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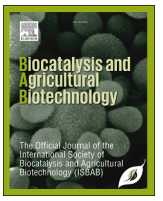

Development, gut health, and longevity of European bee on the provision of biosugar syrup

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ABSTRACT

Syrup containing monomeric sugars was prepared from banana-peel waste by hydrolyzing it with cellulose as well as pectinase to feed honeybees instead of using sucrose syrup in the period when there is no nectar in flowers. This syrup was provided to the caged workers with and without the addition of monomeric sugars (glucose, fructose) for a period of two weeks. It was observed that worker bees belonging to experiment No. 2 (pollens + 25% w/v glucose in BPW sugar syrup) had a significant increase in longevity. In addition to this, morphometric and histological analyses were also done. Morphometric studies confirmed reasonable increases in different body parameters of worker bees. Their hypopharyngeal gland became developed with a mean acinal surface value of 0.019 ± 0.0 mm^2 as compared to the control's 0.0124 ± 0.0 mm^2. Sections of the midgut (stomach) and small intestine up to 8 μm were prepared and observed under camera fitted microscope. Bees of experiment No. 2 had peritrophic membranes in close contact with rabdorium-like control bee's midgut, nuclei stained hyper chromatic, considerable increase in gastric cells discharging, and compact and homogeneous mass of semi-digested pollens in the central part of the intestinal lumen which indicates the improved digestion. The mean midgut lumen diameter was 450 ± 34.6 μm^2 as compared to the control's 240.0 ± 30.5 μm^2 and the mean intestinal lumen diameter was 223.3 ± 33.3 μm^2 as compared to the control's 113.3 ± 38.4 μm^2 which is an indication of tissue development. In the end, it is concluded that the use of biosugar syrup is good alternative to sucrose sugar syrup for honeybees.

1. Introduction

Additionally, to contribute to pollination, honeybees make honey, which has high economic value, and these are also a source of other goods like propolis, pollen, and the collection of waxy resin from leaf buds. All these, three products are used as foodstuff as well as in the medicinal and dietary supplements industries. In beekeeping practice, it is common to use sugar syrup to feed bees, but also different types of carbohydrate products available on the market, such as: invert syrup, high fructose corn syrup (HFCS), and starch syrup (Ohe von der and Schönberger, 2000; Ohe von der and Schönberger, 2002; Rybak-Chmielewska and Konopacka, 2005; Ceksteryte and Racys, 2006a,b; Rybak-Chmielewska et al., 2006a,b; Rybak-Chmielewska et al., 2006a,b; Rybak-Chmielewska, 2007; Leblanc et al., 2009; Sammataro and Weiss, 2013a,b; Semkiw and Skubida, 2016; Matescu et al., 2015; Krainer et al., 2015; Abou-Shaara, 2017). For winter feeding, beekeepers can use sugar syrup, sucrose invert syrup, starch syrup, or high fructose corn syrup (HFCS) (Jachimowicz and El Sherbiny, 1975; Skubida, 1998; von der Ohe and Schonberger, 2002; Liebig, 2005; Ceksteryte and

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Racys, 2006a,b; LeBlanc et al., 2009; Brodschneider and Crailsheim, 2010; Sammataro and Weiss, 2013a,b; Krainer at al., 2016). Molasses (a mixture of water and pure sucrose from sugar beet or sugar cane) has been used as bee food for many years (Free and Spencer-Booth, 1961; Barker, 1974; Bobrzecki, 1976; Gromisz, 1985). Carbohydrates present in the bee diet may be absorbed by the gut to sustain the bee or metabolized by gut bacteria prior to absorption (Baxter et al., 2019). It has been observed that large amounts of fruit waste are generated every year around the world. These wastes are either used uneconomically or disposed of as such, thus creating a serious pollution problem (Christy et al., 2014; Wadhwa and Bakshi, 2013). This cellulosic biomass (fruit waste) can be converted into monomeric sugars by chemical pretreatments or enzymatic hydrolyses which have two prominent fates; either is to be used by ethanologenic microbes for ethanol production, or it can also be used as sugar syrup for honeybees in dearth. Cho et al. (2017) experimented with the use of banana peel waste and proved it as a future alternative feed for bees. Fig. 1 explains the high yield of royal jelly production by developing the hypopharyngeal glands of experimental workers on the provision of biosugar syrup. Therefore, the present research study had been designed to valorize the potential of banana peel waste for monomeric sugar production for *Apis mellifera* workers in the dearth period (see Fig. 2) (see Table 1). As enzymes were used in the current study and these are living entities, so the syrup produced in this way was named as biosugar syrup.

