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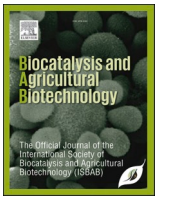

Increased bee venom production in *Apis mellifera* workers on the provision of probiotics and organic acids

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ABSTRACT

Bee venom of honeybees has great importance in Apitherapy. It cures amyotrophic lateral sclerosis, diseases related to central nervous system (CNS), rheumatoid arthritis and inflammations. The potential of honeybee's can be exploited by improving their diets. Recently, the use of synbiotics (simultaneous use of probiotics and prebiotics) has been increased as apipromoters. The current research study was conducted to evaluate the potential of probiotics (*Lactobacillus rhamnosus*, *Lactobacillus brevis* and *Bacillus clausii*) and acidifying agents (Lactic acid and acetic acid) on development of bee sting and its associated gland. Changes in the morphology of this gland affect the amount of venom produced. Significant increases in stings lengths were observed when honeybees were fed with (Pollen + *L. rhamnosus* in 50% (w/v) sucrose in distilled water) and (Pollen + *L. rhamnosus* in 50% (w/v) sucrose in 2.91% acetic acid) with mean values of 2.36 ± 0.04 and 2.38 ± 0.05 respectively compared with control's 2.35 ± 0.06 were observed. Significant increase in sting width was observed when bees were fed with (Pollen + *B. clausii* in 50% w/v sucrose in 2.99% lactic acid), (Pollen + *B. clausii* in 50% w/v sucrose in1.96% acetic acid), (Pollen + *L. brevis* in 50% w/v sucrose in distilled water) and (Pollen + *L. brevis* in 50% w/v sucrose in 2.99% lactic acid) with mean values of 1.84 ± 0.07, 1.79 ± 0.05, 1.87 ± 0.04, 1.78 ± 0.04 compared to control's 1.71 ± 0.03. In addition to this, width of stylet also increased significantly when bees were provided with (Pollen + *B. clausii* in 50% w/v sucrose in distilled water), (Pollen + *B. clausii* in 50% w/v sucrose in 2.99% lactic acid), (Pollen + *B. clausii* in 50% w/v sucrose in 2.91% acetic acid), (Pollen + *B. clausii* in 50% w/v sucrose in1.96% acetic acid), (Pollen + *L. brevis* in 50% w/v sucrose in distilled water), (Pollen + *L. brevis* in 50% w/v sucrose in 2.99% lactic acid), (Pollen + *L. brevis* in 50% w/v sucrose in 2.91% acetic acid), and (Pollen + *L. brevis* in 50% w/v sucrose in1.96% acetic acid) with mean values of 65.00 ± 1.58, 57.50 ± 3.23, 62.00 ± 2.00, 65.00 ± 0.00, 65.00 ± 1.58, 60.00 ± 1.58 compared to control's 55.00 ± 3.54. Rest of the observed parameters also depicted reasonable increases. Supplementation of honeybee's feed with organic acids and probiotics resulted in overall sting development.

1. Introduction

To tell about the exact beginning point of Apitherapy is difficult task to resolve due to dating back to Greece, ancient Egypt, and also has been practiced in China for 3–5, 000 years. Bee poison therapy (BPT) using bee poison to treat various diseases has been used in

