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HONEY AS ANTIBACTERIAL AGENT USED AGAINST *Bacillus Spp.* ISOLATED FROM LOCALLY PRODUCED JUICE

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ABSTRACT

The identification and characterization of *Bacillus spp.* will provide insights into the potential sources of contamination and contribute to developing effective prevention measures. Therefore, the objective of the present study was to investigate and diagnose the prevalence of *Bacillus spp.* contamination of locally produced juices, as well as to evaluate the antibacterial ability of honey against *Bacillus spp.* One hundred random samples were collected from juices locally made in different places like *Ta'meem, Al-Andalus, Al-Ramadi Market, Al-Mal'ab, and Al-Sufaya*. Various agricultural media were used to isolate strains of *Bacillus spp.* and determined based on biochemical and cultural characteristics. Additionally, to assess the antibacterial properties of honey, samples were used at (75, 50, and 25%) dilutions and undiluted. The results showed that different species of *Bacillus spp* highly contaminated the locally produced juices. The most prevalent species were *B. cereus*, *B. subtilis*, and other *Bacillus spp.* the most contaminated types of juice were grape and banana juices. The results also showed that natural honey exhibited the highest antibacterial activity against *B. cereus.* At the same time, it didn't affect *B. coagulans* and *B. sphaericus*. The findings will help raise awareness about the quality of juice products in the region and provide insights into potential public health risks associated with *Bacillus* spp. contamination. Honey can be used instead of other medications to treat various diseases, particularly those brought on by bacteria resistant to antibiotics.

INTRODUCTION

Juice is the liquid phase or concentrate of liquids derived from one or multiple fruits (Sharif and Abdulqader, 2011). Fruit juices are used for their medicinal importance and nutritional value; the juices contain many vitamins, minerals, and beneficial fibers for the body (Durak *et al*., 2010). Fruit juices characterized by their distinct taste and flavor, which have made them desirable and preferred by everyone to consume most of the time, especially during the hot summer months, to compensate for the body's loss of fluids and provide a refreshing feeling (Vantarakis *et al*., 2011). Fruit juices may be polluted during the fruit growth and harvesting, harmful microorganisms have the potential to infiltrate fruit surfaces, which increases fruit contamination, or the outer peel of some fruits may be contaminated with soil and animal feces, which leads to juice contamination (Iqbal *et al*., 2015; Sedeeq *et al*., 2022). Furthermore, the contamination of natural juices can come from various sources, such as during the preparation of juices, starting from the water used during washing, the utensils used for cutting in the case of natural juices, as well as the hands of the workers and the containers in which the liquid is stored, such as pitchers and bags (Esmael *et al*., 2023). Juices may be polluted with microorganisms from machines, raw materials, and environments, as well as during juice transportation and storage (European Union, 202; Tenea *et al*., 2023). Microorganisms responsible for juice contamination are aerobic and spore-forming bacteria, specifically Gram-positive stain belonging to *Bacillus* spp. These commonly found in various environments, including food products (Sharif and Abdulqader, 2011; Jesus *et al*., 2021). Some *Bacillus* species have the potential to cause foodborne illnesses, making it crucial to assess their presence and levels of contamination in popular consumables, such as fruit juices (Ahmed and Al-Taleb, 2018; Dubey *et al*., 2021). Regardless of their types, most natural juices are not subjected to sterilization or pasteurization processes (Dietrich *et al*., 2021). The fresh taste of juices, particularly those made by street vendors, holds more incredible allure for consumers (Iqbal *et al*., 2015). Therefore, they are an essential source of various microorganisms, including bacteria and fungi (Al-Qudah *et al*., 2023). This makes it one of the most important sources of food poisoning, as foods with high sugar content and low acidic (pH) juices may be exposed to contamination by acid-resistant bacteria, which produce some gases and acids such as Clostridium, *Bacillus*, Kebsiella, E. coli, Vibrio cholera, Albicans and Leuconostoc (Sharif and Abdulqader, 2011; Vantarakis *et al*., 2011; Jovanovic *et al*., 2021).

Bacillus spp. constitutes approximately 8% of the contamination in some fresh fruit juices in Nineveh province (Sharif and Abdulqader, 2011). Additionally, *Bacillus* spp. was responsible for 39.5 % in 2017 of food poisoning cases in the United States (Choi *et al*., 2020), and approximately 42% in Asian countries (Sharif and Abdulqader, 2011). In 1984, Cerny and his group isolated strains of the *Bacillus* type from apple juice. They explained can grow in acidic environments at a pH of (5.5 to 2.5) (Cerny *et al*., 1984). Microorganisms resistant to antibiotics significantly jeopardizing public health (Sharif and Abdulqader, 2011; Tenea *et al*., 2023). Few new antibiotics are being created in contrast to the increasing rates of bacterial resistance (Iqbal *et al*., 2015). The creation of new antibacterial tactics is urgently needed. In 2018, fresh Garlic juice inhibited Pseudomonas aeruginosa virulence factors in Anbar city (Lahig *et al*., 2018). Egyptians and Greeks have used honey to heal diseases and inhibit the growth of bacteria (Lotfi, 2008; Gundogan and Avci, 2014). The antibacterial property of honey is caused by a presence of an antioxidant, cenamic acid, and flavonoids (Rahman *et al*., 2010). According to the honey's geographic origin and floral source, there may be noticeable changes in the inhibitory activity of the substance (Lusby *et al*., 2005). This study was conducted as a local study in AL-Ramadi city to study the contamination of locally produced juice with spore-forming bacteria due to their direct importance on human health and their potential to cause food poisoning. Due to *Bacillus* developing higher antibiotic resistance, treating infectious diseases caused by this bacterium has become challenging. As a result, the present research aims to assess the antibacterial effectiveness of a natural honey sample against the isolated bacteria, given its widespread availability and ease of use.