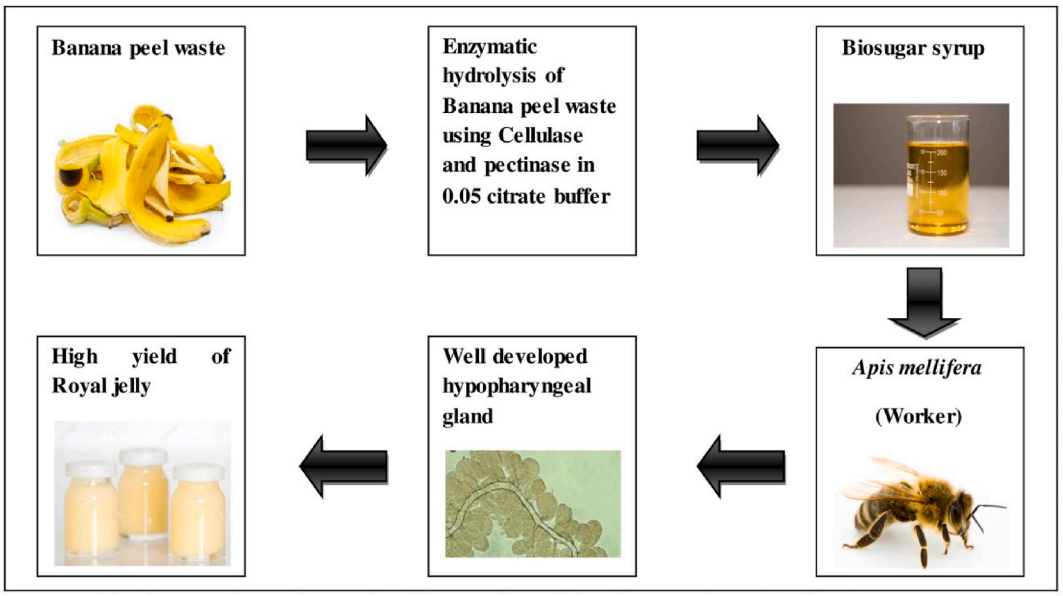

Fig. 1. Figureexplaining the fruitful outcomes of biosugar syrup provision.

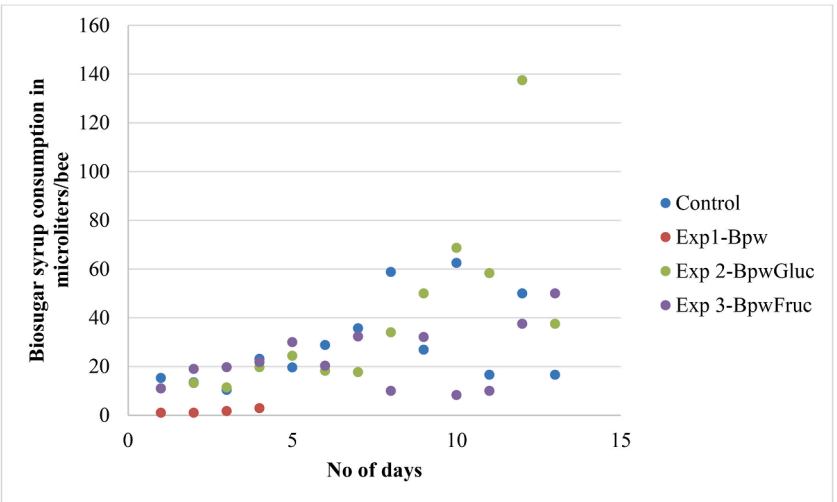

Fig. 2. Biosugar syrup consumption records of worker bees.

Table 1
Treatment details of experimental groups provided with biosugar syrup.

