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Isolation and diagnosis of *Enterobacter* spp. and *Staphylococcus aureus* from *Musca domestica* and effect of *Mentha spicata* extract in inhibiting their growth

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Abstract

Background and Objectives: This study was conducted to examine and isolate bacteria from the External surface of the adult house fly *Musca domestica* body from four regions in Kerbala/Iraq during March 2023, and to test the biological effectiveness of the cold aqueous extract of *Mentha spicata* leaves in the growth of four species of bacteria isolated from the external surface of the insect's body Houseflies.

Materials and Methods: Adult house flies were collected from different residential regions in the governorate and transported to the laboratory, and laboratory experiments were conducted on them. Diagnostic and biochemical tests were investigated for these isolates, the effect of cold aqueous extract of *Mentha spicata* leaves on the bacterial isolates using agar well diffusion method was also determined.

Results: The results of the isolates obtained showed the presence of many bacteria, including *Escherichia coli*, which had the highest percentage of isolates, *Klebsiella pneumonia*, *Enterobacter* spp., *Shigella*, *Staphylococcus aureus*, and *Staphylococcus sciuri*.

It was demonstrated that cold aqueous extract of *M. spicata* has antibacterial effects which showed inhibition of the growth diameters of the bacterial species for both *S. aureus* and *Enterobacter* spp., reduced growth of *E. coli* and *K. pneumonia*, which were under the influence of different concentrations of cold aqueous extract of *M. spicata* leaves, was absent or weak.

Conclusion: The obtained results indicated that the housefly contributes to the infection of diseases caused by pathogenic bacteria and are potential vectors of a wide range of pathogenic organisms, This study examines the importance of the mint plant in treating tow species of pathogenic bacteria, *Enterobacter* spp. and *S. aureus*, which works to reduce the inflammatory reaction in humans.

Keywords: Bacterial pathogens, House fly, mechanical vectors, *Mentha spicata*, biological effectiveness

Introduction

The housefly insect, *Musca domestica* belongs to the Diptera order of the Muscidae family. This insect is considered a medical and veterinary insect due to its importance in transmitting pathogens, including bacteria that are transmitted between animals and humans (Hussein and John 2017) [22] such as Bacteria, Fungi, Viruses, Tapeworms, and Protozoa (Getachew *et al.* 2007; Hogiet and Amendt 2008) [16, 21], as house flies are mechanical vectors for many infectious diseases such as Salmonellosis, Shigellosis, Cholera, and skin infections (Nazari *et al.* 2017), and for many pathogens, including *Staphylococcus* bacteria, *Enterococcus* spp., *Escherichia coli*, *Shigella* spp., *Bacillus anthracis*, *Corynebacterium* spp., *Chlamydiales* and some other parasitic organisms (Olsen 1998; Nmorsi *et al.* 2007) [30, 29], as this insect is tight related, the prevalence and transmission of several acute conditions that cause gastroenteritis and trachoma among young children and infants in developing countries, bacterial resistant to antibiotics in hospitals (Graczyk *et al.* 2001) [18].

And that the continuous movement of house flies and their frequency on different places of animal waste, dirt, and human resources such as food and excremental waste for the purpose of feeding them, which leads to the spread of diarrhea, and this in turn works to increase the number of house flies is high (Pava-Ripoll *et al.* 2015) [31] as well as being one of the main pests causing health problems in the environment (Graczyk *et al.* 2005; Service 2012; Sarwar 2016) [19, 34, 33], and Service (2012) [34] explained that the transmission of diseases by adult

houseflies is carried out by multiple mechanisms, namely: mouth parts, body hair of the insect, feet contaminated with pathogens, and one of the most important methods of transmission is the vomit and excrement of the adult insect on food during their feeding, and this was confirmed by a study Al-Maaly (2020) ^[3] of the transfer of house flies to bacterial pathogens, as these adults were collected from the animal barns of the College of Veterinary Medicine at the University of Baghdad for the year 2018, as six bacterial species were isolated from the external surface of *Musca domestica*, these species included *E. coli* with a percentage of 46.6%, *Klebsiella* with a percentage of 6.6%, *Salmonella* with a percentage of 6.6%, *Pseudomonas* with a percentage of 3.3%, *Streptococcus* with a percentage of 50% and *Staphylococcus* with a percentage of 20%.

The study of Al-Rahimy *et al.* (2023) ^[4], confirmed the registration of ten species of insects belonging to the family of Muscidae of medical importance in Kerbala Governorate in four locations (City Center, Al-Hindiya District, Al-Husseiniya District. and Ain Al-Tamr District), where the largest number of these were recorded Species in the Al-Hindiya region, where they numbered 236 insects, and the highest percentage of insects of medical importance was recorded at 45.91%, while in residential areas the percentage of insect numbers reached 32.8%. As this study confirmed that the number of collected species varied according to the collection areas and their locations, as the Al-Hindiya District recorded the largest number of species, and this study noted that the *M. domestica* insect represented the highest percentage, amounting to more than 50% compared to the rest of the species of medical importance belonging to the family Muscidae.