MATERIALS AND METHODS

Collecting Samples, Isolating *Bacillus spp.*, and Enumerating Isolates

Randomly one hundred samples were collected from various natural juices prepared from natural fruits by street vendors, these juices are orange, apple, banana, grape, and cocktail from different places in the city of Ramadi, which are Ta'meem, Al-Andalus, Al-Ramadi Market, Al-Mal'ab, and Al-Sufaya region. The samples were transferred directly to the laboratory using sterilized containers under cool and sterile conditions. The fruit juice samples were examined in the bacteriology laboratory in AL-Ramadi Teaching Hospital. The pH of each sample was measured directly using a pH meter, and decimal dilutions of the juice samples were prepared by adding (1 mL) of each juice sample to (9 mL) of sterilized distilled water, resulting in dilutions of 10-6. Dilutions of 10-5 and 10-6 were subjected to the pasteurization process in a water bath at 80 ºC for 30 minutes to kill non-spore-producing green cells and isolates of *Bacillus* species. We used growth media provided by Hi Media / India, which are as follows:

- Nutrient agar medium.
- Nutrient agar medium supplemented with 10% egg yolk (lipid degradation).
- Pseudomonas water agar medium (indole production).
- Glucose phosphate agar medium (methyl red and Voges-Proskauer test).
- Nutrient agar medium with starch (starch hydrolysis).
- SIM medium for motility and H2S and indole production.
- Nitrate broth medium (nitrate reduction).
- Simon's citrate medium (citrate utilization).
- Red phenol broth medium with maltose sugar (maltose sugar fermentation).
- Nutrient gelatin medium (gelatin hydrolysis).

All agricultural sectors attended as stated in (Koneman *et al*., 1997). (1) ml of the last two dilutions, 10-5 and 10-6 were transferred to sterilized Petri dishes. Nutrient agar medium was added gently with stirring, and the dishes were incubated at 37 ºC for 24 hours. Thin smears of bacteria were prepared from the growing colonies possessing characteristics of *Bacillus* colonies, which appeared as significant, branched, flat colonies. The spots were stained using Gram staining to study and confirm the microscopic features. Colonies that appeared as positive rods with the Gram stain were inoculated onto nutrient agar media to complete their diagnosis using their biochemical characteristics. The diagnostic scheme for *Bacillus* spp., as mentioned by (Koneman *et al*., 1997), is illustrated in Figure (1). In this study, 265 isolates of the *Bacillus* spp. were obtained from juice samples that were laboratory tested.

Determination of Anti-bacterial Activity of Honey

Natural Honey sourced from the market was gathered, produced by various commercial companies using domesticated bees, and made available for sale. The honey samples were then carefully stored in a dark location at a temperature range of 23-25°C. Honey samples were tested for antibacterial properties at (100, 75, 50, and 25) % dilutions. The antibacterial activity was evaluated against various isolates of *Bacillus* species using the disc diffusion method (Bauer and Kirby, 1966), following the National Committee for Clinical Laboratory Standards guidelines.

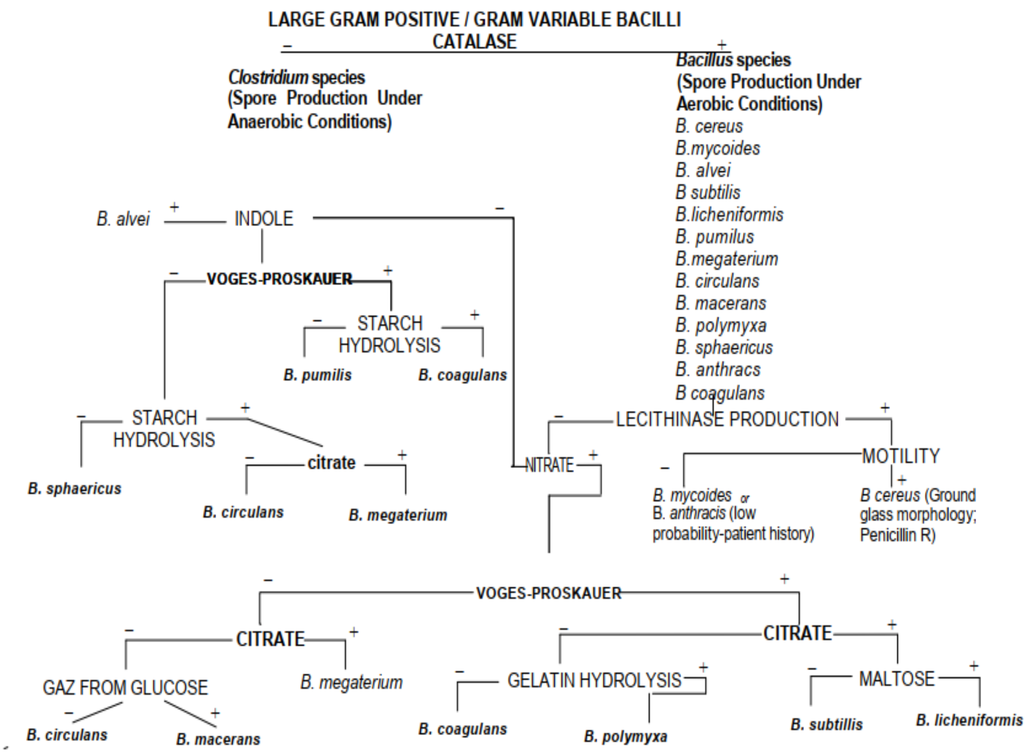

Figure (1): The diagnostic of *Bacillus* spp. as mentioned in (Koneman *et al*., 1997)