Group name	Experimental code	Treatment
Control	–	Pollens +50% (w/v) sucrose in distilled water
Experiment 1	Exp1-Bpw	Pollens + BPW sugar syrup
Experiment 2	Exp2-BpwGluc	Pollens +25% (w/v) glucose in BPW sugar syrup
Experiment 3	Exp3-BpwFruc	Pollens +25% (w/v)fructose in BPW sugar syrup

2. Materials and methods

To have bees of the same age for the experimental trials, the combs (frames) with large sealed brood areas were selected and placed in an incubator at 34 °C according to Williams et al. (2013). Combs (frames) were collected from the honeybee research garden at Punjab University (31.49758° N; 074.29679 E) and Incubated at 34 °C for hatching brood. 24-h post-incubation, newly emerged workers (0–24h) were gathered in cages made of plastic jars after Hasan et al. (2022). Each cage with an initial number of 50 workers was selected for the trial following Evans et al. (2009) and Martín-hernández et al. (2009). These cages were kept in an incubator at 33 °C temperature and 70% humidity. The experiment was conducted from 5 to 10–2020 to 20-10-2020.

2.1. Preparation of banana-peel powder

Banana peels were collected and cut into tiny pieces using a knife. These pieces were dried in sun for a period of 2–3 days and they were finally dried in an electric oven at 60 °C for about 1 day. These pieces were then crushed in a grinder and a fine powder was obtained. This powder was saved in an airtight plastic container till further use.

2.2. Enzymatic hydrolysis

From enzymatic hydrolysis of BPW, BPW-ABF (an alternative bee feed) was produced following Cho et al. (2017). For this purpose, enzymatic hydrolysis was performed in an Erlenmeyer flask in citrate buffer (0.05 M, pH 4.8).Citrate buffer was made and diluted to 0.05M with pH 4.8 following Ghose (1987). In citrate buffer, dry matter 1% (w/v). 8.4 mg each of cellulase (C2730, CAS Number: 9012-54-8, Lot No: SLBS6244, Sigma Aldrich) and pectinase (Lot No: GD 1610662, Uni-chem) were added per gram of BPW and incubated at 45 °C for 24 h.

2.3. Measuring rate of sugar syrup consumption by worker honeybee

Carbohydrate consumption by caged worker honeybees was measured by estimating the change in sugar syrup weight/volume for a specific duration of time after (Barker, 1974). When the feeder was filled with sugar syrup its initial mass was recorded as (Mass-i). The feeders were then fitted to cages, date and time were recorded as initial time (Time-i). The number of worker honeybees was also recorded as workers' initial. After every 24h, the feeders were removed from cages, and the date and time of removal were recorded as time final (Time-f) and the mass of the feeder was measured as mass final (Mass-f). The rate of sugar syrup consumption was measured by subtracting the mass final from the mass initial. By dividing consumed feed by hours, hourly cage consumption was calculated. The rest of the worker bees were considered workers final. So, by dividing the consumed sugar syrup in an hour by the worker's final, the worker's consumption of sugar syrup in an hour was calculated. In the end, by multiplying the sugar syrup consumed hourly by workers by 24, daily workers' consumption of sugar syrup was calculated. Feed consumption was calculated in tabulated form by the process described above for all groups in an experiment for two weeks.

After two weeks of administration of biosugar syrup, except for experiments No. 2 and 3, all other experimental groups including the control group showed a hundred percent mortalities. So the live five workers of these experimental groups were taken from cages and kept in a freezer at −20 °C for 2 h to put them to endless sleep following (Naug and Gibbs, 2009).

2.4. Morphometric analyses of worker's body parts

Body weight was measured using an electronic balance (Shimadzu AY 220) in (mg) and body length was recorded using a millimeter scale. The resting seven characters pertaining forewing length, forewing width, hindwing length, hindwing width, femur length, tibia length and width of hind metatarsus were measured using stage micrometer and the obtained values were converted into millimeters.