Antibiotics are a commonly used method to eliminate pathogenic bacteria, as they are of great importance for maintaining human health. However, the repeated and indiscriminate use of antibiotics in treating bacterial infections has led to an increase in the number of bacterial strains with multiple drug resistance, which has led to a decrease in the effectiveness of antibiotics in treating bacterial infections. It is considered one of the biggest challenges that humans face due to the toxic reactions it causes in the body. Therefore, there is an urgent need to develop strategies that are important to limit the spread and development of resistant bacterial strains. One of the most important of these strategies is the introduction of plant extracts in laboratory experiments to eliminate Pathogenic bacteria, as scientists were previously interested in medicinal plants for a long time and they were introduced as a means of treating It is used in many cases because it does not contain side effects, has a rapid therapeutic effect, and is able to put the human immune system in good condition. It also works as an inhibitor and killer of the growth of pathological bacteria inside the human body.

The mint plant, *Mentha spicata*, belongs to the Lamiaceae family, which consists of three main components, which are essential oils, flavonoids, and terpenes (Monoterpenes and sesquiterpenes) (Bozovic *et al.* 2015; Kapp *et al.* 2020) ^[6, 7], and it is a medicinal plant used by Humans treat diseases, which also shows vital activity against pathogenic bacteria and fungi (Mezaal 2013; Snoussi *et al.* 2015) ^[8, 9].

The presence of house flies in residential areas constitutes a great danger to human health because of their ability to transmit pathogens between individuals, including bacteria, so the current research came to educate people about the

dangers of house flies and eliminate them in correct and safe ways for public health, one of these methods is the use of plant extracts that are safe for human health and the environment, including the cold water extract of the mint plant *Mentha spicata*.

Materials and Methods

Description of the study area

The study was conducted in the Kerbala governorate, located to the southwest of Baghdad, with coordinates 44.03332° E and 32.61667° N (Figure 1). The governorate consists of three main districts (Kerbala district and includes the districts of Al-Husseiniya and Al-Hur), while it includes (Al-Hindiya district center " Tuwaireej " Al-Khairat and Al-Gadol Al-Gharbi sub-districts) and (Ain al-Tamr district), it is distinguished by its soft land, and it is about thirty kilometers away from the Euphrates River in the west. It relies for irrigation, drinking, and agriculture on the waters of streams and the Husseinia River branching from the Euphrates River. Its climate is semi-desert, it is hot in summer and relatively cold in winter, and the amount of rain decreases in warm seasons, so the climatic and environmental factors seem very suitable for the development of flies.

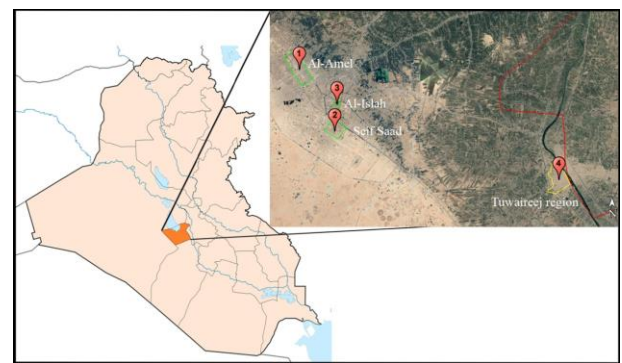


Fig 1: Map of Iraq in which the position of Kerbala governorate and its four residential districts

Collect housefly samples

Samples of adult house flies were collected from four residential areas in Kerbala governorate (Al-Amel neighborhood, Saif Saad neighborhood, Al-Islah neighborhood, and Al-Hindiya district) using an insect net during March 2023, at times from 7 am to 5 pm the day to determine the presence of Bacteria on the bodies of house flies, and were transferred to the laboratory to conduct experiments on them, as (80) adults of house flies were taken from the samples collected from the regions (20 adults randomly from each region), to find the relationship between the spread of flies and the transmission of bacterial diseases, and The insects included in the study were diagnosed at the University of Kerbala/ College of Education for Pure Sciences - Department of Biology by Prof. Dr Rafid Abbas Al-Essa specializes in Medical Entomology.

Preparation of plant extracts

Collecting plant samples

The leaves of the mint plant *M. spicata* were collected from the markets of Karbala Governorate during January 2023. They were dried in places with good ventilation and lighting, away from sunlight. They were ground with a

grinder and placed in opaque glass. The name of the plant was recorded on them and kept in the refrigerator. Until use.

Diagnosis of plant samples

The plant under study was identified by Assis. Prof. Dr. Khansa Shaheed Abdel-Aali at the University of Karbala - College of Science - Department of Biology, as the mint plant *Mentha spicata*.