Filter paper discs were saturated with (25) µl of each honey dilution and Streptomycin (utilized as a positive control). The tested organism cultures were streaked on nutrient agar plates using a sterile cotton swab. The discs were put on the plates and incubated for 24 hours at 37°C. The widths of the obtained inhibition zones were measured as reported by (CLSI, 2006).

Statistical Analysis was performed by SPSS, version-25(Yamin and Kurniawan. 2009). Values were expressed as means ± standard deviation. Associations between *Bacillus* spp. and the types of the juices were examined by Chi-square analysis. A p-value of 0.05 or less was recognized as statistical significance.

RESULTS AND DISCUSSION

The results show that 265 isolates of *Bacillus spp*. were obtained from juice samples, which are distributed in the following tables:

Table (1) indicates a significant difference in the percent of *Bacillus spp*. in local apple juice among the studied regions, except for *B. coagulans*. The highest percent in local apple juice was 33.3% of *B. cereus* and *B. coagulans* in Al-Mal'ab and Al-Sufaya; *B. subtilus* showed the same percent in Al-Mal'ab *and B. mycoides* in Al-Sufaya. The lowest percent was 14.2% of *B. coagulans*, *B. sphaericus*, and *B. mycoides* in the Al-Ramadi Market region. Meanwhile, in local apple juice, *B. sphaericus* appeared only in the Al-Andalus and Al-Ramadi Market regions.

Table (1): A comparison of *Bacillus* spp. isolated from locally produced apple juices at different places of Al-Ramadi city.

No.	Juice Type	Isolation region	Isolates No.	*B. cereus* NO.(%)	*B. subtilis* NO.(%)	*B. coagulans* NO.(%)	*B. sphaericus* NO.(%)	*B. mycoides* NO.(%)
1-	Local apple Juice	Ta'meem	14	4 (28.5)	4 (28.5)	3(21.5)	0 (0.0)	3(21.5)
2-		Al-Andalus	8	2 (25)	2 (25)	2 (25)	2(25)	0 (0.0)
3-		Al-Ramadi Market	14	4 (28.5)	4 (28.5)	2 (14.2)	2 (14.2)	2(14.2)
4-		Al-Mal'ab	9	3 (33.3)	3(33.3)	3 (33.3)	0 (0.0)	0 (0.0)
5-		Al-Sufaya	6	2(33.3)	0 (0.0)	2 (33.3)	0 (0.0)	2 (33.3)
Total			51	15	13	12	4	7
P-value				0.012*	0.003*	0.054	0.0021*	0.045*

Table (2) shows a significant difference in the percent of *Bacillus spp*. in local grape juice among studied places. The highest contamination in local grape juice for *B. cereus* was (55.5%) in Al-Sufaya and (35.3%) in the Ta'meem region. The lowest contamination percent was 10.5% for *B. mycoides* in the Al-Ramadi Market region. On the other hand, no contamination was observed for *B. sphaericus* in local grape juice in Ta'meem, Al-Mal'ab, and Al-Sufaya regions.

Table (2): A comparison of *Bacillus spp*. isolated from local grape juice at different places of Al-Ramadi city.

No.	Juice Type	Isolation region	*Isolates No.*	*B. cereus* NO.(%)	*B. subtilis* NO.(%)	*B. coagulans* NO.(%)	*B. sphaericus* NO.(%)	*B. mycoides* NO.(%)
1-	Local Grape Juice	Ta'meem	17	6 (35.3)	5 (29.4)	4 (23.5)	0 (0.0)	2 (11.7)
2-		Al-Andalus	12	4 (33.3)	2(16.7)	2(16.7)	2(16.7)	2(16.7)
3-		Al-Ramadi Market	19	5 (26.3)	6 (31.5)	4 (21)	2 (10.5)	2 (10.5)
4-		Al-Mal'ab	8	2 (25)	2 (25)	2 (25)	0 (0.0)	2 (25)
5-		Al-Sufaya	9	5 (55.5)	2 (22.2)	2 (22.2)	0 (0.0)	0 (0.0)
Total			65	22	17	14	4	8
P-value				0.002	0.0036	0.014	0.017	0.024

Table (3) indicates a significant difference in the percent of *Bacillus spp*. in local banana juice, except for *B. coagulans* and *B. cereus*. The highest percentage of natural banana juice (41.6%) was for *B. subtilis* in Al-Malab, and the lowest appearance (10%) was in the Ta'meem region. As for *B. coagulans*, it showed the highest occurrence in AL-Andalus (38.5%) and appeared in the same percentage (25%) in Al-Mal'ab and Al-Sufaya regions. *B. sphaericus* had the highest occurrence in Ta'meem (33.3%) and the lowest occurrence in Al-Andalus (14.3%).