2.5. Measurement of hypopharyngeal glands

To expose the hypopharyngeal glands, the heads of five workers were separated by cutting with a sharp razor blade. The isolated glands were then preserved in 10% formaldehyde solution. Glands were placed on a cavity glass slide with a drop of formalin and coverslipped. The length and width of 15 acini were recorded under a binocular Bio-plus microscope (AVI-505) on hundredfold magnification. Acinal surface area was calculated by applying the formula

$$\text{Acinal surface} = \pi \times \frac{\boldsymbol{a} \times \boldsymbol{b}}{\mathbf{2}}$$

where:

‘a’ is length, ‘b’ is width, and $\pi = 3.14$ according to Maurizio (1954).

2.6. Dissection and histological studies of honeybees

Before dissection, bees were placed on blotting paper for a while to get all preserving material absorbed from the bee body surface. Fine needles, scissors, forceps, a petri plate filled with melted wax, and a wire about 150 mm long were used in dissection. A binocular dissecting microscope was used to separate bee body parts more clearly following Carreck et al. (2015). First of all, the proboscis, wings, and legs were cut down and saved for morphometric analyses. Later on, the bee was embedded in wax by making a furrow with a heated wire. After exposing a ventral side of the bee, two lacerations were made superficially along the lateral side of scleritis that started at the posterior end of the abdomen and terminated close to the petiole. The stomachs, as well as the intestines of bees, were then excised and preserved in 10% formalin. This procedure was done for every experimental worker.

2.7. Histological analysis

Histological changes in the stomach and intestine of experimental bees were examined by Bzymas et al. (2012).For this, formalin-fixed tissues were dehydrated in grades of ethyl alcohol (30, 50, 70, 90, and 100%), then transferred to xylene for tissue clearing and embedded in the embedding agent. Blocks were cut using a rotary microtome (model MRS, Japan) in 8 μm thick sections and mounted on slides. After drying for some period, slides were first deparaffinized by keeping these in xylene, secondly rehydrated in ethanol grades, and then stained with H&E. Slides were again passed through graded alcohol and cleared using xylene and cover-slipped. These preparations were examined under a high-power microscope (Utech Schenectady, NY12305).

2.8. Statistical analysis

At the termination of the experiment, the difference between the means of feed consumption rates, bee longevity, hypopharyngeal gland development, midgut, and intestinal lumen diameters was calculated and compared through two-way ANOVA without replication, *t*-test, and one-way analysis of variance (ANOVA) respectively using SPSS and SAS 9.1 (see Table 2).

3. Results

3.1. Feed consumption and longevity

Control bees which were fed with pollen and sugar syrup for two weeks, depicted the average value of bee feed consumption as 27 μl/bee. Whereas, mean values for sugar syrup consumption in Exp1-Bpw, Exp2-BpwGluc and Exp 3-BpwFruc were 1.3, 36 and 22, respectively ($F = 1.809$, $P = 0.167$, $DF = 3$). Bio-sugar syrup consumption increased in Exp17-BpwGluc on days 3, 5, 9, 10, 11, 12, and 13, and Exp 3-BpwFruc on days 2, 3, 5, 9, and 13 (Table 3 and Fig. 3). Maximum increase in sugar syrup consumptions up to 25% was recorded for the bees fed with pollens +25% (w/v) glucose in BPW sugar syrup as compared to the control value and the difference was found statistically significant. Whereas the bees fed with pollens + BPW sugar syrup and pollens +25% (w/v) fructose in BPW sugar syrup consumed 95 and 20% lesser sugar syrup as compared to the control value.

Control bees had a mean mortality value of 4. Whereas worker bees of Exp1-Bpw, Exp 2-BpwGluc, and Exp 3-BpwFruc showed mean mortality values of 8.3, 3.2, and 3.3, respectively. In the case of the control group, 100% mortality was recorded. Whereas Exp1-Bpw, Exp 2-BpwGluc and Exp 3-BpwFruc depicted percentage mortalities 100, 92 and 94%, respectively ($p = 0.013 < 0.05$, $F = 4.192$, $DF = 3$). In the case of experiments 2 and 3, the mortalities decreased by 20 and 17.5% significantly (Table 3, Fig. 3).

