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Duality Effect of Bee Products in Dealing with Yoghurt Starter, Probiotics and Food Borne Pathogenic Bacteria.

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ABSTRACT

This research explores the dual effects of various organic bee products, including propolis (BP), bee wax (BW), Acacia (AH) and Clover honey (CH), bee pollen (P), bee venom (BV), and royal jelly (RJ), on lactic acid, probiotics, and foodborne pathogens bacteria. The study delves into the phytochemical attributes of these organic bee products, such as total phenols (TPC), total flavonoids (TFC), and antioxidant capacity (TAC), Fourier Transform Infrared Spectroscopy (FTIR) analyses unveil distinctive spectra for each bee product. The antimicrobial activity of organic bee products extracts were determined by using the disk diffusion method.

According to the findings, propolis had the highest levels of total flavonoids, phenolic, and antioxidant capacity among all organic bee products. Bee wax was shown to be the next most abundant source of TPC and TFC, although acacia honey and pollen had higher TAC levels than bee wax.

The results showed that, the low concentrations of organic bee products led to active the bacteria species used in fermented dairy products (yoghurt starter, probiotics, and *Lactobacillus casei*). On the other hand, the highest concentrations of honeybee products inhibited all foodborne pathogens bacteria. The dual nature of honeybee products, acting as positive modulators of beneficial bacteria and demonstrating antimicrobial efficacy against pathogens. Results suggested the potential application of honeybee products as natural agents for enhancing beneficial bacteria growth and as alternatives to synthetic preservatives against foodborne pathogens. This research contributes valuable insights into the multifaceted effects of honeybee products, opening avenues for potential applications in the food industry and health-related fields.

KEYWORDS: Bee products, probiotics bacteria, pathogenic bacteria, antimicrobial activity, FTIR.

1. INTRODUCTION

Fermented dairy contains probiotic Lactobacillus bacteria that could promote gastrointestinal health and reduced symptoms of certain digestive illnesses through various beneficial impacts in the gut such as enhance the integrity of the intestinal barrier, and modulate the gut microbiota composition. These effects can help alleviate symptoms of various gastrointestinal disorders, such as irritable bowel syndrome and inflammatory bowel disease (Plaza-Díaz et al 2017 and Nowak et al. 2021). Also, *Lactobacillus species* have immune-modulating capabilities, as they are able to boost the immune system, increase the production of anti-inflammatory short-chain fatty acids, and modulate the inflammatory response (Del Piano et al 2010).

Additionally, certain Lactobacillus species played an important role in lactose digestion by secreting the enzyme beta-galactosidase, which breaks down lactose into simpler sugars that are more easily absorbed and digested (Dahiya & Nigam, 2023).

Honeybees produce a variety of beneficial substances that have long been valued for their nutritional and medicinal properties (Cornara et al.,2017). Bee products such as honey, bee pollen, propolis, bee wax, and royal jelly contain a diverse community of microorganisms, including species of *Lactobacillus* (Ahmad et al., 2023 and Ben-Miled et al.,2023).

Fourier transform infrared (FTIR) spectroscopy is commonly technique used for detecting phytochemicals functional groups. Little studies focused on identifying functional categories in honeybee products where they were as follows. The band at about 3300-3400 cm−1 represents the O-H stretching of substances such as phenolic acids, flavonoids, terpenoids, and sugars, which are prevalent phytochemicals found in beeswax, honey, pollen, and propolis. The 1600-1500 cm−1 area displays bands related to aromatic ring vibrations, suggesting the existence of substances such as flavonoids and phenolic acids.

Absorption bands between 1736-1708 cm−1 indicate the presence of ester carbonyl groups, often seen in terpenoids, phenolic acids, and flavonoids. Bands between 1245-1100 cm−1 represent C-O and C-C stretching vibrations in polysaccharides, sugars, triglycerides, and lignin derivatives, indicating the presence of substances such as carbohydrates, fatty acids, and phenolic polymers. The fingerprint areas below 1200 cm−1 display several bands related to C-O, C-C, and ring vibrations, which may be used to identify certain phytochemical classes such as terpenes, phenolics, lipids, and polysaccharides (Escriche, et al., 2012; Abbas et al., 2017; Bunaciu & Aboul-Enein 2022 & Damto et al.,2023).

The use of bee products in fermented dairy milk is an area of growing research interest due to their nutritional and health-promoting properties. Certain bee products have been shown to enhance the physicochemical and microbiological characteristics of fermented dairy when used as ingredients (Darwish, et al., 2022, Carmen et al.,2019).

Honey is a commonly used bee product in yoghurt production due to its antimicrobial effects and ability to support the growth of beneficial bacteria (Liu et al., 2023). Honey and bee pollen, have been found to harbor *Lactobacillus kunkeei*, *Fructobacillus fructosus*, and other beneficial microbial species and activate them (Shaban et al.,2021, Basuny et al., 2023; Spyridon and Elisavet, 2023; Tsadila et al,2023 &Yasser et al,2023).

Recent study has revealed that adding low dosage of some bee products could boost the activity of probiotic bacteria in the gut. Propolis extract is another addition that can increase the nutritional profile of fermented goods. Bengi et al., (2023) demonstrated that treating kefir grains with propolis extract enhanced total phenolic content and antioxidant activity over untreated controls. The propolis-fortified kefirs also displayed suppression of foodborne pathogens Salmonella enteritidis and Listeria monocytogenes. This antibacterial action was related to bioactive components in propolis such as polyphenols, flavonoids, and coumarins (Nefzi et al., 2023).

Pollen is a good choice that enhanced the properties of fermented milk. Kostić et al., (2020) observed that introducing 1% pollen into milk boosted its protein, fat, carbohydrate, mineral and total phenolic contents compared to milk alone. Royal jelly has gained interest as a functional ingredient in fermented dairy products (Guldas, 2016). It has been found that when mixed with bee pollen and bee bread, increased the antioxidant activity and proteolytic powers of the products, enhancing their organoleptic properties (Reza et al., 2011; Amira et al., 2022; Adhiyamaan and Parimalavalli, 2022). Bee venom has been investigated that dietary bee venom improved antioxidant capacity and affected fatty acid metabolism (Kim et al., 2019).

Food-borne diseases result from the contamination of food by many pathogenic bacteria likewise (*Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi*) which may contaminate food at any stage of its production, delivery, or consumption. These bacteria may be transmitted to food from the surrounding environment. They may be transmitted to it from water, air, or soil, in addition to being transmitted to food if it is Storing or manufacturing it in a contaminated environment. These bacteria cause a wide range of diseases like diarrhea, food poisoning, nausea and cancers and may lead to death (Anum et al., 2023 and Oluwarinde et al., 2023). The 2019 World Bank report on the economic burden of the foodborne diseases indicated that the total productivity loss associated with foodborne disease in low- and middle-income countries was estimated at US$95.2 billion per year, and the annual cost of treating foodborne illnesses is estimated at US$15 billion (World Health Organization,2019).

