concentration	Mortality and smut%	Impossible without the emergence of%
1	Pseudomonas fluorescens	4-10	%15.62	%6.25
		6-10	%12.5	%12.5
		8-10	%9.37	%3.12
2	Serratia marcescens	4-10	%3.12	%12.5
		6-10	%12.5	%3.12
		8-10	%9.37	% 6.25
3	Control	%0		
	LSD Value		* 3.95	* 3.07
P≤0.05 *0\021				

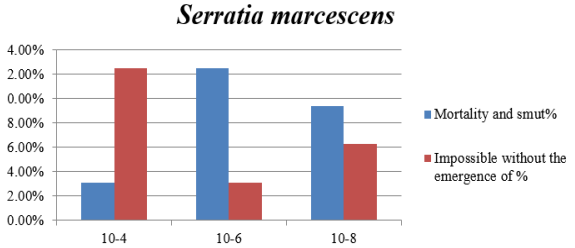

Figure (2): Shows pathology of Serratia marcescens bacteria on the larvae of G. mellonella L.

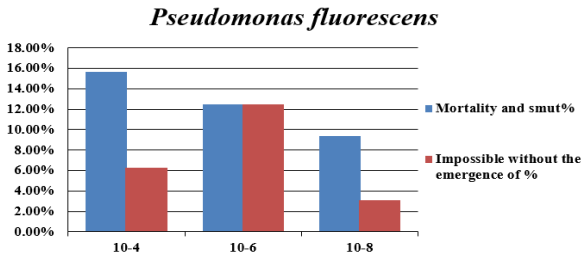

Figure (3): Shows pathology of Pseudomonas fluorescens bacteria on the larvae of G. mellonella L.

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Figure (4): Effect of bacterial concentrations on the larvae of G. mellonella L.

The color of the larvae and blackish changed after being treated with Pseudomonas fluorescens bacteria with a concentration of 10-4.

The color of the larvae changed after being treated with Serratia marcescens bacteria at a concentration of 10-6.

The results of the current study were consistent with those of a study conducted by [23], in the use of Pseudomonas chlororaphis bacteria belonging to the Pseudomonas fluorescens group, which was found to produce cellular toxins Exolysin (ExlA) that have the potential to kill the larvae of G. mellonella when injected with it, as the results were consistent with Péchy-Tarr et al. [24] as the study showed that the lowest dose of P. fluorescens CHA0 or Pf-5 has the potential to kill the larvae of tobacco moths Manduca sexta and Galleria mellonella larvae.

The study agreed with in the use of Serratia marcescens bacteria as it was found that the immune system to Galleria mellonella larvae works to subtract from the bacteria quickly, but then collapses to the bacteria.

Ruiu [25] also found that Serratia marcescens have the ability to produce protein toxins such as SepA, SepB, SepC, a group of insect-neutral protein toxins, and can also produce certain extra cellular enzymes such as lipases, proteases and chitinases, Some species of Serratia marcescens bacteria have the ability to suppress immune cells by reducing the adhesion of the insect's immune control cells.

Statistical analysis also showed the presence of significant differences and at the level of 0.05 depending on the concentrations used and the duration of exposure, and the results indicate that the first larval stages are severely affected by the incompleteness of their defense systems, especially blood cells formed, which are of the prohaematocytes and which do not participate in the phagocytosis of bacteria and foreign objects entering the cavity of the body.

G. mellonella L. larvae have been used to pathology of bacteria isolated from domestic flies as they have already been used to determine the virulence of different types of pathogens affecting humans, and the larva has many advantages, including having advanced cellular defenses that perform the same function as macrophages, neutrophils and are not subject to the restrictions used in the study of mammal models.

[6], explained the ability of the great waxworm to resist

various microorganisms, and that these insects have an effective defense system against microorganisms for survival, which consists of a cellular and mechanical defense system and that the cellular defense agent responds to pathogens and parasites by phagocytosis and node formation [7], because they produce induced proteins specific to the lymph and effective against various protein toxins induced by the pathogen [8].

One of the most important symptoms that appeared on the larvae of G. mellonella L. is the lack of movement and activity of larvae and the appearance of spots on the body of the larva where it becomes dark color and then turn black then occurs death, the cause of black spots on the larva is evidence of hyper melanin, as it is an indicator of the attack of bacteria to the immune system of the larva, which stimulates the phenoloxidase system responsible for the process of melanosis and pathogen resistance [26]. As for pupa, one of the most important symptoms is the failure of adult emergence, but if they emerge, they are distorted.

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