3.2. Morphometric analyses

Control bees that were fed with pollen and sugar syrup for two weeks depicted the mean values (mm) of 116.03 ± 5.97, 14.00 ± 0.00, 9.20 ± 0.20, 3.17 ± 0.03, 6.63 ± 0.09,1.87 ± 0.03, 2.47 ± 0.09, and 2.97 ± 0.03, 1.12 ± 0.03for body weight, body length, forewing length, forewing width, hind wing length, hind wing width, femur length, tibia length, and metatarsus width,

Table 2
Details of sugar syrup consumption by bees fed with bio-sugar (produced form BPW).

Treatments	Mean sugar syrup consumption (μl)/bee	F-value	P-value	DF
Control	26.9857	1.809	0.167	3
Exp1-Bpw	1.3			
Exp2-BpwGluc	35.8286			
Exp3-BpwFruc	21.5714			

Means were compared applying two factor ANOVA without replication at $\alpha = 0.05$.

Table 3
Mortality record of bees after feeding with bio-sugar (produced form BPW).

Treatments	Mean mortalities	F-value	P-value	DF
Control	4	4.192	0.013	3
Exp1-Bpw	8.3333			
Exp2-BpwGluc	3.2857			
Exp3-BpwFruc	3.3571			

Means were compared applying two factor ANOVA without replication at $\alpha = 0.05$.

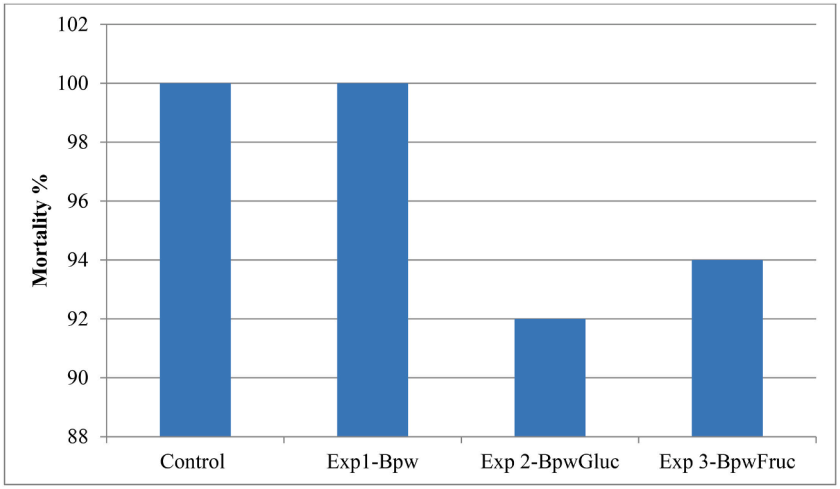

Fig. 3. Mortality records of worker bees fed on biosugar syrups.

respectively. Following two weeks of the feeding of bio-sugar syrup made from BPW, i.e., Exp 2-BpwGluc and Exp 3-BpwFruc, the mean ± SE values for these experimental bees were: body weight (120.62 mg ± 8.33 and 102.27 mg ± 14.54), body length (14.40 mm ± 0.24 and13.67 mm ± 0.33), forewing length (9.34 mm ± 0.09 and 9.35 mm ± 0.10), forewing width (3.20 mm ± 0.00 and 3.17 mm ± 0.03), hind wing length(6.50 mm ± 0.03 and 6.57 mm ± 0.03),hind wing width (1.85 mm ± 0.03 and 1.83 mm ± 0.03), femur length (2.48 mm ± 0.04 and 2.43 mm ± 0.03), tibia length (3.00 mm ± 0.03and 3.05 mm ± 0.03) and metatarsus width(1.08 mm ± 0.03 and 1.15 mm ± 0.03). In the case of experiment 16, 100% mortality was observed (Table 4).

3.3. Hypopharyngeal gland development

Control bees which were fed with pollen and sugar syrup for two weeks depicted the mean ± SE value of acinal surface area as 0.012447 ± 0.0009 mm^2. The acinal surface area of HPG from bees of Experiment No.2 was found as 0.01955 ± 0.001 mm^2, ($p = 0.0001$, $DF = 47$). Different treatments of organic acids and probiotics affected the hypopharyngeal gland positively by increasing acinal surface area, Table 5.

Table 4
Different morphometric characteristics of bees supplemented with BPWand monomeric (glucose, fructose) sugars.