Prepare the cold aqueous extract

The cold aqueous extract of the mint plant *M. spicata* was prepared according to the method of Riöse *et al.* 1987^[10]. The extract was sterilized by taking 1 g of the dry extract and dissolving it in 2 ml of sterile distilled water, thus obtaining the standard concentration of 200 mg/ml. Membrane filters (0.22 µm) were used, which is a method for sterilizing the plant extract as it works to prevent the passage of germs through it. From the standard concentration, the following dilutions were prepared (400, 600, 800) mg/ml used in the research.

Isolation and identification of bacteria

In the laboratory, house flies were transferred individually from the net into sterile tubes to prevent cross-contamination between flies and killed by placing them at -20 °C, putting 1 ml of sterile physiological saline into each tube, and gently agitating by inversion for one minute. After placing the fly inside it, then the fly was removed from the saline solution using sterile forceps, and the solution containing the germs that were washed from the external surface of the fly was taken (Deeb 2015)^[11], and the samples taken from each residential area were cultivated by inoculating the general media such as Nutrient agar medium, Nutrient broth with a drop of the solution by means of a flame sterilized Henley inoculation loop, and these media were incubated in an aerobic condition at a temperature of 37 °C for a period of 24 hours, as the readings were taken according to growth in the presence of bacterial colonies and the presence of turbidity, distinct colonies were selected and then sub cultured from the general medium to the differential and special media to reach pure culture and grow pure bacterial cultures, where they were cultured on MacConkey agar, S.S. agar, *Pseudomonas* selective medium with the aim of isolating *Enterobacter* iucea, Blood agar, Eosin methylene blue (the selective medium for *E. coli* bacteria) and Mannitol salt agar in order to isolate *Staphylococcus aureus*. These media were incubated at a temperature of 37 °C for 48 hours, and then the colonies were examined in pure form under the microscope after being stained by Gram (Cheesbrough 2005)^[13].

Chemical tests

Several chemical tests were conducted to identify the types of bacterial growth, including: Catalase test, oxidase test, Coagulase test, Urease test and IMVIC test (Quinn *et al.* 2004; Cheesbrough 2005)^[32, 13].

Identification of isolated bacteria using the Vitek-2 device: Bacterial isolates were biochemically diagnosed according to the Vitek-2 automated system report, and according to the manufacturer's instructions (BioMerieux Company/France). The bacterial suspension was prepared by transferring several of bacterial colonies to be diagnosed

from a pure culture that had been previously purified at the age of (18-24) hours into 3 ml of sterile saline (0.45% sodium chloride solution), and the turbidity was controlled using Turbidity control device (Densi-Chek) to 0.5 McFarland for Gram-negative bacteria and 0.63 McFarland for Gram-positive bacteria. Gram stain, as Gram-negative bacteria are diagnosed with a cassette (GN-ID) Gram Negative Identifier and Gram-positive bacteria with a cassette (GP-ID) Gram Positive Identifier, It is a completely closed system, it is not necessary to add any automatic reagents. The card was placed on the cassette designed for use with the Vitek-2 system, placed in the device, automatically filled in a sealed vacuum chamber, incubated at 35.5 °C and automatically subjected to colorimetry (With a new reading) every 15 minutes for a maximum incubation period of 8 hours. Data were analyzed using the Vitek-2 database by actively identifying the organism starting 180 min after the start of incubation (Karagos *et al.* 2015; Ghali 2022)^[23, 46].

Antibacterial activity of plant extract

The biological activity of the antibacterial plant extract of the cold-water extract of the mint plant *M. spicata* was calculated using the agar well diffusion method (Shen *et al.* 2006)^[1]. Mueller-Hinton agar plates were inoculated with bacterial strains of *S. aureus*, *E. coli*, *K. pneumonia*, *Enterobacter* sp. and *Shigella* Spread on all sides of the dish using a sterile glass L-shape spreader. Before inoculation, the bacterial strains were placed in physiological saline solution, suspended in Suspension solution, and then adjusted to 0.5 McFarland standard turbidity (Matuschek *et al.* 2014)^[15].

Wells with a diameter of 5 mm were prepared using a sterile cork borer, and these wells were filled with 0.1 ml of plant extract of each concentration (200, 400, 600, 800) mg/ml, and sterile distilled water (D.W.) was added to one well, Just to be used as a negative control, as added 0.1 ml of Ampicillin (1 mg/0.1 ml) for Gram-negative bacteria and Ciprofloxacin for Gram-positive bacteria were also added to another well to be used as a positive comparison.

The plates were then incubated at 37 °C for 24 hours, and the diameter of the inhibition zone was measured using a ruler (Ibekwe *et al.* 2001)^[25].

Statistical Analysis

The data were analyzed using the (SAS) program, and the results were compared using the Least Significant Difference (LSD) value and the Duncan's Test at the probability level of 0.01 (SAS, 2012).