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traditional medicine (Billingham et al., 1973; Liu and Tong, 2003). Bee venom is a well-known pharmacological activity product in the bee hives. It is made from venom glands associated with stinging equipment in workers and queens, stored and introduced by means of a stinging apparatus during the stinging process (Schmidt and Bchmann, 1999). In the first two weeks of an adult worker's life, production increases, peaking when worker bees participate in hive defense and foraging. Bee venom is a transparent liquid that easily dries out at room temperature. It is characterized by a tasteless natural irritating odor, bitterness, a hydrolysis mixture of protein and pH 5.0 to 5.5, and bees are used for defense. Honeybee venom (HBV) is soluble in water, insoluble in alcohol and ammonium sulfate. Bee venom contains many volatile compounds that may be lost during the collection process. It is a rich source of enzymes, peptides and biogenic amine (Schmidt and Bchmann, 1999). 18 active substances are found in honey bee venom. Mellitin (a basic 26-amino--acid polypeptide that constitutes 40–60% of dry *Apis mellifera* venom) is the most common substance which is the most effective anti-inflammatory drugs known, and 100 times strong than hydrocortisone. The strong anti-inflammatory drug is a Adolapin (An analgetic, anti inflammatory polypeptide present in bee venom) that inhibits Phospholipase A2, cyclooxygenase, degrades the cell membrane phospholipids. It also inhibits blood clotting and reduces blood pressure. Hyaluronidase causes capillaries dilation, which causes inflammation (Alvarez-Fischer et al., 2013). Histamine is conscientious for allergic reactions. Protease inhibitors take action like anti-inflammatory medicine and prevent bleeding. Other biochemical agents, like compound X, apamine and mast cell degranulating Protein (cationic 22-amino acid residue peptide with two disulfide bridges derived from bee venom) act to make tissues softer and assist the fluid's flow. In addition, there are calculable neurotransmitters norepinephrine, Dopamine, and serotonin (Bennton and Mulfinger, 1989). The health of honeybee colony can be improved with the use of probiotics and prebiotics (Hasan et al., 2022). The quality of bee venom is greatly dependent on environmental factors like heat, humidity, harvest time and the production method (Ghany Zidan et al., 2018; Hussein et al., 2019). Even some researches confirm the influence of honeybee's age (Owen et al., 1990; Owen, 1978; Owen et al., 1974), Supplementary feeding (Abusabbah et al., 2016; Nowar, 2016) and season (Owen and Anne, 1982; Owen and Sloley, 1988). The use of synbiotics (simultaneous use of probiotics and prebiotics) is gaining special attention these days to exploit the honeybee's potential. It is well known that provision of probiotic and prebiotic's addition to honeybee's feed can play a key role in improvement of health of worker honeybees as well as their complete colony. Acids perform their functions like prebiotics. Acidifying agents such as organic acids result in the lowering of pH of intestine and therefore provide selective site for the growth of only beneficial microbes while inhibiting the growth of pathogenic bacteria. It has been observed experimentally that growth of pathogenic bacteria decreases when the honeybees are provided with sugar syrups with pH between 3 and 6.5. Application of probiotics and organic acids in combination forms exerts synergistic effects as microbe can't grow in low pH and the host is benefitted by colonization of healthy and beneficial micro flora in it's gut that will ultimately improve host immunity (Patruica et al., 2013). The present investigation was attempted to appraise the potential of organic acids (lactic and acetic) and probiotics (*L. rhamnosus*, *L. brevis* and *B. clausii*) on degree of development of sting of *Apis mellifera*. Fig. 1 explains the one liner of the whole experimental philosophy.

2. Materials and methods

2.1. Ethics statement

Our research study did not necessitate special permits and experiments were accomplished at Institute of Zoology (**31.50103 N; 074.30746 E**), university of the Punjab Lahore, Pakistan. Honeybees were taken from (**31.49758° N; 074.29679 E**) honeybee research garden which is possession of Institute of Zoology and it is neither privately owned nor protected in any sense. The research animal *Apis mellifera* is not endangered or protected species at Pakistan.

2.2. Experimental set up

Comparatively new frames with numerous quantity of sealed brood of *Apis mellifera Ligustica* were selected to be kept in an incubator (33 °C Temperature and 65% Relative Humidity) and subsequently to have workers of 0–24 h age for experimental trials following Williams et al. (2013). Freshly emerged workers were placed in plastic jar cages made after Hasan et al. (2022a, 2022b) with number of fifty honeybee workers in each experimental cage following Martin-hernadez et al. (2009) in both control as well as experimental groups. Plastic jar cages were also equipped with pollen provider and feeder. Two organic acids: lactic acid (Catalogue Number: 100366, Merck), acetic acids (Lot No: 91400, sigma Aldrich) and three probiotics (*L. rhamnosus*, *L. brevis* and *B. clausii*) were provided to caged workers in the current research experiments.