Parameter	Mean ± SE			P-value	DF
	Control	Exp 2-BpwGluc	Exp 3-BpwFruc		
BW	116.03 ± 5.97	120.62 ± 8.33	102.27 ± 14.54	0.4453	2
BL	14.00 ± 0.00	14.40 ± 0.24	13.67 ± 0.33	0.1695	
FWL	9.20 ± 0.20	9.34 ± 0.09	9.35 ± 0.10	0.6958	
FWW	3.17 ± 0.03	3.20 ± 0.00	3.17 ± 0.03	0.4408	
HWL	6.63 ± 0.09	6.50 ± 0.03	6.57 ± 0.03	0.2184	
HWW	1.87 ± 0.03	1.85 ± 0.03	1.83 ± 0.03	0.8227	
FL	2.47 ± 0.09	2.48 ± 0.04	2.43 ± 0.03	0.8298	
TL	2.97 ± 0.03	3.00 ± 0.03	3.05 ± 0.03	0.3149	
MTW	1.12 ± 0.03	1.08 ± 0.03	1.15 ± 0.03	0.3317	

Differences between values of respective characteristics have been found statistically nonsignificant.
Abbreviations: BW = body weight, BL = body length, FWL = forewing length, FWW = forewing width, HWL = hind wing length, HWW = hind wing width, FL = femur length, TL = tibia length, MTW = metatarsus width.

Table 5
The means compared by applying Two Sample *t*-test for assessing hypopharyngeal gland development.

Treatment	Mean ± SE	P-value	DF
Control	0.012 ± 0.0009	<0.0001	47
Exp 2-BpwGluc	0.019 ± 0.001		

3.4. Histological analyses

3.4.1. Midgut

In the case of the control group, it was found that the wall of the midgut was wrinkled properly, the peritrophic membrane was in contact with rabdorium, nuclei were stained well, and columnar epithelial cells were arranged in a good array. Gastric cells were visible. Mean lumen diameter was 240.00 ± 30.55 μm² (Plate2, Fig C and C1, Table 6, P = 0.0030, D.F = 2).

Exp2-BpwGluc = lumen diameter was 450 ± 34.641 μm², peritrophic membranes were in close contact with rabdorium-like control, nuclei stained hyperchromatic, and gastric cells were dispersed in huge numbers (Plate2, Fig D and D1, Table 6, P = 0.0030, D.F = 2).

3.4.2. Intestine

Control = Homogeneous mixture of pollen was clanged with peritrophic membranes and both of these were very close to the intestinal epithelium. The mean lumen diameter was 113.33 ± 38.44 μm² (Plate 1, Fig. A and A1, Table 6, P = 0.0027, D.F = 2).

Exp2-BpwGluc = Compact and homogeneous mass of pollen feed were in the central part of the lumen. The mean lumen diameter was 223.3333 ± 33.333 μm² (Plate1, Fig. B and B1, Table 6, P = 0.0027, D.F = 2).

4. Discussion

The purpose of this study was to valorize the potential of banana peels to produce syrup for experimental workers following the methodology of Cho et al. (2017). In the current experiments, pollens + biosugar syrup, pollens +25% (w/v) glucose in BPW sugar syrup, and pollens +25% (w/v) fructose in BPW sugar syrup were provided to *Apis mellifera* workers as sucrose alternatives. The biosugar syrup produced by the enzymatic hydrolysis of banana peel waste can be proved as a good alternative feed for honeybees when there is no nectar in flowers.

Table 6
Midgut and intestinal lumen diameters of bees fed with (BPW).