Table (3): A comparison of *Bacillus spp*. isolated from local banana juices at different places of Al-Ramadi city.

No.	Juice Type	Isolation region	*Isolates No.*	*B. cereus* NO.(%)	*B. subtilis* NO.(%)	*B. coagulans* NO.(%)	*B. sphaericus* NO.(%)	*B. mycoides* NO.(%)
1-	Local banana Juice	Ta'meem	20	2(10.0)	2 (10.0)	6 (30)	4 (20)	6 (30)
2-		Al-Andalus	13	2(15.3)	2 (15.3)	5(38.4)	2 (15.3)	2 (15.3)
3-		Al-Ramadi Market	18	3(16.6)	3(16.6)	4(22.2)	4 (22.2)	4 (22.2)
4-		Al-Mal'ab	12	0 (0.0)	5 (41.6)	3 (25)	2(16.6)	2(16.6)
5-		Al-Sufaya	8	0 (0.0)	2 (25)	2 (25)	2 (25)	2(25)
Total			71	7	14	20	14	16
P-value				0.056	0.032*	0.055	0.036*	0.025*

Table (4) illustrates a significant difference in the percentage of *Bacillus spp*. in local orange juice. The highest percentage of *B. subtilus* was found in local orange juice in Ta'meem (75%) and 50% in Al-Sufaya. *B. cereus* showed the highest percentage in Andalus (33.3%). *B. coagulans* appeared only in Al-Mal'ab (25%). *B. mycoides* appeared in all the studied regions except in the Al-Mal'ab region, and it appeared in Al-Sufaya with a percentage of 50%.

Table (4): A comparison of *Bacillus* spp. isolated from local citrus juice at different places of Al-Ramadi city.

No.	Juice Type	Isolation region	*Isolates No.*	*B. cereus* NO.(%)	*B. subtilis* NO.(%)	*B. coagulans* NO.(%)	*B. sphaericus* NO.(%)	*B. mycoides* NO.(%)
1-	Local citrus Juice	Ta'meem	8	0 (0.0)	6 (75)	0(0.0)	0 (0.0)	2 (25)
2-		Al-Andalus	6	2(33.3)	2(33.3)	0(0.0)	0 (0.0)	2(33.3)
3-		Al-Ramadi Market	12	2(16.6)	4(33.3)	0(0.0)	2(16.6)	4(33.3)
4-		Al-Mal'ab	8	2 (25)	2 (25)	2(25)	2 (25)	0 (0.0)
5-		Al-Sufaya	4	0 (0.0)	2 (50)	0(0.0)	0 (0.0)	2 (50)
Total			38	6	16	2	4	10
P-value				0.008*	0.021*	0.037*	0.025*	0.017*

Table (5) shows a significant difference in the proportion of *Bacillus* spp. in local cocktail juice, except for *B. sphaericus*. The highest percentage of *B. cereus* was observed in AL-Andalus, reaching 50 %, and it did not show any contamination in Al-Mal'ab or AL-Sufaya. *B. subtilis* had the highest percentage (50%) in the AL-Andalus and Al-Sufaya regions, and it did not show any contamination in the Ta'meem and Al-Mal'ab regions. Meanwhile, *B. coagulans* appeared only in Al-Ramadi Market with 18.1% and Al-Mal'ab with 22.2%. As for *B. sphaericus*, it recorded a non-significant increase in Ta'meem and Al-Sufaya by 50%. The highest percentage of *B. mycoides* was observed in the Al-Mal'ab region, reaching 44.4 %.

Table (5): A comparison of *Bacillus spp*. isolated from local cocktail juice at different places of Al-Ramadi city.

No.	Juice Type	Isolation region	*Isolates No.*	*B. cereus* NO.(%)	*B. subtilis* NO.(%)	*B. coagulans* NO.(%)	*B. sphaericus* NO.(%)	*B. mycoides* NO.(%)
1-	Local cocktail Juice	Ta'meem	8	2 (25)	0(0.0)	0 (0.0)	4 (50)	2 (25)
2-		Al-Andalus	6	3 (50)	3 (50)	0 (0.0)	0 (0.0)	0 (0.0)
3-		Al-Ramadi Market	11	2(18.1)	2(18.1)	2(18.1)	3(27.2)	2(18.1)
4-		Al-Mal'ab	9	0 (0.0)	0(0.0)	2(22.2)	3(33.3)	4(44.4)
5-		Al-Sufaya	6	0 (0.0)	3 (50)	0 (0.0)	3 (50)	0 (0.0)
Total			40	7	8	4	13	8
P-value				0.033*	0.036*	0.024*	0.056	0.048*

Table (6) shows the antibacterial activity of diluted honey against *Bacillus spp*. isolated from fruit juices. The honey sample used in this study exhibited antibacterial agent against *B. cereus*, *B. subtilis*, *B. coagulans*, *B. sphaericus*, and *B. mycoides*. Streptomycin served as a positive standard, and all the bacterial samples displayed inhibition zones when exposed to this antibiotic. Notably, Natural Honey exhibited the highest antibacterial effect against *B. cereus*. No activity was found against *B. coagulans* and *B. sphaericus* at a 25% dilution. Additionally, no activity was found against *B. subtilis* and *B. mycoides* at 25% and 50% dilutions of natural honey, respectively. Biological Honey samples showed antibacterial activity against all isolates at 100% and 75% dilutions. Perhaps because this particular brand of honey is so pure.