Results and Discussions

Results

Bacterial culture and isolation

The results of bacterial cultures on the general culture media were shown, as 80 bacterial isolates were obtained and gave positive results out of (80) samples shown in Table (1), as the results of the primary diagnosis of bacteria isolated from the external surface of the body of the housefly were shown from four Residential areas in Kerbala governorate, which showed bacterial growth in the broth and nutrient agar in all study regions, As the number of positive samples (+) for the study regions was 20 samples for each region as in Table (1) (s = 1 means the samples taken from the Al-Amel neighborhood, s = 2 means the samples taken from the Seif

Saad neighborhood, and $s = 3$ means The samples taken from the Al-Islah neighborhood, and $s = 4$ means the

samples taken from the Tuwareej region).

Table 1: Results of primary bacterial culture of samples in broth and nutrient agar

Sample media	s1=20	s2=20	s3=20	s4=20
Nutrient broth	+	+	+	+
Nutrient agar	+	+	+	+
No. positive samples	20	20	20	20

The results of the bacterial isolation of *E. coli* were (40) cases out of (80) positive samples, a percentage of them was (50%), while *Klebsiella* bacteria were (25) cases out of (80) positive samples and their percentage was (31.25%), *Enterobacter* bacteria were (20) cases out of (80) positive samples, and their percentage was (25%), and *Shigella*

bacteria were (15) cases out of (80) positive samples, and their percentage was (18.75%). *Staph. aureus* bacteria was (10) cases out of (80) positive samples and its percentage (was 12.5%), while *Staph. sciuri*, so it was (35) cases out of (80) positive cases, and its percentage was (43.75%) Figure 2.

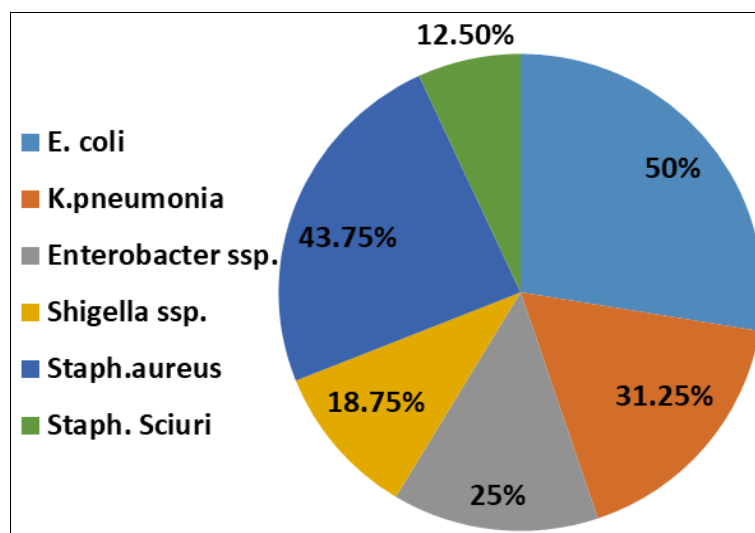


Fig 2: Frequency of Bacterial Isolates

In the current study, Gram-negative bacteria recorded the highest percentage of isolation compared to Gram-positive bacteria, as *E. coli* and *S. aureus* are among the most common pathogens, and this result was similar to the results of other researchers in their previous studies, they concluded that *E. coli* are the pathogen responsible for urinary tract infections for both males and females of different ages, followed by *S. aureus* (Al-Behadliy *et al.* 2020) ^[12].

Diagnosis of bacterial isolates

Primary diagnosis according to cultural characteristics on selective and differential media

The study of the cultural characteristics of bacterial growth on different culture media, specific and selective, according to the primary classification of the growing bacteria, is shown in table (2) according to the companies producing the used nutritious media data. Bacterial groups classified as *Shigella* have been isolated on S.S. agar according to (Nashiro *et al.* 2005) ^[27], *Staphylococcus* on mannitol salt agar (M.S.A.) according to (Simone and Jaine 2007) ^[35], and *Enterobacteriaceae* such as *Escherichia coli* and *Klebsiella* sp. and *Enterobacter* sp. on MacC. agar.

Bacteriology Aspect: Morphological and biochemical identification of isolated bacteria

The primary diagnosis of the isolated bacterial genera was based on some basic criteria that included the shape of the colonies, their color and appearance characteristics on the general culture media, and some differential media such as MacConkey agar media, which helps in the development and isolation of Gram-negative bacteria only, as well as the use of several biochemical tests. For primary diagnosis of isolated bacterial species according to the methods described (Collee *et al.* 2007) ^[14] and as shown in Table (2).

Bacterial identification using the Vitek-2 system

Bacterial isolates were biochemically diagnosed according to the Vitek-2 automated system results in the report and according to the manufacturer's instructions (BioMerieux Company France). The final diagnosis of the isolated bacterial species was made using cards (GP-ID and GN) 64 specialized tests for diagnosing negative and positive bacteria gram stains, which is characterized by ease of work and accuracy of diagnosis. The probability of diagnosis for positive and negative Gram-stained isolates was high ranging from (87% - 99%).