2. MATERIAL AND METHODS

2.1. Organic bee products:

Propolis (P), bee wax (BW), honey (Acacia honey (AH), Clover honey (CH)**)**, bee pollen (BP), bee venom (BV) and royal jelly (RJ) used in this work was obtained from Agricultural Research Center - Agricultural Experiments Administration in Dakhla, New Valley.

2.2. Yoghurt Starter:

Yoghurt starter consisted of *Loctobacillus delbrueckii* subsp *bulgaricus* (EMCC 111.2) and *Streptococcus salivarius* subsp *thermophilus* (EMCC 11044) and *Loctobacillus casei* ware obtained from Cairo Microbiological Resource's Center (MIRCEN) Faculty of Agriculture, Ain Shams University

2.3. Probiotic bacteria:

B*ifidobacterium animals, Bifidobacterium bifidum LMG 10645 and Bifidobacterium breve.* were obtained from Cairo Microbiological Resource's Center (MIRCEN) Faculty of Agriculture, Ain Shams University.

2.4. Food borne pathogenic bacterial species:

Staphylococcus aureus, E. colli 0157 and *Salmonella typhi* were obtained from Department of Microbiology, Faculty of agriculture Minia University.

2.5. Screening of antibacterial activity:

Screening of antibacterial activity was performed by standard disc diffusion method (Saeed *et al.,* 2007). Hundred sterilized discs of filter paper (6 mm diameter) were soaked in water extract of organic bee products and (RJ) (0.6, 0.8, 1.0%), honey (HA, and HC) (5.0, 10.0, 15.0 %), (BP) (0.6 ,0.8, 1%), (P) (0.5, 1.0, 1.5, %), (BW) (0.3 ,0.5, 1.0 %), (BV) (0.01, 0.02, 0.03 %) separately for 1-2 minutes and then used for screening. The potency of each disc was Mueller-nutant agar (NA) for common food borne pathogens, MRS agar was used for lactic acid bacterial and MRS with *L. cysteine* was used for bifidobacterial (Süle et al., 2014) were used for the preparation of inoculum. 8 colonies of tested each. The inoculated tubes were incubated at 35-37^{o} C for 24 hours (Saeed et al., 2007). A sterile cotton swab was dipped into the standardized bacterial test suspension to inoculate entire surface of a media agar plate.

Discs of organic bee products and honey were placed on the surface of inoculated plates with the help of sterile forceps. The inoculated plates were incubated at 35-37C^{o} for 24 hours. After incubation inhibition zone diameters were measured to the nearest millimeter (mm). Completely randomized design was used in all experiments.

2.6. Determination of total flavonoid contents (TFC):

The total flavonoid contents (TFC) were determined using catechin as a reference using the aluminum chloride method (Chang *et al.*, 2002).

2.7. Determination of Total Antioxidant Capacity (TAC):

Total Antioxidant Capacity (TAC) was determined according to the method as described by (Ozyurt *et al*. ,2007).

2.8. Total phenolics content (TPC):

TPC was measured using a Folin-Ciocalteu assay according to (Papanov et al.,2019).

2.9. Fourier Transform Infrared Spectroscopy (FTIR)_:

All spectra were obtained using an FTIR (Omnic spectra) spectroscopy (Singh *et al.*, 2011).

2.10. Statistical analysis:

The statistical analysis was performed employing the Costat software program. The Duncan's multiple range test using tow-way analysis of variance (ANOVA) at 5% significant level ($P < 0.05$) (Snedecor and Cochran, 1980).

3. RESULTS AND DISCUSSION

3.1. Phytochemical Attributes:

Some phytochemical contents (TPC), (TFC) and (TAC) were measured and summarized in Table 1. The results showed that, P. and BP had the greatest phenolic content, (TPC) (3015.00 and 2459.00mg /100g) with no significant differences between them, followed with BW (907mg/100g) and AH (203.8mg/100g) and RJ (110.2mg/100g) with significant differences between them. Also (TFC) were in descending order P> BP >BW> AH>BV and (TAC) with significant references between the treatment. P, BP, CH and AH had maximum TAC their values are significant when compared with BW. These results are in agreement with (Nagai et al., 2001 and Nakajima et al., 2009). Who demonstrated that propolis extracts and Bee wax are the most potent antioxidant among organic bee products. Also, (Botezan *et al.*, 2023) revealed that honey includes a diversity of phytochemicals that vary on the floral source. Common antioxidants include phenolic acids, flavonoids, ascorbic acid, and carotenoids. According to (Tutun et al., 2021) Pollen is rich in antioxidants such phenolic compounds, ascorbic acid, carotenoids, and flavonoids. As shown in Table 1 pollen demonstrates powerful antioxidant. Common antioxidants in propolis include caffeic acid phenethyl ester, artepillin, and flavonoids. The antioxidant contents are quite low in BW compared to other organic bee products. (Yulian et al.2023). On the other hand, (Abdel-Wahab, 2023) determined TPC of royal jelly to be 115.23 mg gallic acid equivalents /100 g. The flavonoid concentration was 34.23 mg catechin equivalents/100 g. Common antioxidants in propolis include caffeic acid phenethyl ester, artepillin, and flavonoids.

Table 1. Phytochemical and antioxidant capacity of some organic bee products

organic bee products	TPC mg/100g	TFC mg/100g	TAC mg/100g
CH	54.2^{cd}	20.75^{d}	417.4^{b}
AH	203.8^{c}	102.9^{c}	505.8^{a}
BW	907^{b}	857.9^{b}	171.6^{c}
BP	2459^{a}	2719^{a}	426.8^{b}
P	3015^{a}	2881^{a}	550.2^{a}
BV	109.9^{c}	35.08^{d}	345^{f}
RJ	110.20^{c}	36.25^{d}	398^{e}

Values with the same letter in the same column are not statistically different (p<0.05). TFC: The total flavonoid contents – TAC: Total Antioxidant Capacity, TPC: Total phenolics content .Clover honey (CH), Acacia honey (AH), bee wax (BW), ,bee pollen (BP) , Propolis (P), Bee venom(BV) and Royal jelly(RJ).

3.2.FTIR-ATR spectrum of the organic bee products:

FTIR-ATR spectrum of the organic bee products is shown in fig (1).