The results in Tables (1, 2, 3, 4, and 5) agree with (Sharif *et al*., 2011), who isolated species of *Bacillus* from locally prepared beverages in different places in Mosul city. Also, these results agree with (Vantarakis *et al*., 2011; Iqbal *et al*., 2015; Van Luong *et al*., 2021), who found that Microorganisms, which may pose potential risks to public health, are present in products made from fresh fruits and vegetables. These results disagree with (Iqbal *et al*., 2015) who indicated that bacteria were most frequently associated with citrus juice and referred to bacterial count as low for some unpasteurized fruit juices and comparatively higher for others. Fruit juices are well-liked drinks vital to human nutrition (Tenea *et al*., 2023).

Table (6): The antibacterial efficacy of natural honey against *Bacillus* isolates is presented as mean zone of inhibition (mm²).

Bacillus SPP.	Honey concentrations				+ve Control Streptomycin
	100%	75%	50%	25%	
B. cereus %	447±1.26	325±3.4	238±1.14	116±1.04	845±5.11
B. subtilis %	169±2.14	120±1.24	-	-	714±3.14
B. coagulans %	288±1.06	232±0.23	123±1.24	-	722±1.7
B. sphaericus %	357±1.73	166±2.42	128±2.09	-	262±3.6
B. mycoides %	128±2.1	172±1.9	-	-	138±1.9

- means the absence of inhibition. +ve: means positive control.

Previously, it was believed that *B. cereus* was the only type of *Bacillus spp*. responsible for human food poisoning, it produces toxins that can lead to diarrhea and vomiting. However, some studies have revealed the presence of other species of *Bacillus* and their role in causing poisoning, such as *B. mycoides*, *B. coagulans*, *B. lichneformis*, *B. subtilis*, and *B. sphaericus* (Sharif *et al*., 2011). *Bacillus spp*. are found in various habitats, including soil, water, and the gastrointestinal tracts of animals (Iqbal *et al*., 2015; Tenea *et al*., 2023). Our results generally indicated that grape and banana juices were the most contaminated juice types. These juices may be exposed to contamination from various sources, including dust, inadequate fruit washing, contamination during juice manufacturing, the use of unsterilized utensils or machines, the addition of additives, and the hands of workers. Also, in their artificial and natural types, apple juice and cocktails were contaminated with bacteria spores, which resist high temperatures and pasteurization processes. The contamination of these juices with such types of bacteria may result from residues of soil that may remain on the surfaces of the fruits used in juice production. Sugar used in sweetening could also be a source of contamination (Sharif *et al*., 2011; Choi and Kim, 2021). As for the orange juice was found to be the least contaminated, this may be attributed to the presence of citric acid and vitamin C, as well as the significant presence of some antioxidants in such juices (Sharif *et al*., 2011; Iqbal *et al*., 2015). The increased bacterial numbers resulted from inadequate hygiene conditions, leading to food spoilage and the risk of foodborne diseases (AL-Ramathny, 2021). These results contradicted those of results by (Jesus *et al*., 2021), who observed that the bacterial counts in fruit juices were within acceptable levels. To the best of my knowledge, this is the first study conducted in AL-Ramadi city concerning the microbiological quality of fruit juices.

Fruit juices are frequently contaminated with germs that may withstand a variety of storage environments and processing methods, such as certain spores of *Bacillus spp*.; the ability of *Bacillus* to survive in acidified vegetable products poses a serious threat to the consumers because of previously documented outbreaks associated with fruit juices (Iqbal *et al*., 2015; Esmael *et al*., 2023). The enhanced acid resistance they establish could result in their ability to survive for extended periods within the digestive system, heightening the disease risk. Moreover, it is essential to consider that microbial growth in acidified environments triggers a cross-protection response against heat, which should be considered while designing pasteurization processes for acidic foods (Deanna and Jeffrey, 2007). It is crucial to establish recommendations to avoid possible food poisoning from juices containing harmful fungi or bacteria. To safeguard consumers from accidental illnesses, juice-producing companies should be subject to monitoring through a series of tests, including the microbiological assessment of all equipment used in the production process, in addition to the final product (Vantarakis *et al*., 2011; Gundogan and Avci, 2014).

Also, this research exposed the antibacterial properties of different concentrations of natural honey samples against *Bacillus spp*., as shown in Table (6). The antibacterial activity of honey samples against other bacteria has been demonstrated in several studies (Badawy *et al*., 2004; Gomashe *et al*., 2014), indicating that honey might be utilized as an alternative to antibiotics to treat various diseases. In the current study, honey samples showed antibacterial activity against *B. subtilis*, *B. coagulans*, *B. sphaericus*, and *B. mycoides* in undiluted, 75, and 50% concentrations, respectively. However, the antibacterial activity started to decline with decreasing the dilution percentage. Numerous researchers have demonstrated that honey exhibits antibacterial action in doses ranging from 3% to 50% and more significant (Wilkinson and Cavanagh, 2005; Lusby *et al*., 2005). The concentration of honey affects antibacterial action; at increasing concentrations, honey becomes more effective as an antibacterial agent. It has been demonstrated that the concentration of honey and the kind of bacteria impact honey's bactericidal effect (Basualdo *et al*., 2007). Less than 18% of honey is water, with the remaining 80% being primarily glucose and fructose, with some maltose and sucrose (Hamouda and Marzouk, 2011). Microorganisms cannot taint honey due to osmotic stress brought on by high sugar concentration and low moisture content. Substances besides sugar are in charge of the antibacterial activity at greater dilutions. Honey, made from glucose by glucose oxidase, contains H2O2, which was shown to be a significant antibacterial component in the 1960s (Iqbal *et al*., 2015; Sultan and Yassen, 2018).