2.3. Probiotics dose maintenance as master stock

Each packing of an industrially available probiotic *B. clausii* (enlistment No: 00100.0001, Tabros pharma, Karachi) contained a

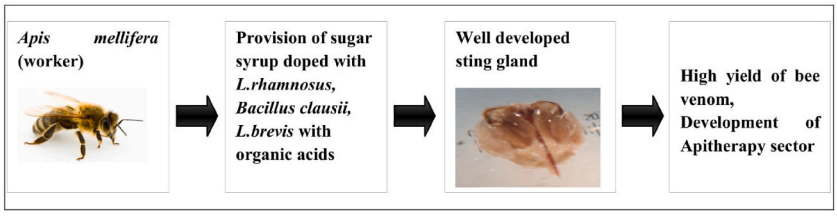

Fig. 1. One liner of the whole experimental philosophy.

suspension of 2 billion colony forming units (C.F.Us), so this packing was divided in 5 equal parts in eppendorf tubes (every tube holding 4×10^8 C.F.U) and subsequently centrifuged at 15,000 rpm to discard the supernatant and then the pellets of cells were saved in 0.9% normal saline. These eppendrof tubes were hoarded in a caravell fridge 400 Liter capacity (MEC-450, Pakistan) for further use. Both of the bacteria: *L. rhamnosus* and *L. brevis* were locally isolated and identified by amplifying their 16S rRNA gene sequence analysis. In case of *L. rhamnosus* and *L. brevis*, sterile MRS broth was inoculated separately with cultures *L. rhamnosus* and *L. brevis*. Colony forming units were calculated after taking 48 h's. It was observed that 10^8 C.F.U show optical density up to 0.5. Again fresh growth of each bacterial species was taken and then these broths (with massive magnitude of bacterial cells) were diluted by adding fresh and sterile MRS broth in these till 0.5 O.D readings attained on VIS spectrophotometer (V-M5, Italy). Afterwards, 2 ml quantity of this diluted broth was poured in eppendrof tubes. After centrifugation at 15000 rpm, the pellets of cells were soaked in normal saline (0.9%) as mentioned above in case of *B. clausii*. Two eppendrofs of each; *L. brevis* and *L. rhamnosus* (having 4×10^8 C.F.U) whereas single eppendrof of *B. clausii* (having 4×10^8 C.F.U), were withdrawn from master stock (having 4×10^8), on daily basis to be administered to the caged workers. To fulfill protein requirements of caged workers, pollens and distilled water were mixed in (9:1) in a beaker and using metallic spatula or gloved finger, pollen was kneaded well to create thick paste of soft dough consistency according to Alaux et al. (2010). Pollen was provided to all experimental bees including control group. The general as well as administration details of probiotics and organic acids are shown in Tables 1 and 2.

2.4. Provision of specific feeds on daily basis

Control group was provided with sugar syrup made in distilled water following Martin-Hernadez et al. (2009). Control groups of both these trials were separate; hence these were named as Control (I) and Control (II). Bothcontrol groups were provided with sugar syrups made in distilled water and pollen (as protein). inEach experimental cage was provided with 4 mL of sugar syrup on daily basis. For exploring the effects of organic acids following Patruica et al. (2013), stock solutions were made as: 2.99% lactic acid, 2.91% acetic acid, 1.96% acetic acid. Sugar syrups were prepared by mixing sucrose in respective stock solutions of organic acids in (2:1). All Probiotics were administered in both ways, i.e. in stock solutions of organic acids as well as by mixing them in sugar syrups using made by sucrose and distilled water in (1:2). In order to provide specific kind of sugar syrup and probiotic, mixtures were blended using Vortex mixer (IRMECO gmbh, 21493 schwarzenbek, Germany). The provision of probiotics and organic acids is shown in Tables 3 and 4. Bees were provided with four ml of sugar syrup daily following procedural steps by Hasan et al. (2022a, 2022b).