3.2.1. FTIR-ATR spectrum of Honey

The honey spectrum's strongest band, with an absorption maximum at 3327 cm-1, is created by the stretching vibrations of the hydroxyl groups in water and carbohydrates (glucose, fructose, and trehalose). It is extremely possible that this absorption band coincides with N–H stretching vibrations (amide A) of proteins, which generally absorb in the spectral range of 3500 to 3300 cm-1, given the substantial abundance of protein-based components in the hemolymph of honey bees. Proteins and lipids' C–H stretching of their methyl and methylene groups is associated with the vibration at 2927 cm-1. The stretching vibrations of C=O and C–N (amide I) at 1636 cm-1 were found as the medium intensity vibration; these vibrations are associated to the presence of peptides and enzymes of globular proteins (Jelena and Lidija, 2020)

3.2.2. FTIR-ATR spectrum Royal jelly (RJ)

The vibrational bands of the components included in the royal jelly were discovered at 3275, 1636, 1547, 1409, 1347, 1239, 1152, 1103, 1079, 1062, 1035, and 992 cm^{-1}. The strongest and broadest band was found, with a peak point positioned at 3275 cm^{-1}. The stretching of O-H groups from water and N-H stretching vibrations from amines created the peak that was noticed at 3275 cm^{-1}. Two bands were detected at 1636 and 1547 cm^{-1}, while the second notable hitting zone was placed between 1500 and 1800 cm^{-1}. The Amide 1 was represented by the band with a peak point at 1636 cm^{-1}, which is associated to C- O stretching vibrations that come from the backbone conformation of proteins. Amide II's N-H bending and C-N stretching vibrations created the peak visible at 1547 cm^{-1}. The Amide-I and Amide-II spectral ranges can be used to check the legitimacy of dietary items as they give information about the secondary structure of proteins. The chemical groups were related to these bands (Lazarevska & Makreski, 2015 and Cebi, et al., 2020).

3.2.3. FTIR-ATR spectrum Bee wax (BW)

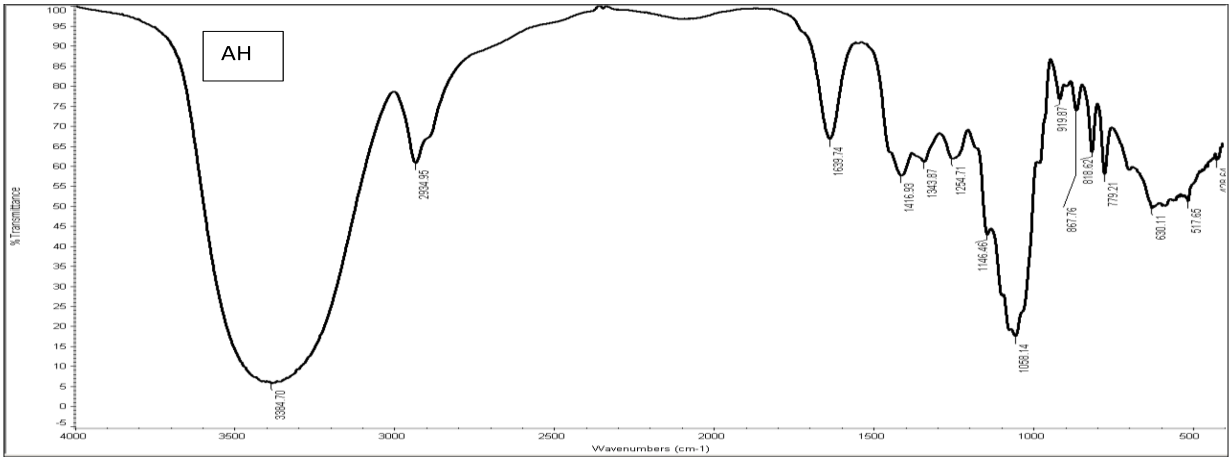
In the wave number range of 3000–2700 cm_1, the FTIR-ATR spectra of beeswax were stretching vibrations of the aliphatic C–H bonds of methyl and methylene groups. Four bands were found in the spectra at about 2956 (CH3), 2916 (CH2), 2871 (CH3), and 2848 cm_1 (CH2). At 1472/1462 cm_1 and 730/720 cm_1, there were significant doublets of scissoring and rocking vibrations that suggested the deformation vibrations of aliphatic C–H bonds. An orthorhombic crystal structure of hydrocarbon chains is proposed by the splitting of the CH2 scissoring and rocking modes. The deformation vibration of the methylene group close to the carboxyl group is seen by the band at 1414 cm_1. Methyl group vibrations arising from symmetrical deformation created a low intensity band at 1378 cm_1 (Alexandra et al., 2020)

3.2.4. FTIR-ATR spectrum bee venom (BV)

The N-H stretching vibration amide band (a band) of the secondary structures of peptides and proteins is responsible for the wide, medium intensity absorption band visible in the FTIR spectrum of BV. This band has an absorption maximum at 3288 cm -1. (Socrates et al., 2004 & Kong and Yu, 2007), As for the low-intensity signal, it emerges at 3060cm-1 (amide b) and corresponds to the same vibrational mode. The latter could also hint to an amide II band overtone (Socrates et al., 2004). Lower intensity analyte signals at 2873 cm-1 and 2855 cm-1, which are attributable to CH2 symmetric stretching, follow greater intensity absorption of odium at 2960 cm-1 and 2928 cm-1, which correspond to CH2 asymmetric stretching vibration.

There are various absorption bands that are particular to the secondary structure of peptides and proteins that occupy the fingerprint area, which is the spectral range between 1700 and 700 cm^{-1}. The amide I (C=O stretching) and amide II (N–H bending and C–N stretching vibrations) bands are ascribed to the most significant vibrations in this area, which occur at 1645 cm^{-1} and 1537 cm-1, respectively. This is in keeping

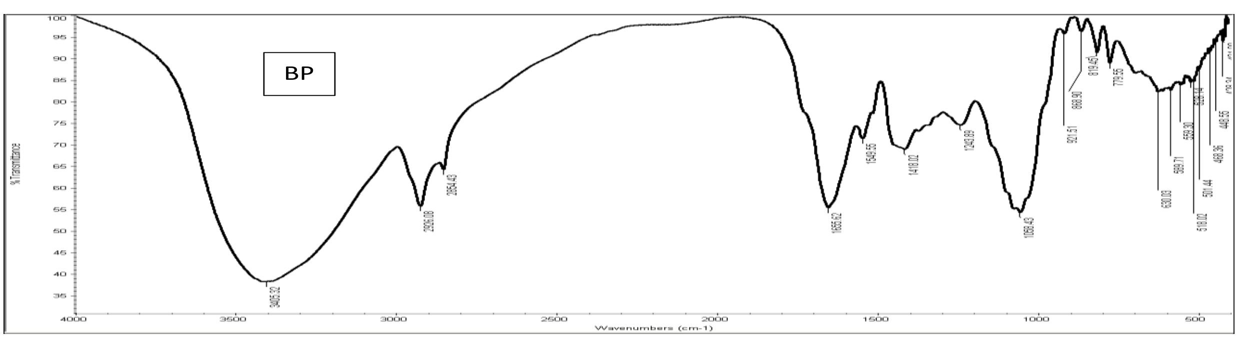
A


AH
% Transmittance
Wavenumbers (cm-1)
3384.70
2934.95
1639.74
1416.93
1343.87
1254.71
1146.46
1058.14
919.87
818.62
867.76
779.21
630.11
517.65