Table 2: Diagnostic and biochemical tests for isolates of *Musca domestica*

		Bacterial Isolates	Gram Stain	M.S.A.	Mac. A	E.M.B.	S.S. Agar	Catalase	Oxidase	Coagulase
80	External surface	<i>E. coli</i>	-rod	It inhibits growth	Pink	Has a metallic green sheen	Pink	+	-	-
		<i>Klebsiella</i>	-rod	It inhibits growth	Mucous sticky pink	Violet in color	Pale pink or off-white color	+	-	-
		<i>Enterobacter</i>	-rod	It inhibits growth	pale pink	Dark violet color	Off-white color	+	-	-
		<i>Shigella</i>	-rod	It inhibits growth	Pale large	colorless	Colorless without a black precipitate	+	-	-
		<i>Staph. aureus</i>	+cocci	golden or yellow	It inhibits growth	No growth appears	It inhibits growth	+	-	+
		<i>Staph. sciuri</i>	+cocci	yellowish grey	It inhibits growth	No growth appears	It inhibits growth	+	-	-

Antibacterial activity of plant extract

The results of a study showed the biological effectiveness of the cold water extract of *M. spicata* leaves against the species of bacteria isolated from the external surface of house flies that were collected from four residential areas in Kerbala Governorate / Iraq and were diagnosed through Morphological and biochemical diagnosis of the bacterial isolates and using the Vitek-2 system. *E. coli*, *K. pneumonia*, *Enterobacter* spp., *Shigella*, and *Staphylococcus* bacteria were treated *S. aureus* and *S. sciuri*.

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The results indicated a positive effect of the aqueous extract of the *M. spicata* on the appearance of average inhibition zone when treated for *Enterobacter* spp. and *S. aureus*. By observing the average diameter of the inhibition zones reached (5.00, 9.00, 18.00, 20.16) mm respectively when treated *Enterobacter* spp., and average diameter of inhibition zones reached (20.00, 25.00, 31.00, 34.40) mm respectively when treated *S. aureus* with concentrations as (200, 400, 600, 800) mg/ml, compared of the inhibition zones when using the antibiotics Ampicillin for Gram-negative bacteria and Ciprofloxacin for Gram-positive bacteria were at average diameters of (6 and 29) mm respectively, shown in table (3).

Table 3: Antibacterial activity of *M. spicata* leaves aqueous extract on the growth on *Enterobacter* spp. and *S. aureus* isolates isolated from the external surface of the body of house flies *M. domestica*

Concentration	Mean±SD	
	<i>Enterobacter</i> spp.	<i>S. aureus</i>
200	5.00±1.00 D	20.00±1.00 D
400	9.00±0.50 C	25.00±0.50 C
600	18.00±1.50 B	31.00±0.00 B
800	20.16±0.76 A	34.40±0.52 A
LSD	1.5846	1.1645
P(value)	0.0001	0.0001

*Averages with similar or common letters don't differ significantly

From the results presented in table (4), it was found the effect of the cold aqueous extract of *M. spicata* had a similar effect to both of the bacterial isolates above, but the effect differed according to the concentrations of the extract. The

results of the statistical analysis indicated the significance of the differences in the results obtained.

This was confirmed by the study of Shen *et al.* (2006) [1] when treating a number of bacterial strains with gum arabic extract, as it had inhibitory activity against them, including *S. aureus* and *Enterobacter* spp. and *K. pneumonia* and *E. coli*.

The results of the current study confirmed the appearance resistance of *E. coli*, *K. pneumonia*, and *Shigella* bacteria to the cold aqueous extract of *M. spicata* leaves when treated with the above concentrations, which resulted in the absence of inhibition zones, and this is what was stated in the study of Attol *et al.* (2022) [36]. Which confirmed the emergence of resistance to *E. coli* isolated from clinical samples using concentrations (0.25-8µg/mL) of both RHAC-ATH and RHAC-AMPH prepared chemical compounds. The study of Saleh *et al.* (2023) [37] also confirmed that *K. pneumonia* isolates It was resistant to mint leaf extract at all concentrations tested.

The study of Dwivedi *et al.* (2012) [38] confirmed the antimicrobial effectiveness of *M. arvensis* leaf extract using agar wells dilution and diffusion methods against various bacterial pathogens such as *S. aureus*, *P. vulgaris*, *E. coli*, *S. typhi*, and *P. aeruginosa*, and *K. pneumonia*, and the extract was effective at different concentrations (1:2, 1:4, 1:8) against most of the bacteria tested, with the exception of *K. pneumonia* and *E. coli*.

Many studies have focused on the antibacterial activity of mint leaf extracts, and the effectiveness of *M. arvensis* and nodal callus leaf extracts against *S. typhi*, *S. paratyphi*, *A. baumannii* and *P. mirabilis*, which confirmed that *M. arvensis* leaf extract has antibacterial activity against *Acinetobacter baumannii* with an inhibition zone of 19 mm (Johnson *et al.* 2011) [39].

Discussions

M. domestica is a pest spread all over the world and the dominant species of flies breed inside animal products, homes, restaurants and therefore the continuous movement of flies back and forth from feces (Or other animal waste) to food and drinking water exposes humans and animals to the risks of infection (Khamesipour *et al.* 2018) [24].