2.5. Statistical analysis

At the end of experiment, differences between means were determined by applying Duncan's multiple range test DMR in one-way analysis of variance (ANOVA) using SAS 9.1 at 95% confidence level.

2.6. Termination of experiment

After two week's oral administration of organic acids and probiotics doped sugar syrups, five bees were randomly selected from each experimental group and these were kept at−20 °C in a freezer for 2 h to put them to endless sleep following Nuag and Gibbs (2009). Subsequently these bees were stored in 10% formalin solution for further examinations. On dissection, sting apparatus of each bee got separated and again placed in formaldehyde solution again, later on these sting apparatuses got divided into their parts and measured under dissecting microscope (ER-59-1828, Carolina biological supply company USA) and High power microscope (Utech schenectady, NY12305) respectively. Sting apparatus parts examined microscopically are shown in Fig. 2. Total eleven characters including sting length, sting width, length of stylet, width of stylet, length of lancet, width of lancet, length of volvular lobe, width of volvular lobe, length of venom sac, width of venom sac, acid gland width were observed under microscope. Sting length, sting width, length of stylet, width of stylet were measured by placing these directly on stage micrometer under dissecting microscope on 40X, where as rest of the seven characters length of lancet, width of lancet, length of volvular lobe, width of volvular lobe, length of venom sac, width of venom sac, acid gland width were measured with the aid of ocular micrometer using high resonance binocular on 100X. The values regarding sting length and width were calculated in millimeters (mm) whereas the values of resting nine characters were calculated in μm^2. Experiments employing three probiotics were conducted in two trials; first trial included groups related to *L. rhamnosus* and organic acids whereas the second trial included groups related to *L. brevis* and *B. clausii* with and without addition of organic acids.

3. Results

Control (I) bees depicted the mean values of 2.35 ± 0.06, 1.71 ± 0.03, 236.25 ± 3.75, 55.00 ± 3.54, 770.00 ± 257.65, 17.50 ±

Table 1
Name and source of Probiotics, administered to the experimental bees in a dose of 1×10^8 C.F.U./ml of sugar syrup.

Probiotic name	Source	Colony forming units/ml of sugar syrup
Lactobacillus rhamnosus (NR_113332)	Microbial Biotechnology Laboratory, institute of Zoology, Punjab University	1×10^8
Bacillus clausii spores (BC-2Bio)	(enlistment No: 00100.0001, TABROS pharma, Karachi)Purchased from local market (TABROS pharma)	1×10^8
Lactobacillus brevis (MF179529)	Immunology Laboratory, institute of Zoology, Punjab University	1×10^8

Table 2
Feeding details of different groups fed on *Lactobacillus rhamnosus* with organic acids.

Experiment	Experimental code	Treatment
Control	–	Pollen+ 50% (w/v) sucrose in distilled water pH: 8
Experiment 1	Exp1-LA	Pollen+ 50% (w/v) sucrose in 2.99% Lactic acid pH: 3.14
Experiment 2	Exp 2-AA(II)	Pollen+ 50% (w/v) sucrose in2.91% Acetic acid pH: 2.95
Experiment 3	Exp 3-AA (I)	Pollen+ 50% (w/v) sucrose in1.96% Acetic acid pH: 3.12
Experiment 4	Exp 4-Lr	Pollen + *L. rhamnosus* in 50% (w/v) sucrose in distilled water pH: 8
Experiment 5	Exp 5-LaLr	Pollen + *L. rhamnosus* in 50% (w/v) sucrose in 2.99% Lactic acid pH: 3.14
Experiment 6	Exp 6-AA(II)Lr	Pollen + *L. rhamnosus* in 50% (w/v) sucrose in2.91% Acetic acid pH: 2.95
Experiment 7	Exp 7-LrAA (I)	Pollen + *L. rhamnosus* in 50% (w/v) sucrose in1.96% Acetic acid pH: 3.12

Table 3
Feeding details of different groups fed on *Bacillus clausii* and *Lactobacillus brevis* with organic acids.