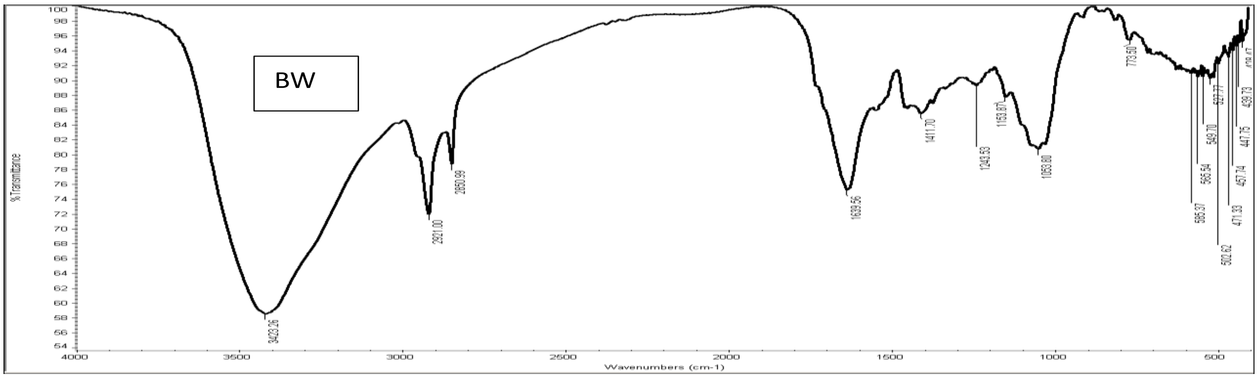
B


RJ
%Transmittance
Wavenumbers (cm-1)

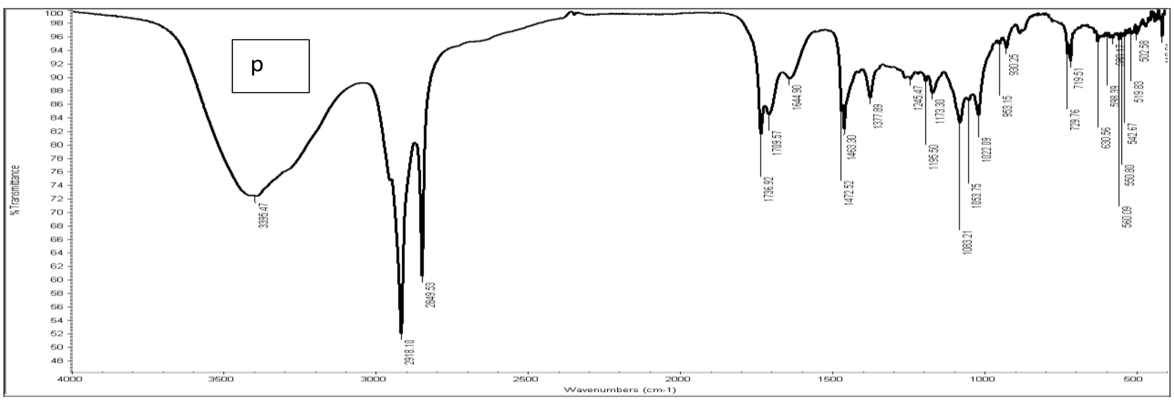
C


BP
%Transmittance
Wavenumbers (cm-1)

D



E



F

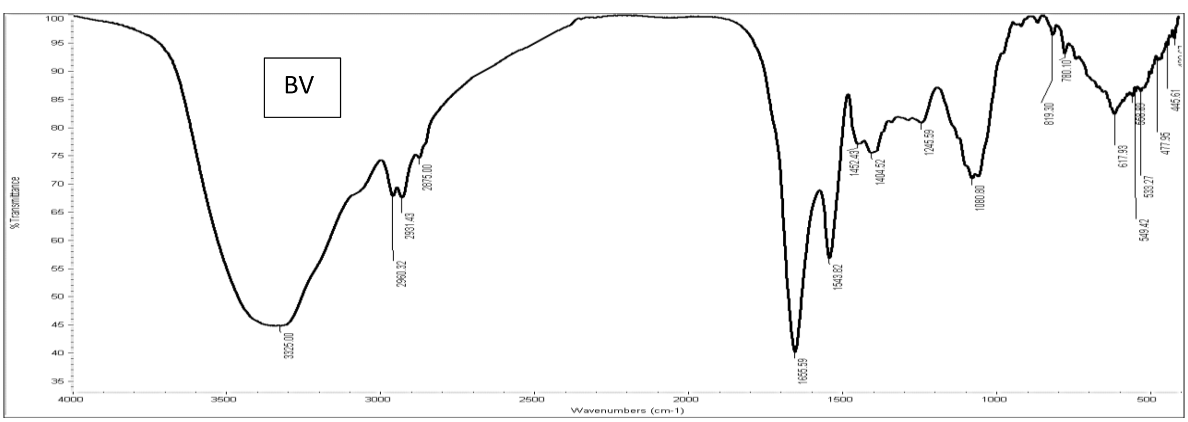


Fig 1. Characteristic FTIR-ATR spectrum of organic bee products

A- Acacia honey (AH), B- royal jelly (RJ), C- bee wax (BW), D- bee venom (BV), E- bee pollen (BP) F- Propolis (P).

with the information that was supplied by on the fundamental spectrum properties of the venom of bees. (Park et al., 2018). The following frequencies correspond to other amide bands: The protein side chain COO-is most likely responsible for the peaks that emerge at 1454 and 1386 cm-1.

The amide III band, which consists of 10% C–O stretching, 10% N–H bending, 30% C–N stretching, and 10% O=C–N bending vibrations, generates a wide medium intensity absorption between 1290 and 1240 cmπ1. The remaining vibrations are classed as diverse vibrations (Tanuwidjaja et al., 2021). The various aromatic structures in the bee venom's C–H in-plane deformation vibration is responsible for the medium intensity absorption measured at 1088 cm-1. Nearby at 805 cm-1, a faint analyte signal is created by the symmetric CNC stretching vibration of proteins (enzymes). There have been observations of amide IV (mainly O=C–N bending) and amide V (N–H bending) bands at 660 cm-1 and 730 cm-1, respectively. (Tanuwidjaja et al., 2021).