The feeding habits of house flies lead to the adhesion of pathogens to parts of its mouth, wings, legs, and other parts of its body, with which it returns to human homes and animal farms where it lives and completes its life cycle. The feces and vomit of the fly may act as a major team for the transmission of pathogens (Pava-Ripoll *et al.* 2015) [31].

This study clarified the importance of the house fly as a mechanical transmitter of high numbers of microorganisms that cause diseases to humans through the external surface

of the insect's body, especially bacteria that reflect the environment in which it lives. Many types of Gram-negative and Gram-positive pathogenic bacteria were isolated from the surface of the insect. The body of the house fly (Al-Maaly 2020) ^[3].

This study also showed that the dominant isolate is *E. coli* bacteria in this study, followed by *S. aureus* bacteria, as many studies have confirmed that *E. coli* and *S. aureus* bacteria are among the most common types isolated from house flies (Ahmed *et al.*, 2013) ^[1].

E. coli is one of the most common bacterial species isolated by Hasssan *et al.* (2022) ^[20]. The reason for this may be attributed to the ways in which house flies feed mainly on human and other animal waste, which is a rich source of these bacteria

E. coli was the most prevalent species of bacteria isolated (50%) in this study, as a similar pattern was observed in a study conducted in two hospitals in Calabar/ Nigeria (Akpan *et al.* 2017) ^[2], and these bacteria also evolved in that It becomes a multi-system pathogen that manifests in various forms as Enteropathogenic, Enterohaemorrhagic, Enterotoxigenic, Meningitis associated and Uropathogenic *E. coli*, and this explains why flies that harbor these pathogens pose a serious threat to society.

The effectiveness of mint leaf extract in showing inhibition zones against *Enterobacter* spp. and *S. aureus* may be due to the presence of active compounds, such as flavonoids, saponins, tannins, and alkaloids, which stimulate the generation of reactive oxygen types (ROS) and protein leakage, leading to damage to the bacterial membrane (Chetia and Saikia 2020) ^[40].

An investigation concerning *M. arvensis* leaf extract with concentrations of 120-200 µg/ml had an antibiotic impact on various clinically isolated bacteria such as *S. aureus*, *P. aeruginosa*, and *S. pyogenes*. The revealed findings had a similar impact to the common antibiotic kanamycin (Sharma *et al.*, 2013) ^[41].

The progress of bacterial resistance to the aqueous plant extract employed in the investigation is due to the existence of secondary plant elements, particularly phenolic elements that are known for their potent antimicrobial properties (Dwived *et al.*, 2012) ^[38].

There are different toxic impacts of various types of plant extracts against microorganisms and their difference depend on the different parts of the plant parts such as the leaf which represents the high source of compounds with biological activity against microorganisms (Thakur *et al.*, 2021) ^[42]. Similarly, Mancuso (2020) ^[43] identified secondary substances in GC-MS analysis reveals that the leaves comprise pharmaceutically significant compounds including alkaloids, flavonols, tannins, steroids, eucalyptol, and menthol. Finally, the water-distilled essential oils occurs in plants have also shown remarkable impact against pathogenic microorganisms (Horvath and Koscova 2017) ^[44].

Conclusions

- The housefly collected from the studied residential areas contributes to the infection of diseases caused by pathogenic bacteria and are potential vectors of a wide range of pathogenic organisms like *Staphylococcus*, *Enterococcus*, and *Enterobacteria* (*Shigella*, *Escherichia coli*, *Klebsiella*).

- *Klebsiella pneumonia* and *Staphylococcus aureus* are pathogenic that isolates from the house fly in the culture of the study are more dangerous and epidemiologically important because they are related to public health.
- Flies are more than a nuisance to the ease of an organism but also a source of serious health risks as mechanical vectors. Therefore, its living and reproduction environment must be controlled, and its population density must be reduced by adopting various methods of disease vector control.
- *M. spicata* plant is one of the plants that has medicinal importance as it contains many effective compounds of therapeutic importance against bacteria. This study examines the importance of the mint plant in treating two species of pathogenic bacteria, *Enterobacter* spp. and *S. aureus*, which works to reduce the inflammatory reaction in humans.