Group name	Experimental code	Detail
Control	–	Pollen+ 50% (w/v) sucrose in DW pH: 8
Experiment 8	Exp8-Bc	Pollen + *B. clausii* in 50% (w/v) sucrose in DW pH: 8
Experiment 9	Exp9-BcLa	Pollen + *B. clausii* in 50% (w/v) sucrose in 2.99% LA pH: 3.14
Experiment 10	Exp10-BcAA(II)	Pollen + *B. clausii* in 50% (w/v) sucrose in2.91% AA pH: 2.95
Experiment 11	Exp11- BcAA (I)	Pollen + *B. clausii* in 50% (w/v) sucrose in1.96% AA pH: 3.12
Experiment 12	Exp12- Lb	Pollen + *L. brevis* in 50% (w/v) sucrose in DW pH: 8
Experiment13	Exp13- LbLa	Pollen + *L. brevis* in 50% (w/v) sucrose in 2.99% LA pH: 3.14
Experiment 14	Exp14-LbAA(II)	Pollen + *L. brevis* in 50% (w/v) sucrose in2.91% AA pH: 2.95
Experiment 15	Exp15-LbAA(I)	Pollen + *L. brevis* in 50% (w/v) sucrose in1.96% AA pH: 3.12

LA: Lactic acid, AA: Acetic acid, DW: Distilled water.

Table 4
Mean values of sting's different parts of worker bees fed on *Lactobacillus rhamnosus* with organic acids.

Parameters	Control	Exp1-LA	Exp 3-AA (I)	Exp 4- Lr	Exp 5-LaLr	Exp 6-AA(II) Lr	Exp 7-LrAA (I)	P-value
Sting length	2.35^{a}±0.06	2.22^{ab} ± 0.12	1.92^{b} ± 0.20	2.36^{a}± 0.04	2.30^{a} ±0.00	2.38^{a} ±0.05	2.34^{a}± 0.02	0.0277
sting width	1.71 ± 0.03	1.72 ± 0.11	1.82 ± 0.05	1.84 ± 0.05	1.92 ± 0.06	1.76 ± 0.06	1.73 ± 0.04	0.2030
length of stylet	236.25 ± 3.75	213.33 ± 12.02	215.00 ± 11.40	236.00 ± 2.92	223.00 ± 8.31	240.00 ± 3.16	235.00 ± 2.24	0.0586
width of stylet	55.00 ± 3.54	56.67 ± 3.33	55.00 ± 2.24	58.00 ± 2.00	59.00 ± 2.45	65.00 ± 2.24	57.00 ± 2.00	0.0979
length of lancet	770.00 ± 257.65	900.00 ± 180.09	940.00 ± 44.27	908.00 ± 20.83	950.00 ± 68.85	970.00 ± 14.4	940.00 ± 32.09	0.8689
width of lancet	17.50^{b} ± 6.29	30.00^{ab} ± 0.00	26.00^{ab} ± 2.45	32.00^{a}±2.00	30.00^{ab} ± 0.00	29.00^{ab} ± 1.00	26.00^{ab} ± 2.45	0.0244
length of volvular lobe	90.00 ± 30.28	150.00 ± 0.00	140.00 ± 3.16	104.00 ± 27.13	138.00 ± 5.83	140.00 ± 4.47	138.00 ± 4.90	0.1201
width of volvular lobe	65.00 ± 21.79	110.00 ± 5.77	98.00 ± 4.90	88.00 ± 22.23	108.00 ± 7.35	102.00 ± 5.83	90.00 ± 8.37	0.3211
length of venom sac	2907.50 ± 265.75	2071.33 ± 879.95	2464.80 ± 721.40	2912.00 ± 203.90	3078.40 ± 100.23	2896.40 ± 329.45	3187.60 ± 132.98	0.6280
width of venom sac	1163.50 ± 90.30	1138.67 ± 49.55	1175.20 ± 28.95	1237.60 ± 84.09	1128.40 ± 73.81	1279.20 ± 135.45	1289.60 ± 40.78	0.6869
acid gland width	43.33 ± 3.33	33.33 ± 3.33	45.00 ± 7.64	47.50 ± 2.50	40.00 ± 0.00	40.00 ± 5.77	45.00 ± 2.89	0.2920

Those not sharing a common alphabet within a respective column are significantly different from each other.