Different honey, however, has demonstrated antibacterial activity because of non-peroxide components. Recently, the antibacterial chemicals bee defensin-1 and methylglyoxal have been found in honey. It has been established that honey's low pH (3.2 - 4.5) contributes to its antibacterial activity (Kwakman *et al*., 2010). Pathogenic bacteria were discovered to be present in significant concentrations in every fruit juice sold by the roadside (Iqbal *et al*., 2015). Honey can also be added to fruit juices as a flavoring or sweetener as a substitute, protecting the fluids from impurities thanks to its antibacterial characteristics (Ebisu *et al*., 1988; Oussama *et al*., 2023). The usage of honey in the commercial non-alcoholic beverage sector has just recently increased, beginning in 1990. In Japan, more than 40 new honey beverages were created, and honey serves as both a flavor enhancer and sweetener in different fruit juices. Apple juice is also employed to categorize fruit juice (Ebisu *et al*., 1988; Vantarakis *et al*., 2011).

CONCLUSIONS

The natural juice was polluted with several *Bacillus* species. The presence of *Bacillus* species in juice is generally seen as undesirable since some strains of the bacterium can degrade food or release toxins that might be dangerous if taken in excessive numbers. These findings can help producers, consumers, and regulatory agencies make wise choices about the consuming and creating of safe, high-quality juice products. The fruit juices can be enhanced with honey, which serves both as a flavor enhancer and a natural sweetener. This combination not only adds a delightful taste but also the antibacterial activity of honey, safeguarding the juices against any potential contaminants. Also, honey can be used as an alternative for treating various of diseases, particularly those brought on by bacteria that are resistant to antibiotics.

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CONFLICT OF INTEREST

The author declares no conflicts of interest regarding the publication of this article.

العسل كعامل مضاد بكتيري استخدم ضد بكتريا *Bacillus* المعزولة من العصائر المنتجة محلياً

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الخلاصة

تحديد وتوصيف *Bacillus spp.* سيوفر رؤى حول المصادر المحتملة للتلوث ويساهم في تطوير تدابير وقائية فعالة. لذلك، كان الهدف من هذه الدراسة هو التحقيق في انتشار *Bacillus spp* في العصائر المصنعة محليًا في مناطق مختلفة من مدينة الرمادي، بالإضافة إلى تقييم قدرة العسل المضادة للبكتيريا *Bacillus spp*. تم جمع مائة عينة عشوائية من عصائر طبيعية متنوعة من مناطق مختلفة في مدينة الرمادي وهي (منطقة التأميم، سوق الرمادي، منطقة الملعب، حي الاندلس ومنطقة الصوفية). استخدمت وسائط زراعية مختلفة لعزل سلالات *Bacillus spp*. وتم تحديدها بناءً على الخصائص البيوكيميائية والزرعية. بالإضافة إلى ذلك، لتقييم فعالية العسل المضادة للبكتيريا، تم استخدام عينات العسل بتخفيف (75، 50، 25٪) وكذلك العسل

غير مخفف. أظهرت النتائج أن العصائر المحلية كانت عالية التلوث من قبل أنواع مختلفة من *Bacillus spp.* وكانت الأنواع الأكثر انتشارًا هي *B. cereus* تليها *B. subtilis* ، ثم أنواع *Bacillus spp* الأخرى. وكان أكثر أنواع العصير تلوثاً هو عصير العنب وعصير الموز مقارنة بالعصائر الاخرى. كما أظهرت النتائج أن العسل الطبيعي أظهر أعلى فعالية كمضاد للجراثيم ضد بكتيريا *B. cereus*. لم يتم العثور على أي نشاط ضد *B. coagulans* و *B. sphaericus* بتخفيف 25٪. ستساعد النتائج في رفع مستوى الوعي حول جودة منتجات العصير في المنطقة وتقديم رؤى حول مخاطر الصحة العامة المحتملة المرتبطة بتلوث بانواع *Bacillus spp*. يمكن استخدام العسل بدلاً من الأدوية الأخرى لعلاج مجموعة متنوعة من الأمراض، لا سيما تلك التي تسببها البكتيريا المقاومة للمضادات الحيوية.

الكلمات المفتاحية: بكتريا، العنب، عزلات، مضاد بكتري، تلوث.

REFERENCES

Ahmed, S., & Al-Taleb, M. (2018). Effect of Red Cabbage Crude Extracts on E. coli and *Staph. aureus* Growth. *Mesopotamia Journal of Agriculture*, 46(3), 229-238. https://doi.org/10.33899/magrj.2018.161503.