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References

1. Ahmed AS, Ahmed KM, Salih SS. Isolation and identification of bacterial isolates from houseflies in Sulaymanya city. J Eng Technol. 2013;31:24-33.
2. Akpan N, Anwan E, Antia U. Housefly (*Musca domestica*) as a carrier of pathogenic microorganisms in Hospital Environments in Calabar, Nigeria. J Sci Technol. 2017;24(2):193-199.
3. Al-Maaly NMH. Isolation and identification of some bacterial pathogens from flies in the animal farm. Plant Arch. 2020;20(1):1243-1246.
4. Al-Rahimy SK, Al-Khafagi AS, Al-Essa RA. Survey and diagnostic to the medical important species that belonging to Muscidae family in Kerbala Governorate-Iraq. AIP Conf Proc. 2023;2414:020009.
5. Malen SD. Serratia infections: from military experiments to current practice. Clin Microbiol. Rev. 2011;24(4):755-791.
6. Bozovic M, Pirolli A, Rango R. *Mentha suaveolens* Eheh. (Lamiaceae) essential oil and its main constituent piperitenone oxide: biological activity and chemistry. J Molecules. 2015;20:8605-8633; doi:10.3390/molecules20058605.
7. Kapp K, Püssa T, Oray A. Chemical composition and antibacterial effect of *Mentha* spp. grown in Estonia. Nat Prod Commun. 2020, 15(12). doi:10.1177/1934578X20977615.
8. Mezaal LA. Study of the effect of volatile oils from some plants on *Pseudomonas aeruginosa* isolated from burn infections. Msc Thesis, College of Science, University of Kerbala; c2013. p. 79.
9. Snoussi M, Noumi E, Trabelsi N, Flamini G, Papetti A, DeFeo V. *Mentha spicata* essential oil: Chemical composition, antioxidant and antibacterial activity against planktonic and biofilm cultures of *Vibrio* spp. strains. J Molecules. 2015;20:14402-14424.

10. Riöse L, Recio MC, Villar A. J Ethnopharmacol. 1987;21:139-152.
11. Deeb DM. Epizootic bacterial of house fly (*Musca domestica*) in animal farms. MSc Thesis. Tishreen University, Agriculture Engineering-Animal Production Specialty (Ruminant and Poultry); c2015. p. 72.
12. Al-Behadli NK, Al-Wazni WS, Alwan AH. Evaluation of some biological activities of Arabic gum (*Senegalia senegal*) aqueous extract *in vivo* and *in vitro*. AIP Conf Proc. 2020;2290:020021-9.
13. Chessbrough M. District Laboratory Practice in Tropical Countries part 1. 2nd ed. Cambridge University Press. 2005, 454.
14. Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney Practical Medical Microbiology Chapter 4. Tests for the Identification of Bacteria. New Delhi: Elsevier; c2007.
15. Matuschek E, Brown DFJ, Kahlmeter G. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in Routine Microbiology laboratories. Clin Microbiol Infect. 2014, 20(4).
16. Getachew S, Gebre-Micheal T, Erko B, Balkew M, Medhin G. Non-biting cyclorrhaphan flies (Diptera) as carriers of intestinal human parasites in slum areas of Addis Ababa, Ethiopia. Acta Trop. 2017;103:186-94.
17. Hassan RS, Aliyu SH, Adam AS, Mienda BS, Muhammad AS. Prevalence and antibiotic susceptibility patterns of *Staphylococcus aureus* in locally pasteurised cow-milk sold at dutse metropolis, Jigawa state, Nigeria. Int. J Biol. Sci. 2021;3(1):29-33. DOI: 10.33545/26649926.2021.v3.i1a.26
18. Graczyk TK, Knight R, Tarnang L. Mechanical transmission of human protozoan parasites by insects. Clin Microbiol Rev. 2001;18(1):128-132.
19. Graczyk TK, Knight R, Gilman R, Cranfield M. The role of non-biting flies in the epidemiology of human infectious diseases. Microbes Infect. 2005;3:231-235.
20. Hasssan AO, Obeagu EI, Olamijewon PB. Evaluation of different microbial pathogens associated with the external surfaces of houseflies and to determine the antibiotic susceptibility pattern of recovered bacterial pathogens in Owo. Int J Curr Res Med Sci. 2022;8(1):1-13.
21. Hogiette JR, Amendt J. Flies. In: Bonnetoy X, Kampen H, Sweeney K, editors. Public Health Significance of Urban Pests. WHO Regional Office for Europe; c2008. p. 209-237.
22. Hussein S, John L. House fly. Featured creatures. University of Florida; c2017. Available from: http://entnemdept.ufl.edu/creatures/urban/flies/house_fly.
23. Karagöz A, Acar S, Körkoca H. Characterization of *Klebsiella* isolates by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and determination of antimicrobial resistance with VITEK 2 advanced expert system (AES). Turkish Journal of Medical Sciences. 2015;45(6):1335-1344. <https://doi.org/10.3906/sag-1401-32>.
24. Khamesipour F, Lankarani KB, Honarvar B, Kwenti TB. A systematic review of human pathogens carried by the housefly (*Musca domestica*). BMC Public Health. 2018;18:1049.
25. Ibekwe VI, Nnanyere NF, Akujobi CO. Studies on antibacterial activity and phytochemical qualities of extracts of orange peels. International Journal Environmental Health and Human Dev. 2001;2(1):14-46.
26. Shen D, Wu Q, Wang M, Yang Y, Lavoie EJ, Simon JE. Determination of the predominant catechins in *Acacia catechu* by liquid chromatography/electrospray ionization-mass spectrometry. Journal of Agriculture Food Chemistry. 2006;54(9):3219-3224.
27. Nashiro Ami, Stoppa GFZ, Cardoso Alsp, Tessri ENC, Castro Agm. Serovars of *Salmonella* spp. isolated from broiler chickens and commercial breeders in diverse regions in Brazil from July 1997 to December 2004. Brazilian Journal of Poultry Science. 2005;3:195-198.
28. Nazari MT, Mehrabi SM, Alikhani MY. Bacterial contamination of adult house flies (*Musca domestica*) and sensitivity of these bacteria to various antibiotics, captured from Hamadan City, Iran. Journal of Clinical and Diagnostic Research. 2017;11(4):dc04-dc07. DOI:10.7860/JCDR/2017/23939.9720.
29. Nmorsi OP, Agbozele G, Ukwandu NC. Some aspects of epidemiology of filth flies: *Musca domestica*, *Musca domestica* vicina, *Drosophila melanogaster*, and associated bacteria pathogens in Ekpoma, Nigeria. Vector-Borne Zoonotic Diseases. 2007;7:107-117.
30. Olsen AR. Regulatory action criteria for filth and other extraneous material III Review of flies and foodborne enteric disease. Regulatory Toxicology and Pharmacology Journal. 1998;28:199-211.
31. Pava-Ripoll M, Miller AK, Tall BD, Keys CE, Ziobro GC. Ingested *Salmonella enterica*, *Cronobacter sakazakii*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*: Transmission dynamics from adult house flies to their eggs and first filial (F1) generation adults. BMC Microbiology. 2015;15:150.
32. Quinn PJM, Carter E, Carter Markey B, Carter GR. Clinical veterinary microbiology. 6th ed. Mosby ANIMP Wolf, London; c2004. p. 13-17.
33. Sarwar M. Life history of house fly (*Musca domestica* Linnaeus (Diptera: Muscidae)), its involvement in disease spread and prevention of vector. International Journal for Research in Applied and Natural Science. 2016;2(7):31-35.
34. Service M. Medical Entomology for Students. 5th edition. Cambridge University Press; c2012. p. 303.
35. Simone CM, Jaine DGOSHP. Formation of biofilms by *Staphylococcus aureus* on stainless steel and glass surfaces and its resistance to some selected chemical sanitizers. Brazilian Journal of Microbiology. 2007;38:538-543.
36. Attol DH, Mihsen HH, Jaber SA, Al-Wazni WS, Eesa MT. Synthesis of organic functionalized Silica from Rice Husk as an antibacterial agent. Springer Nature. 2022, 22(02194-5).
37. Saleh BH, Al-ugaili DN, Oraibi AG, Ibrahim RA. Antibacterial and cytotoxic activity of *Mentha arvensis* L. leaves Methanolic extract *in vitro*. Journal Pure Applied Microbiology. 2023;17(2):1221-1230. doi:10.22207/JPAM.17.2.53.
38. Dwivedi D, Khandelwal G, Patidar RK, Singh V. Antimicrobial activity of *M. arvensis* against clinical isolates of human cariogenic pathogens-an *in-vitro*