6.29, 90.00 ± 30.28, 65.00 ± 21.79, 2907.50 ± 265.75, 1163.50 ± 90.30, 32.50 ± 11.09 for Sting length, sting width, length of stylet, width of stylet, length of lancet, width of lancet, length of volvular lobe, width of volvular lobe, length of venom sac, width of venom sac, acid gland width respectively, (see Table 4). Control (II) bees depicted the mean values of 2.35 ± 0.06, 1.71 ± 0.03, 236.25 ± 3.75, 55.00 ± 3.54, 770.00 ± 257.65, 17.50 ± 6.29, 90.00 ± 30.28, 65.00 ± 21.79, 2907.50 ± 265.75, 1163.50 ± 90.30, 43.33 ± 3.33 for Sting length, sting width, length of stylet, width of stylet, length of lancet, width of lancet, length of volvular lobe, width of volvular lobe, length of venom sac, width of venom sac, acid gland width respectively, (see Table 5).

Exp1-LA, Exp 3AA (I), Exp 4- Lr, Exp 5-LaLr, Exp 6-AA(II)Lr, Exp 7-LrAA (I) depicted mean values of 2.22 ± 0.12, 1.92 ± 0.20, 2.36 ± 0.04, 2.30 ± 0.00, 2.38 ± 0.05, 2.34 ± 0.02 for Sting length ($p = 0.02$). Exp 4- Lr and Exp 6-AA (II)Lr showed significant increases in sting length. However all experiments: Exp 3AA (I), Exp 4- Lr, Exp 5-LaLr, Exp 6-AA(II)Lr, Exp 7-LrAA (I) showed reasonable increases in all other parameters, (see Table 4). Similarly, Exp9-BcLa, Exp11- BcAA (I), Exp12- Lb, Exp13- LbLa, Exp14-LbAA(II) and Exp15-LbAA(I), showed significantly increased values of 1.84 ± 0.07, 1.79 ± 0.05, 1.87 ± 0.04, 1.78 ± 0.04, 1.69 ± 0.08, 1.78 ± 0.04 for sting width ($p = 0.0398$), 65.00 ± 1.58, 57.50 ± 3.23, 62.00 ± 2.00, 65.00 ± 0.00, 65.00 ± 1.58, 60.00 ± 1.58, 58.00 ± 3.39 for width of stylet ($p = 0.0215$). However, reasonable increases in all other parameters (width of lancet, length of volvular lobe, width of

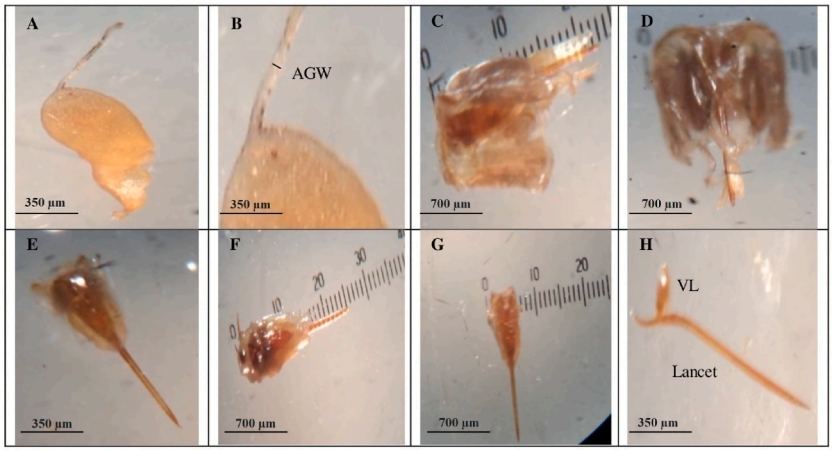

Fig. 2. A: venom sac with acid gland, **B:** AGW: acid gland width, **C:** length of sting on stage micrometer, **D:** width of sting, **E:** stylet of sting, **F:** stylet length on stage micrometer scale, **G:** width of bulb of stylet, **H:** lancet with volvular lobe.