3.2.5. FTIR-ATR spectrum Bee pollen (BP)

Around 1,660 cm−1 (Amide I) and 1,550 cm−1 (Amide II and lignin), roughly 1,460 and 1,410 cm−1 (mainly from lipids and proteins, respectively), and approximately 1,200 cm−1 are the predominant absorption bands in this spectral range. At this stage, a wide and organized absorption band predominantly connected to carbs develops. For instance, Fig. 3a–c illustrates a single spectrum for every taxon from dataset B, which was obtained from a collection of pollen grains belonging to the same plant species within a100×100 μm2 window following area normalization and baseline subtraction in the 1,800–850 cm−1 range (Anjos et al.,2017).

3.2.6. FTIR-ATR spectrum of Propolis (P)

Two unique peaks were found in the propolis FT-IR spectra at 2918 and 2851 cm_1, which correspond to the symmetric and asymmetric stretching vibrations of the C–H of CH2 groups in saturated hydrocarbons. Moreover, additional peaks in the 1100–1600 cm_1 range revealed the presence of flavonoids and lipids in propolis.(Cebi et al 2020).

Honey: - Main peaks indicate presence of glucose, fructose, sucrose and other oligosaccharides. Peaks at 1020-1150 cm-1 correspond to C-O stretching vibrations of sugars. Peaks at 2900 cm-1 are due to C-H stretching vibrations. - Peaks in 1540-1640 cm-1 range suggest presence of proteins/amino acids(Stojko, 2019 and Ciulu et al., 2020). Bee pollen has similar sugar-related peaks as honey at 1000-1200 cm-1 area:- Peaks at 1540-1650 cm-1 confirm presence of proteins. - Fingerprint area below 800 cm-1 exhibits distinct peaks for diverse plant sources of pollen. (Bunaciu & Aboul-Enein, 2022).

Bee venom: - Strong bands at 1650-1650 cm-1 are attributable to Amide I, II and III stretches of phospholipase A2 enzyme. - Peaks at 2930-2850 cm-1 correspond to C-H lengths of fatty acid residues. - Absorption at 1230-1300 cm-1 area detect C-O stretches in lipids/phospholipids (Calegari et al.,2020). Royal jelly: - Sugar-related peaks are less severe than other goods due to decreased carbohydrate content. - Peaks centered at 1650-1540 cm-1 predominantly derive from Amide I, II bands of protein fraction. - Minimal peaks below 800 cm-1 suggest low amounts of other biomolecules (El-Guendouz et al., 2020). Bee wax: -Strong peaks at2920, 2850 cm-1 are due to C-H asymmetric/symmetric lengths of hydrocarbon chains. - Absorption bands about 1740, 1470 cm-1 emerge from C=O, C-H deformation modes in esters- Fingerprint area is unique to check wax consistency (Escriche et al., 2012).

3.3. Antimicrobial Effect of organic bee products

3.3.1. Effect of organic bee products on benefits bacteria (yoghurt starter, (L *delbrueckii bulgaricus, S. thermophilus*)

Data presented in Tables 2 and Fig (2) shows the antibacterial activity of different organic bee products extracts by disk diffusion method against the yoghurt starter strains. The results showed that, the low concentrations of different organic bee products have not any antimicrobial effect on yoghurt starter strains (*Loctobacillus delbrueckii bulgaricus* and, *Streptococcus thermophilus),* probiotic bacteria (*B. animals, B. bifidum, B. breve) and L. casei.* And it was observed that all tested organic bee

Table 2. Antibacterial test results of organic bee products and 2 kinds of honey (acacia honey HA, clover honey HC) against yoghurt starter, probiotic bacteria and *B. breve*

Honey product	Concent. (%)	Inhibition zone diameter by mm at different bacteria					
		L delbrueckii bulgaricus	***S. thermophilus***	***B. animals***	***B. bifidum***	***B. breve***	***L. casie***
	5.00	0.00	0.00	0.00	0.00	0.00	-
HC	9.00	0.00	0.00	0.00	0.00	0.00	-
	15.00	7.00^{d}	6.00^{cd}	5.00^{ef}	8.00^{cd}	8.00^{c}	7^{cd}
	5.00	0.00	0.00	0.00	0.00	0.00	-
HA	9.00	0.00	0.00	0.00	0.00	0.00	-
	10.00	9.00^{c}	9.00^{b}	7.00^{d}	10.00^{ab}	9.00^{bc}	9^{b}
	0.60	0.00	0.00	0.00	0.00	0.00	-
RJ	0.80	0.00	0.00	0.00	0.00	0.00	6^{d}
	1.00	5.00^{e}	7.00^{c}	12,00^{a}	8,00^{cd}	10.00^{ab}	8^{bc}
	0.30	0.00	0.00	0.00	0.00	0.00	-
BW	0.50	0.00	0.00	7.00^{d}	7.00^{d}	9.00^{bc}	-
	1.00	7.00^{d}	9.00^{b}	10.00^{bc}	10.00^{ab}	11.00^{ab}	11^{a}
	0.01	0.00	0.00	0.00	0.00	0.00	-
BV	0.02	0.0	0.00	7.00^{d}	9.00^{bc}	10.00^{ab}	9^{b}
	0.03	9.00^{c}	7.00^{c}	10.00^{bc}	9.00^{bc}	10.00^{ab}	11^{a}
	0.60	0.00	0.00	0.00	0.00	0.00	-
BP	0.80	0.00	0.00	7.00^{d}	5.00^{e}	7.00^{c}	-
	1.00	6.00^{de}	5.00^{d}	9.00^{c}	11.00^{a}	10.00^{ab}	8b^{c}
	0.50	0.00	0.00	0.00	0.00	0.00	-
P	1.00	11.00^{b}	9.00^{b}	6.00^{de}	7.00^{d}	8.00^{c}	8^{bc}
	1.50	12.00^{a}	11.00^{a}	11.00^{ab}	8.00^{bc}	10.00^{ab}	11^{a}

Values with the same letter in the same column are not statistically different ($p<0.05$). CH: Clover honey, AH: Acacia honey, BW: bee wax, BP: bee pollen and P: Propolis; BV: Bee venom

products led to activate all six bacteria species used in fermented dairy products (yoghurt starter, probiotics, and *L. casei*).