Al-Qudah, M.A., Rahahleh, R.J., Alraei, W.Y., Aljaraedah, T.Y., Abu-Harirah, H.A., Amawi, K., and El-Qudah, J.M. (2023). Evaluation of the Antimicrobial Activity of Bacteriocin-Producing Lactic Acid Bacteria Isolated from Human Intestine against Pathogenic Microorganisms. *Tropical Journal of Natural Product Research*, 7(6), 3182-3190. http://www.doi.org/10.26538/tjnpr/v7i6.18.

AL-Ramathny, Z. F. (2021). Economic Analysis of Grape Production Farms in Salah El-Din Governorate for The 2019 Productive Season (Balad District As A Model). *Mesopotamia Journal of Agriculture*, 49(4), 1-10. https://doi.org/10.33899/magrj.2021.131238.1140.

Badawy, O. F. H., Shafii, S. S. A., Tharwat, E. E., *et al*. (2004). Antibacterial activity of bee honey and its therapeutic usefulness against Escherichia coli O157:H7 and Salmonella typhimurium infection. *Revue Scientifique et Technique-International Office of Epizootics*, 23, 1011-1022. https://doi.org/10.20506/rst.23.3.1543.

Basualdo, C., Sgroy, V., Finola, M. S., *et al*. (2007). Comparison of the antibacterial activity of honey from different provenances against bacteria usually isolated from skin wounds. *Veterinary Microbiology*, 124, 375-381. https://doi.org/10.1016/j.vetmic.2007.04.039.

Bauer, A.W., & Kirby, W.W.M. (1966). Antibiotic susceptibility tests by standard single disc method. *American Journal of Clinical Pathology*, 45, 493-496. https://doi.org/10.1093/AJCP/45.4_TS.493.

Cerny, G., Hennlich, W., & Poralla, K. (1984). Fruchtsaftverderb durch Bacillen: Isolierung und charakterisierung des verderbserregers. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, 179(3), 224-227. https://doi.org/10.1007/BF01041898.

Choi, W., & Kim, S.S. (2020). Outbreaks, germination, and inactivation of *Bacillus cereus* in food products: A review. *Journal of Food Protection*, 83(9), 1480-1487. https://doi.org/10.4315/0362-028X.JFP-19-429

CLSI. (2006). Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute. 16th Informational Supplement M100-S16. *Wayne*, PA. https://clsi.org/media/tc4b1paf/m10033_samplepages-1.

Deanna, M. M., & Jeffrey, S. B. (2007). Acid-alkaline balance: role in chronic disease and detoxification. *Alternative Therapies*, 13, 62-65. https://europepmc.org/article/med/17658124.

Dietrich, R., Jessberger, N., Ehling-Schulz, M., Märtlbauer, E., & Granum, P.E. (2021). The Food Poisoning Toxins of *Bacillus cereus*. *Toxins*, 13, p.98. https://doi.org/10.3390/toxins13020098.

Dubey, S., Sharma, N., Thakur, S., Patel, R., &Reddy, B.M. (2021). *Bacillus cereus* food poisoning in Indian perspective: A review. *Journal of Food Science and Technology*, 10, 970-975. https://doi.org/org/10.3390/toxins13020098.

Durak, M.Z., Churey, J., Danyluk, M.D., & Worobo, R.W.(2010). Identification and haplotype distribution of *AlicycloBacillus* spp. from different juices and beverages. *International Journal of Food Microbiology*, 142(3), 286-291. https://doi.org/10.1016/j.ijfoodmicro.2010.07.003.

Ebisu, T., Maeda, N., Matsubara, H., *et al*. (1988). Honey powder. Composition as additives for foods and Pharmaceuticals. *Japanese Patent Number*, 63, 157-943. https://doi.org/10.1016/j.tifs.2019.03.019.

Esmael, A., Al-Hindi, R. R., Albiheyri, R. S., Alharbi, M. G., Filimban, A. A., Alseghayer, M. S., ... & Teklemariam, A. D. (2023). Fresh produce as a potential vector and reservoir for human bacterial pathogens: Revealing the ambiguity of interaction and transmission. *Microorganisms*, 11(3), 753. https://doi.org/10.3390/microorganisms11030753.

European Union. (2021). The European Union One Health 2020 Zoonoses Report. *European Food Safety Authority Journal*, 19, p.e06971. https://doi.org/10.2903/j.efsa.2019.5926.

Gomashe, A. V., Narad, M. V., & Gulhane, P. (2014). In vitro assessment of the antimicrobial potential of honey against enteric pathogens. *International Research Journal of Science and Engineering*, 2, 153-157. https://doi.org/10.4314/ejhd.v18i2.9945

Gundogan, N., & Avci, E. (2014). Occurrence and antibiotic resistance of Escherichia coli, Staphylococcus aureus, and *Bacillus cereus* in raw milk and dairy products in Turkey. *International Journal of Dairy Technology*, 67(4), 562-569. https://doi.org/10.1111/1471-0307.12149.

Hamouda, H. M., & Marzouk, D. S. (2011). Antibacterial activity of Egyptian honey from different sources. *International Journal of Microbiological Research*, 2, 149-155. https://doi.org/10.3923/jm.2011.488.495.

Iqbal, M.N., Anjum, A.A., Ali, M.A., Hussain, F., Ali, S., Muhammad, A., *et al*. (2015). Assessment of microbial load of un-pasteurized fruit juices and in vitro antibacterial potential of honey against bacterial isolates. *Microbiology Journal*, 9, 26. https://doi.org/10.2174/1874285801509010026.