volvular lobe, length of venom sac, width of venom sac, acid gland width) were observed in Exp13- LbLa, Exp14-LbAA(II) and Exp15-LbAA(I), (see Table, 5).

4. Discussion

The main purpose of current study was to assess the role of organic acids and probiotics experimentally on *Apis mellifera* sting development under controlled conditions. The developmental degree was evaluated by measuring parts of sting. Eleven characters regarding sting length, sting width, length of stylet, width of stylet, length of lancet, width of lancet, length of volvular lobe, width of volvular lobe, length of venom sac, width of venom sac, acid gland width were recorded under microscope. Changes in the morphology of this gland affect the amount of venom produced (Brizola-bonacina et al., 2006). The morphology of the gland was analyzed along with the amount of venom produced and the genetic characteristics of the bees (Brizola-bonacina et al., 2006). The quality of bee venom is greatly dependent on environmental factors like heat, humidity, harvest time and the production method (Ghany Zidan et al., 2018; Hussein et al., 2019). Even some researches confirm the influence of honeybee's age (Owen et al., 1990; Owen, 1978; Bachmayer et al., 1972; Owen et al., 1974), Supplementary feeding (Abusabbah et al., 2016; Nowar, 2016) and season (Owen and Anne, 1982; Owen and Sloley, 1988). Nowar (2016) experimented to valorize the potential of pollen substitutes on the production of honeybee venom. Results depicted that parameters regarding sting gland got affected by the provision of pollen substitutes. The percent increases in main components noticed were phospholipase A2 (16.4%), melittin (48.7%) and apamin (2.0%). Experiment 4 (Pollen + *L. rhamnosus* in 50% w/v sucrose in distilled water) and 6 (Pollen + *L. rhamnosus* in 50% w/v sucrose in 2.91% acetic acid), showed significant increases in sting lengths. However overall sting apparatus development in case of reasonable increases in most of the characters was observed when *L. rhamnosus* was used as probiotics. In case of second group, significant increase in sting width observed in Exp9-BcLa, Exp11- BcAA (I), Exp12- Lb and Exp13- LbLa when provided with diets Pollen + *B. clausii* in 50% w/v sucrose in 2.99% lactic acid, Pollen + *B. clausii* in 50% w/v sucrose in 1.96% acetic acid, Pollen + *L. brevis* in 50% w/v sucrose in distilled water, Pollen + *L. brevis* in 50% w/v sucrose in 2.99% lactic acid respectively. Whereas, width of stylet and width of volvular lobe in all experimental groups from 8 to 15 (when *L. brevis* and *B. clausii* were provided as probiotics with and without organic acids. Feeding of diet like Pollen+ 50% w/v sucrose in 1.96% acetic acid, Pollen + *L. rhamnosus* in 50% w/v sucrose in distilled water, Pollen + *L. rhamnosus* in 50% w/v sucrose in1.96% acetic acid, Pollen + *B. clausii* in 50% w/v sucrose in distilled water, Pollen + *B. clausii* in 50% w/v sucrose in 2.99% lactic acid, Pollen + *L. brevis* in 50% w/v sucrose in 2.99% lactic acid, Pollen + *L. brevis* in 50% w/v sucrose in 2.91% acetic acid, and Pollen + *L. brevis* in 50% w/v sucrose in 1.96% acetic acid resulted in reasonable increase in acid gland width. All experimental groups showed development of sting compared with control group. At the end, we consider it an essential to endorse the statement that probiotics and organic acids resulted in improvement of bee condition. The future research studies regarding quantity of bee venom production can be done after organic acids and probiotics treatments to check the degree of development by its supplementations.