The highest all concentration tested from all organic bee products gave antibacterial effect with significant differences between them according to type of product and the bacteria spesies as shown in Table 2 and Fig 2. The highest effect was observed when bacteria treated with Propolis with concentration 1.5% against yoghurt starters bacteria (*L delbrueckii bulgaricus* and *S. thermophilus)* followed by BW, P with concentration 1%, and BV The other products are differed according to type of bacteria. The least one was RJ and AH with no significant differences between them and are significant between them and the other treatments.

Yoghurt's probiotic capacity was boosted by the propolis extract's beneficial interactions with lactic acid bacteria, despite its antibacterial activity. A viable replacement for yoghurt manufacturing is propolis. AH, CH with concentration 5 and 9%, BW with 0.3 and 0.5%, and RJ with 0.6 and 0.8% have not any effects on bacteria of yoghurt starters. And can be use with these concentration as additive materials without any effects on the starter of yoghourt.

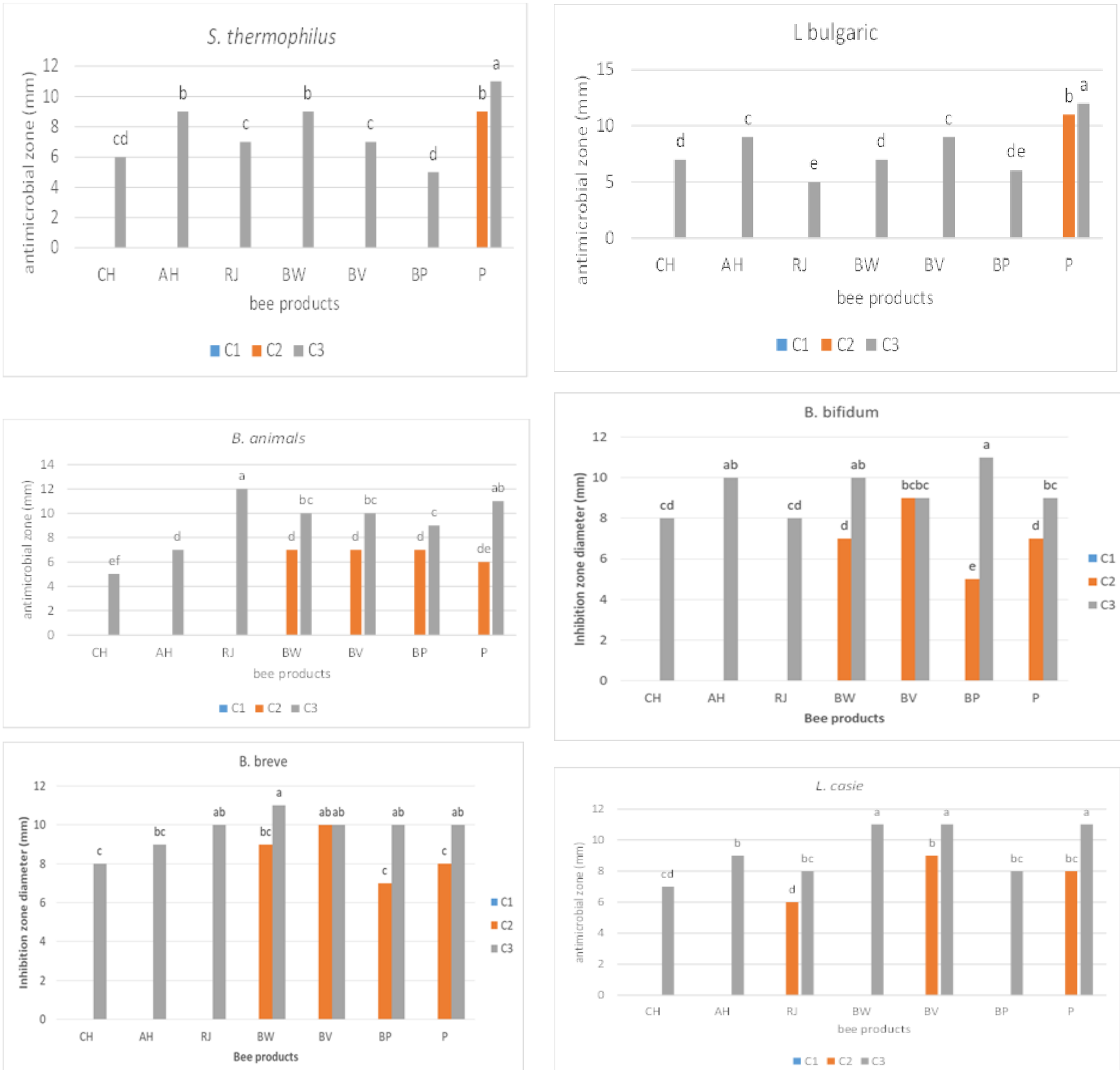


Fig 2. Antibacterial results of organic bee products and 2 kinds of honey (acacia honey HA, clover honey HC) against yoghurt starter, probiotic bacteria and *B. breve*

3.3.2. Effect of organic bee products on the probiotic's bacteria (*B. animals, B. bifidum and B. breve and Lactobacillus casei,*

As shown In Table 2 and Fig 2 The organic bee products with the lowest concentration from different organic bee products have not any effect on the growth of the three strains of probiotic bacteria and *Lactobacillus casei* which use in fermented dairy products revealing positive effects on beneficial bacteria even at low concentrations of organic bee products and the two types of honey. The highest concentration from every product caused inhibition in the growth of probiotic bacteria and differed according to the type of product and species of bacteria. it was observed that all tested organic bee products led to activate all three bacteria species used in fermented dairy products (probiotics, and *L. casei*).

The highest all concentration tested from all organic bee products gave antibacterial effect with significant differences between them according to type of product and bacteria species as shown in Table 2 and Fig 2. The highest effect was observed with RJ and Propolis with concentration 1 % and 1.5% against *B. animals* and against *B. bifidum and B.breve* followed by AH, CH, with highest concentration used. The other products are differed according to species of bacteria. Many studies were carried out on the nutritional and health-promoting benefits of using organic bee products in fermented dairy milk. It has been demonstrated that the physicochemical and microbiological properties of fermented dairy can be improved by using specific organic bee products as additives.

3.3.3. Effect of organic bee products on food borne pathogenic bacteria:

Data reported in table (3) and fig (3) reveals the antibacterial activity of honey and organic bee products extracts by disk diffusion technique against the three strains of food borne pathogenic bacteria exclusively; the greatest concentrations of honeybee products inhibited all foodborne pathogens bacteria. The antimicrobial activity of bee product extracts showed that the highest inhibition zone for foodborne pathogens bacteria were recorded against *Staphylococcus aureus* by BW (1%) & BV (0.3) (13mm), BP (1%) (14mm) and p (1.5 %) 15mm followed by *E. Coli* by CH 10% &AH 10% (13mm), BP (1%) (12 mm) and P (1.5) (13 mm).