	Control	Exp2-BpwGluc	DF	P-value
Midgut	240^{b} ± 30.551	450^{a} ± 34.641	2	0.0030
Intestine	113.33^{b} ± 38.442	223.33^{a} ± 33.333	2	0.0027

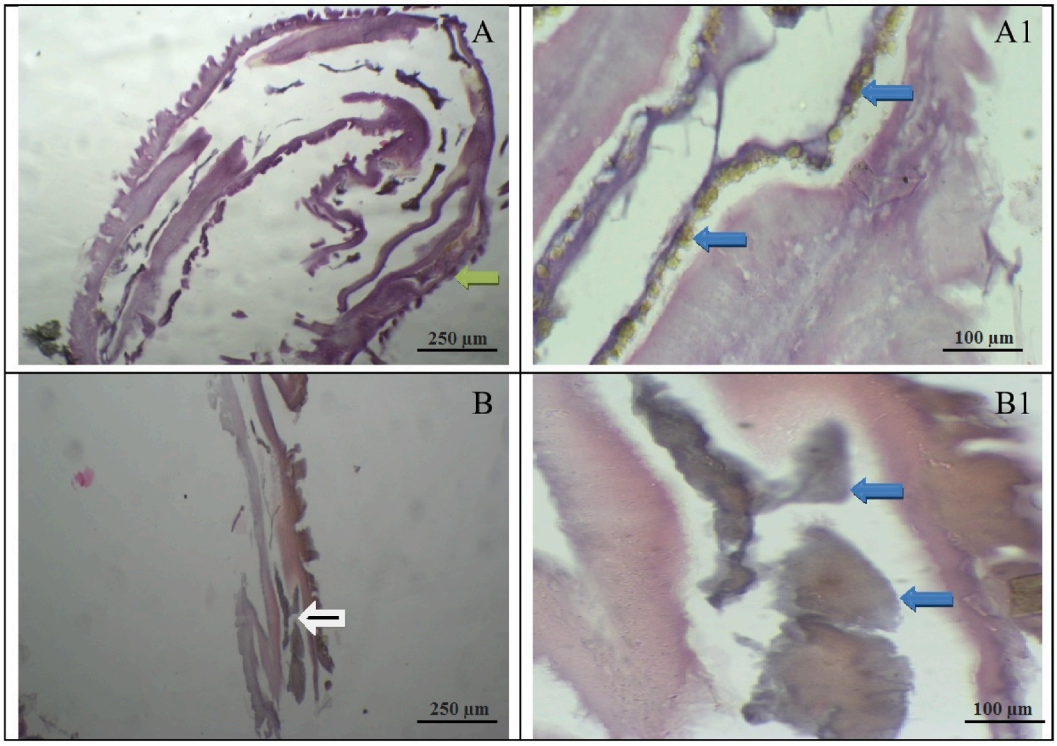

Plate 1. Intestinal histology of worker bees fed on biosugar syrup. Green arrow: muscle layer, Blue arrow: pollen wrapped in peritrophic membranes, Black and white arrow: the highlighted portion in (A1 and B1). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