5. Conclusions

The supplementation of acidifying agents and probiotics to the sugar syrup of honeybees proved fine for pollinator's overall health especially sting developments. It is environment-friendly and good methodology to improve honeybee's efficiency. Our findings of the present study will be helpful to obtain higher bee venom yields. Future studies on the assessment of various other organic acids and probiotic blends at pilot and commercial scales are needed for obtaining exact quantitative results at higher scales.

Table 5
Mean values of sting's different parts of worker bees fed on *Lactobacillus brevis* and *Bacillus clausii* with organic acids.

Parameters	Control	Exp8-Bc	Exp9-BcLa	Exp11- BcAA (I)	Exp12- Lb	Exp13- LbLa	Exp14-LbAA(II)	Exp15-LbAA(I)	P-value
Sting length	2.35 ± 0.06	2.26 ± 0.13	2.19 ± 0.14	2.15 ± 0.17	2.25 ± 0.09	2.31 ± 0.07	1.84 ± 0.17	2.00 ± 0.18	0.1772
sting width	1.71^{abc}±0.03	1.62^{c} ±0.05	1.84^{ab} ± 0.07	1.79^{abc}±0.05	1.87^{a}±0.04	1.78^{abc}±0.04	1.69^{bc}±0.08	1.78^{abc}±0.04	0.0398
length of stylet	236.25 ± 3.75	226.00 ± 12.08	217.50 ± 12.50	213.00 ± 15.78	224.00 ± 9.14	224.00 ± 9.67	186.00 ± 17.20	197.00 ± 15.38	0.1696
width of stylet	55.00^{b} ± 3.54	65.00^{a} ±1.58	57.50^{ab} ± 3.23	62.00^{ab} ± 2.00	65.00^{a}±0.00	65.00^{a} ±1.58	60.00^{ab} ± 1.58	58.00^{ab} ± 3.39	0.0215
length of lancet	770.00 ± 257.65	932.00 ± 56.69	867.50 ± 66.13	878.00 ± 35.83	886.00 ± 36.82	910.00 ± 35.21	758.00 ± 85.29	690.00 ± 173.61	0.7323
width of lancet	17.50 ± 6.29	28.00 ± 2.00	30.00 ± 0.00	33.00 ± 3.00	29.00 ± 2.45	32.00 ± 2.00	30.00 ± 0.00	24.00 ± 6.00	0.0908
length of volvular lobe	90.00 ± 30.28	143.00 ± 2.00	140.00 ± 4.08	134.00 ± 6.78	116.00 ± 9.27	128.00 ± 8.00	128.00 ± 8.00	108.00 ± 27.46	0.2758
width of volvular lobe	65.00^{b} ± 21.79	154.00^{ab} ± 22.05	115.00^{ab} ± 9.57	116.00^{ab} ± 6.78	112.00^{ab} ± 5.83	114.00^{ab} ± 2.45	121.00^{ab} ± 5.57	102.00^{a} ±25.96	0.0400
length of venom sac	2907.50 ± 265.75	2802.80 ± 101.83	2619.50 ± 227.25	2646.80 ± 149.00	2132.00 ± 533.54	3213.60 ± 170.22	3047.20 ± 155.87	3073.20 ± 216.66	0.1302
width of venom sac	1163.50 ± 90.30	1123.20 ± 108.27	962.00 ± 81.53	1076.40 ± 73.81	868.40 ± 224.51	1216.80 ± 23.83	1279.20 ± 68.00	972.40 ± 129.64	0.2228
acid gland width	32.50 ± 11.09	36.00 ± 2.45	40.00 ± 0.00	30.00 ± 8.37	32.00 ± 9.17	56.00 ± 5.10	37.00 ± 9.95	40.00 ± 3.16	0.2675

Those not sharing a common alphabet within a respective column are significantly different from each other.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

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