The otherwise the maximum inhibition zone was obtained against *Salmonella typhi* by RJ. (1%) (12 mm). On the other hand, the maximum inhibition zone was reported against *Staphylococcus aureus* by BW (1%), BV (0.3) (13mm), BP (1%) 14mm) and p 1.5 %) 15mm. Throughout the history of traditional medicine, organic bee products have been used to treat and prevent illnesses.

Honey can directly modify the gut microbial balance because it comprises oligosaccharides, which increase the growth of probiotic lactobacilli and bifidobacteria and boost human metabolic function (Kajiwara et al., 2002 and Mohan et al., 2017). It is generally documented that BP is high in riboflavin, pantothenic and nicotinic acids in fact, riboflavin concentration in BP is the highest of any plant-based substance.

It has been demonstrated that the physicochemical and microbiological properties of fermented dairy can be improved by using specific organic bee products as additives. Because of its antibacterial properties and capacity to promote the growth of good bacteria, honey is a frequently utilized bee product in the making of yoghurt. (Simova et al., 2002 and Feknous & Boumendjel 2022). Propolis extract is another additive that can boost the nutritional profile of fermented products (Maria et al., 2017& Soycan et al., 2022). Additionally, propolis extract has been found to enhance diet degradation in rumen fluid and increase cumulative in vitro gas production, indicating its potential as a dietary additive for ruminants (Maria et al., 2017).

Also, propolis considered as a natural food additive for human consumption, it has been recognized for its bioactive compounds and antioxidant and antimicrobial properties (Özer, 2020). The propolis-fortified kefirs also demonstrated inhibition of foodborne pathogens *Salmonella Enteritidis* and *Listeria monocytogenes*. The propolis-fortified kefirs also displayed suppression of foodborne pathogens *Salmonella enteritidis* and Listeria monocytogenes. This antibacterial action was related to bioactive components in propolis such as polyphenols, flavonoids, and coumarins (Candan et al., 2003).

The main antibacterial compounds in honey are phytochemicals and flavonoids derived from flower nectar. Honey can inhibit the growth of common foodborne pathogens like *Salmonella enteritidis*, *E. coli, Listeria monocytogenes, and Staphylococcus aureus.* (Soltan & Patra 2020; Darwish et al., 2022).

Bee pollen also shows promise as an ingredient in fermented milk (Michal et al., 2023). Bee pollen can enhance nutritional value of fermentation products and increased its and biological activities, such as antioxidant and antimicrobial properties (William et al., 2021;

Table 3. Antibacterial test results of organic bee products and 2 kinds of honey (acacia honey HA, clover honey HC) against food borne pathogenic bacteria.

Honey product	Concent. (%)	Inhibition zone diameter by mm		
		Staphylococcus aureus	***E. coli***	***Salmonella typhi***
	5.00	0.00	0.00	0.00
HC	9.00	0.00	$10\ .00^{cd}$	0.00
	15.00	11.00^{de}	$13.oo^{a}$	10.00^{bc}
	5.00	0.00	7.oo	0.00
HA	9.00	7.00^{g}	9.00^{de}	0.00
	10.00	10.00^{ef}	13^{a}	10.00^{bc}
	0.60	0.00	7.00^{ef}	0.00
RJ	0.80	7.00^{g}	8.00^{e}	$9c^{d}$
	1.00	9.00^{f}	10^{cd}	12.00^{a}
	0.30	0.00	9.00^{de}	0.00
BW	0.50	9.00^{f}	10.00^{cd}	9.00^{cd}
	1.00	13.00^{bc}	10.00^{cd}	10.00^{bc}
	0.01	0.00	0.00	0.00
BV	0.02	10.00^{ef}	11.00^{bc}	10.00^{bc}
	0.03	13.00^{bc}	12.00^{ab}	12.00^{a}
	0.60	0.00	9.00^{de}	0.00
BP	0.80	12.00^{cd}	11.00^{bc}	8.00^{d}
	1.00	14.00^{ab}	12.00^{ab}	12.00^{a}
	0.50	11.00^{de}	11.00^{bc}	10.00^{bc}
P	1.00	13.00^{bc}	11.00^{bc}	11.00^{ab}
	1.50	15.00^{a}	13.00^{a}	12.00^{a}

Values with the same letter in the same column are not statistically different ($p<0.05$). CH: Clover honey, AH: Acacia honey, BW: bee wax, BP: bee pollen and P: Propolis; BV: Bee venom

Vaida et al., 2022), Lactic acid fermentation of bee pollen using starter cultures, such as *Lactobacillus rhamnosus* and *Lactococcus lactis*. The fermentation process increases the content of bioactive compounds, including phenolic compounds and flavonoids, which contribute to the antioxidant activity of bee pollen Additionally, fermentation improves the antibacterial and antifungal activities of bee pollen (Oktay, 2014; Vilma et al.,2020 and Setyawan et al., 2023).

As for bee venom several studies looked at the effects of bee venom, specifically the main component melittin, on beneficial probiotic bacteria for gut and overall health. The studies showed that low, non-toxic doses of melittin helped the probiotic bacteria *Lactobacillus plantarum* and *Bifidobacterium longum* grow better and be more metabolically active by increasing their survival, reproduction, and release of short-chain fatty acids. Additionally, melittin promoted the adhesion of probiotic bacteria to human epithelial cells in vitro, an important property that can help probiotics more effectively colonize the gut. However, while the studies suggest melittin may have therapeutic potential through these mechanisms, more research is still needed to fully understand its actions and potential health applications (Markowiak and Śliżewska 2017; Guha, et al.,2021; Juśkiewicz et al, 2021; Elkomy et al.,2023; Li et al., 2023 & Pandey et al., 2023).