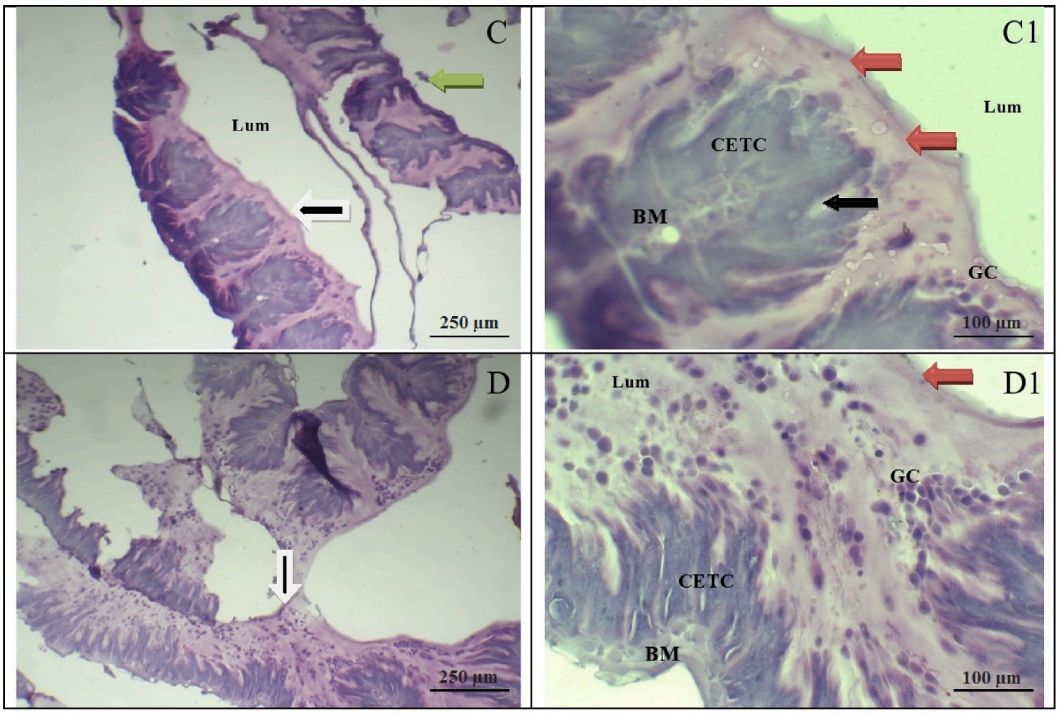

Plate 2. Midgut histology of worker bees fed on biosugar syrup. Green arrow: muscle layer, Red arrow: peritrophic membranes, Blue arrow: pollen wrapped in peritrophic membranes, Black and white arrow: the highlighted portion in (C1 and D1), Orange arrow: cytoplasmic vacuolization, BM: basement layer, CETC: columnar epithelial cells, Lum: lumen, GC: gastric cells.. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Crude fibers, sugars, crude protein, and ash contents are present in banana peel waste. These also include a rich quantity of phosphorus, calcium, iron, magnesium, sodium, copper, potassium, manganese, and zinc so the peel waste can be proved as good animal feed (Hassan et al., 2018). All these ingredients ensure the good body development of animals as confirmed in the current experiments regarding the overall body development of worker bees and especially the hypopharyngeal gland. Biosugar syrup was consumed more willingly by bees in experiment No. 2. In the case of experiments No 2 and 3, increases in syrup consumption were observed when the bio-sugar syrup was mixed with glucose and fructose respectively. In addition to this, the significant increase in the acinal surface of the hypopharyngeal gland in experiment No. 2 (Pollen + 25% (w/v) glucose in BPW sugar syrup). Well-developed hypopharyngeal glands produce more royal jelly than less developed ones.

Morphometric analyses help in predicting the productivity of a colony in such a way that honeybees having big-sized wings and legs have the ability to gather more quantity of nectar and pollen for colony population and brood rearing (Mostajeran et al., 2006). Similarly, lengths of fore and hind wings, the width of fore and hind wings, tongs' length, tibia, femur, and metatarsus have been related to honey production (Hasan et al., 2022).

To discuss the histological analyses it is stated that, in insects, most of the digestion and absorption of nutrients and chemicals takes place in the midgut. In this region of most insects, food is surrounded by a cell layer of a semipermeable membrane called the peritrophic membrane (PM), which has a number of functions (Cruz-Landim, 1985).It was found that in experiment 2, the peritrophic membranes (Some Functions of peritrophic membranes) were in close contact with rabdorium, increase in gastric cell discharge was observed. The mean lumen diameters of the stomach and intestine were 450 $\pm$ 34.641 μm^2 and 223.3333 $\pm$ 33.333 μm^2 respectively. No complete vacuolation of columnar cells was seen. Regenerations crypts were saved.

Life span no doubt provides a permanent clue about the health of honeybees. Two prominent factors on which the life span of bees depends include immunity and nutrition according to Li et al. (2019). Our results regarding the longevity of bees are similar to Cho et al. (2017) in which bees when fed solely on bio-sugar syrup exhibited 100% mortality, and the addition of monomeric sugars to biosugar syrup resulted in decreased mortality of bees. The reason is that the biosugar syrup produced by banana peels by enzymatic hydrolysis contained a little bit amount of monomeric sugars in it which did not fulfill the carbohydrate requirements of honeybees as an energy source. And when glucose and fructose were added to biosugar syrup, the mortality rate of bees became decreased.

5. Conclusion

It is concluded that the biosugar syrup proves a good alternative to sucrose syrup and can be presented to honeybees in times when there is no nectar in flowers. The basic requirement in promoting this methodology is to make it understandable to end users: the farmers and beekeepers, so it can easily be accustomed.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

Acknowledgment

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