Jesús, R. L. O. D., Ibáñez, A. T. G., Puebla, I. R., García, N. P., Siclán, M. L. S., & Romero, L. D. C. (2021). Microbiological quality and presence of enteropathogenic bacteria in orange juice sold in popular markets. *Food Science and Technology*, 42, e09621. https://doi.org/10.1590/fst.09621.

Jovanovic, J., Ornelis, V.F.M., Madder, A., and Rajkovic, A. (2021). *Bacillus cereus* food intoxication and toxicoinfection. *Comprehensive Reviews in Food Science and Food Safety*, 20, 3719-3761. https://doi.org/10.1111/1541-4337.12785.

Koneman, E., Allen, S., Janda, W., & Winn, W. (1997). Color Atlas and Textbook of Diagnostic Microbiology (5th ed.). *Lippincott-Raven Publishers*, Philadelphia, USA. https://books.google.iq/books/about/Color_Atlas_and_Textbook_of_Diagnostic_M.html?id=OHhhQgAACAAJ&redir_esc=y

Kwakman, P. H. S., Te Velde, A. A., de Boer, L., *et al*. (2010). How honey kills bacteria. *European Food Safety Authority Journal*, 24, 2576-2582. https://doi.org/10.1096/fj.09-150789.

Lahig, H., Hassan, M., & Yassir, L. (2018). Molecular Detection of Pseudomonas aeruginosa and Study the Effect of Fresh Garlic juice on Some Virulence Factors of this Bacteria. *Journal of University of Anbar for Pure Science*, 11(2), 9-17. https://doi.org/10.37652/JUAPS.2017.141584.

Lotfi, A. (2008). Use of honey as a medicinal product in wound dressing (human and animal studies): a review. *Research Journal of Biological Sciences*, 3, 136-140. https://doi.org/rjbsci.2008.136.140.

Lusby, P.E., Coombes, A.L., & Wilkinson, J.M. (2005). Bactericidal activity of different honeys against pathogenic bacteria. *Archives of Medical Research*, 36, 464-467. https://doi.org/10.1016/j.arcmed.2005.03.038.

Oussama, B. K., Fatima, S., Djilali, B., & Rym, B. (2023). The combined effect of Rosmarinus officinalis L essential oil and Bacteriocin BacLP01 from *LactoBacillus plantarum* against *Bacillus subtilis* ATCC11778. *Tropical Journal of Natural Product Research*, 7(3), 2551-2557. https://doi.org/10.48317/IMIST.PRSM/morjchem-v9i3.22091.

Rahman, M.M., Richardson, A., & Sofian-Azirun, M. (2010). Antibacterial activity of propolis and honey against Staphylococcus aureus and Escherichia coli. *African Journal of Microbiology Research*, 4, 1872-1878. https://doi.org/10.3390/molecules24112047.

Sedeeq, A., Tofeq, O. M., & Ali, S. S. (2022). An Investigation into The Effect Of Using Different Harvesting Methods on The Indicators of Potato Crop Loss. *Mesopotamia Journal of Agriculture*, 50(3). https://doi.org/10.33899/magrj.2022.1 33350.1168.

Sharif, A.Y. &Abdulqader, M.A. (2011). Isolation and Diagnosis of *Bacillus* from Locally Prepared Juices in Mosul City. *Journal of Education and Science*, 24(4). https://doi.org/10.20546/ijcmas.2019.801.206.

Sultan, M., & Yassen, M. (2018). Relationship Between Bacteria and Fungi Number In Rhizosphere and Bulk Soil. *Mesopotamia Journal of Agriculture*, 46(3), 295-306. https://doi.org/10.33899/magrj.2018.161508.

Tenea, G. N., Reyes, P., Molina, D., & Ortega, C. (2023). Pathogenic Microorganisms Linked to Fresh Fruits and Juices Purchased at Low-Cost Markets in Ecuador, Potential Carriers of Antibiotic Resistance. *Antibiotics*, *12*(2),236. https://doi.org/10.3390/antibiotics12020236.

Yamin, S., and Kurniawan, H. (2009). SPSS Complete: Teknik Analisis Statistik Terlengkap dengan Software SPSS. *Jakarta*: Salemba Infotek. https://inlislite.uin-suska.ac.id/opac/detail-opac?id=22708.

Van Luong, T. S., Moir, C., Bowman, J. P., & Chandry, P. S. (2021). Heat resistance and genomics of spoilage *AlicycloBacillus spp*. isolated from fruit juice and fruit-based beverages. *Food Microbiology*, 94, 103662. https://doi.org/10.1016/j.fm.2020.103662 .

Vantarakis, A., Affifi, M., Kokkinos, P., Tsibouxi, M., &Papapetropoulou, M. (2011). Occurrence of microorganisms of public health and spoilage significance in fruit juices sold in retail markets in Greece. *Anaerobe*, 17(6), pp.288-291. https://doi.org/10.1016/j.anaerobe.2011.04.005.

Wilkinson, J. M., & Cavanagh, H. M. (2005). Antibacterial activity of 13 honeys against Escherichia coli and Pseudomonas aeruginosa. *Journal of Medicinal Food*, 8, 100-103. https://doi.org/10.1089/jmf.2005.8.100