4. CONCLUSION

These studies suggest that the biologically active compounds in organic bee products can beneficially modulate probiotic bacteria and lactic acid bacteria even at low concentration, non-antimicrobial doses. This may represent a natural approach for improving probiotic lactic acid bacteria, function and survival in the gut. On the other hand, organic bee products demonstrate

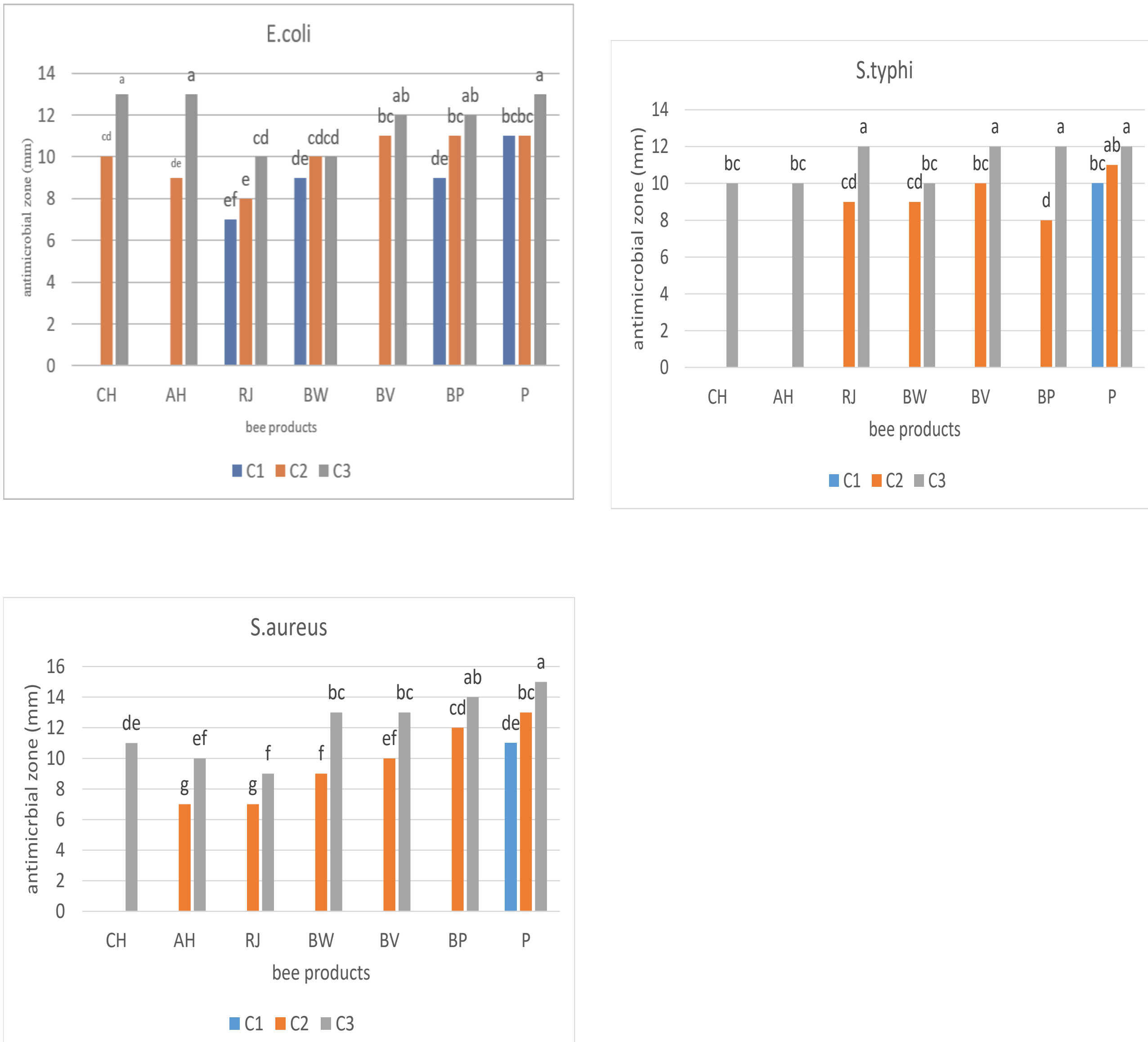


Fig 3. Antibacterial test results of organic bee products and 2 kinds of honey (Acacia honey AH, Clover honey CH) against food borne pathogenic bacteria.

antimicrobial effects against common foodborne bacteria, offering potential natural alternatives to synthetic preservatives in some food applications at high antimicrobial doses.

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الملخص العربي

التاثير المزدوج لمنتجات نحل العسل في التعامل مع بكتيريا حمض اللاكتيك وبكتيريا البروبيوتيك والبكتيريا المسببة للأمراض المنقولة بالغذاء.

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يبرز هذا البحث التأثير المزدوج لمنتجات النحل العضويه المختلفة، والتي تشمل البروبوليس(BP) ، وشمع النحل(BW) ، و عسل السنط (AH) وعسل البرسيم(CH) ، وحبوب لقاح النحل(P) ، وسم النحل(BV) ، والغذاء الملكي(RJ) ، على بكتريا حمض اللاكتيك، والبروبيوتيك، والبكتيريا المسببة للأمراض المنقولة بالغذاء. حيث تطرقت الدراسه لتقدير مركبات الفيتوكيميكال لمنتجات النحل حيث تم تقدير الفينولات الكليه(TPC) ، و الفلافونويد الكليه(TFC)، وكذلك مضادات الأكسدة(TAC) ، كما كشفت تحليلات الأشعة تحت الحمراء الطيفية (FTIR) عن أطياف مميزة لكل منتج من منتجات النحل. وايضا تم تحديد النشاط المضاد للميكروبات لمستخلصات هذه المنتجات باستخدام طريقة الانتشار بالقرص.

ووفقا للنتائج، حقق البروبوليس أعلى مستوي في كمية كلا من المواد الفينولية الكليه و الفلافينويد الكليه، و كذلك مضادات الأكسدة بين جميع منتجات النحل. تبين أن شمع النحل هو المصدر التالي الأكثر وفرة لـ TPC وTFC، على الرغم من أن عسل السنط وحبوب اللقاح يحتويان على مستويات TAC أعلى من شمع النحل.

وقد أظهرت النتائج أيضا أن التركيزات المنخفضة من منتجات النحل أدت إلى تنشيط أنواع البكتيريا المستخدمة في منتجات الألبان المتخمرة (yoghurt starter, probiotics, and Lactobacillus casei) من ناحية أخرى، فإن جميع التركيزات العاليه لكل منتجات نحل العسل قد ثبطت نمو البكتيريا المسببة للأمراض المنقولة بالغذاء. هذه الطبيعه المزدوجة لمنتجات نحل العسل تجعلها تعمل كمحفزات طبيعيه للبكتيريا المفيدة بينما تظهر تثبيطا واضحا للميكروبات الضاره. تشير النتائج إلى إمكانية تطبيق منتجات نحل العسل كعوامل طبيعية لتعزيز نمو البكتيريا المفيدة وكبدائل للمواد الحافظة الصناعية ضد مسببات الأمراض المنقولة بالغذاء. من خلال نتائج هذا البحث يمكن استغلال التأثيرالمزدوج لمنتجات النحل العضويه المختلفة ، كتطبيقات جديده في صناعة الأغذية والمجالات المتعلقة بالصحة.