- study. International Journal of Pharmaceutical Science Research. 2012;3(5):1355-1360.
39. Johnson M, Wesely EG, Kavitha MS, Uma V. Antibacterial activity of leaves and inter-nodal callus extracts of *M. arvensis* L. Asian Pacific Journal of Tropical Medicine. 2011;4(3):196-200. doi:10.1016/s1995-7645(11)60068-0.
 40. Chetia J, Saikia LR. Antimicrobial activity assay and phytochemical study of different aerial parts of *M. arvensis* L. collected from Dibrugarh, Assam. Journal Science Research. 2020;64(1):103-112. doi:10.37398/JSR.2020.640115.
 41. Sharma U, Agnihotri R, Ahmad S, Mahajan S, Sharma R. Antibacterial activity of some medicinal plants of family Lamiaceae from Braj region. Global Journal of Medicinal Plant Research. 2013;1:72-76.
 42. Thakur S, Walia B, Chaudhary G. *Mentha arvensis* (Pudina): A Review based upon its medicinal properties. Research Journal of Pharmacognosy and Phytochemistry. 2021;13(3):143-148. doi:10.52711/0975-4385.2021.00024.
 43. Mancuso M. The antibacterial activity of *Mentha*. Herbs spices. Intechopen; c2020. doi:10.5772/intechopen.92425.
 44. Horvath P, Koscova J. *In vitro* antibacterial activity of *Mentha* essential oils against *Staphylococcus aureus*. Folia Veterinaria. 2017;61(3):71-77. doi:10.1515/fv-2017-0030
 45. SAS. Statistical Analysis System, User, Guide. Statistical. Version 9.1th ed. SAS. Institute Incorporated Cary. N.C. USA; c2012.
 46. Ghali DW. Association of the IL-4 gene polymorphisms with developing of renal diseases and bacterial urinary tract infections. 2007; MSc Thesis. University of Kerbala, College of Science; c2022. p. 114.