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Original article

Effects of sugar feeding supplemented with three plant extracts on some parameters of honey bee colonies

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ABSTRACT

Sugar feeding is crucial to bee colonies during periods without natural nectar resources. The health and the development of bee colonies are affected by the sugar feeding type. Also, some materials can be added to the sugar feeding to boost the ability of bee colonies to withstand parasites. Three materials (mint, cinnamon, and chamomile) are used commonly to control bee parasites (e.g. *Varroa* mites). In the present study, the effects of these materials on the development and health of bee colonies were assessed. Sugar candy supplemented with these materials plus sugar candy only as a control group were tested. Bee colonies were fed with these feeding types weekly. Then, some parameters were evaluated. The results showed the suitability of the tested feeding types to bee colonies. Building of wax foundations was accelerated in cinnamon group. This group had also the lowest infestation rates with *Varroa* mites, suggesting a specific role of cinnamon in *Varroa* control. The colony development was significantly better in chamomile group than the other groups. Mint group showed no variations than the control group in most parameters. All feeding types showed safety to bees based on morphological characteristics and bee survival results. Practically, cinnamon is advised when building of wax combs is required while chamomile is recommended when increasing strength of colonies is needed. The role of cinnamon in controlling *Varroa* is recommended for further investigations.

1. Introduction

Honey bees, *Apis mellifera* L., require sugar and protein feeding to survive. The bees depend on natural sources especially nectar as source of carbohydrates (Brodschneider and Crailsheim, 2010) and pollens as sources of protein (Huang, 2012). Honey bees require high amounts of nectar per year (Brodschneider and Crailsheim, 2010) especially bee colonies contain thousands of individuals (Southwick and Heldmaier, 1987). However, flowering plants are not available for bee colonies all seasons which expose bees to starvation. Therefore, beekeepers provide their colonies with sugar feeding (i.e. sugar syrup or sugar candy) (Abou-Shaara, 2017). The main aim of such artificial sugar feeding is to prevent the decline of bee colonies due to the absence of nectar sources. The periods between honey seasons and winter are the main times for providing bees with sugar feeding. Indeed, colony losses are regularly reported during winter (Brodschneider et al., 2010; Nguyen et al., 2010; Spleen et al., 2013; Al-Ghamdi et al., 2016). Also, exposing bees to starvation at any time of the year can destroy bee colonies.

There are different sugar types that can be used for bee feeding. However, sucrose is the prefect alternative to nectar (Barker, 1977) than other alternatives including grape syrup (Barker and Lehner, 1978) and sugarcane juice (Carrillo et al., 2015). Moreover, sucrose has high sweetness (Hough and Phadnis, 1976). Fermentation can occur to liquid feeding unlike sugar candy, suggesting the benefits of candy over syrup (Abou-Shaara, 2017). Also, candy showed better results in enhancing survival of bees than liquid feeding (Abou-Shaara, 2017; Abou-Shaara et al., 2017). On the other side, there are some plant materials including mint, cinnamon, and chamomile that can be utilized to enhance bee health of bee colonies.

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These materials can assist in controlling bee pathogens including *Varroa* mites and *Nosema* (Abd El-Wahab et al., 2012; Castagnino and Orsi, 2012; Goswami and Khan, 2013; Kotwal and Abrol, 2013; Abou-Shaara, 2014; Abou-Shaara, 2018a). Also, feeding bees on such materials has shown improvement to bee health. However, supplying sugar candy with these materials on regular basis has not been studied to find out the potential effects on comb building, colony development and infestation rates with *Varroa* mites. Moreover, the potential effects of these materials on the survival of honey bees have not been investigated to find out any side effects.

In addition to these points, nectar or its alternatives (i.e. carbohydrates) are required during the development of bee larvae (Rortaiset al., 2005; Brodschneider and Crailsheim, 2010). It's known that bee larvae are supplied with feeding by workers bees; hence, plant extracts can reach to larvae during feeding and may cause effects on bee morphology. This point has not been studied sufficiently. In the present study, the potential effects of using sugar candy supplemented with plant extracts (mint, cinnamon, and chamomile) on some parameters of bee colonies were studied to find out the best plant extract. Also, the safety of these plant extracts to bees was evaluated by assessing survival ability of bee workers and effects on bee morphology.

2. Materials and methods

2.1. Preparing sugar candy and bee colonies

Sugar was firstly dissolved in warm water (1 kg/1000 ml water) then 4 g of dried plant materials: chamomile flowers (Family: Asteraceae; *Matricaria chamomilla*), mint leaves (Family: Lamiaceae; *Mentha Spicata*), and cinnamon (Family: Lauraceae; *Cinnamomum Zeylanicum*) were mixed with the sugar syrup separately, and subsequently filtered to remove plant parts. These syrup stocks were used to prepare the candy by mixing them with powdered sugar, and fresh sugar syrup stocks were prepared each two weeks. Thus, three candy treatments (chamomile, mint, and cinnamon) plus the control treatment (i.e. sugar candy prepared using sugar syrup only) were compared in this study. These treatments were presented weekly for bee colonies (300 g per colony), and four colonies were allocated per each treatment (a total of 16 colonies).

The colonies contained only three combs (one brood comb, one food comb, and an empty comb) at the beginning of the experiment and headed by young Carniolan hybrid queens (<1 year old). The experiments were performed during summer 2020 after clover season in Damanhour, Egypt. Under normal conditions, bee colonies depend on the artificial feeding (sugar feeding) during this period after the harvest of clover honey. So, few nectar/pollen resources mostly from roadside trees and herbs occurred during the experiment period. This means that the bees depended primarily on the test candy types as sources of carbohydrates during the study.

2.2. Preference of bees for feeding type

Five colonies with equal strength and not among the 16 colonies used in the other experiments were used to test the preference of bees for feeding types. The four feeding types (100 g from each feeding type) were added for the five colonies above frames. Then, the consumption of each feeding by bees was assessed after 72 h (= 100 − weight of feeding after 72 h) and the most preferred feeding type was specified.

2.3. Building wax foundation

The abilities of bees feed on the test feeding types to build wax foundations were assessed after three weeks from the start of the experiment. Each colony was provided with a wax foundation (~20 × 40 cm) and after 72 h the percentage of the drawn wax area was calculated: (the drawn area ÷ total area) X100, and compared between treatments.

2.4. Colony development

Empty combs were added to the colonies according to their need during the study. The development of bee colonies was assessed after 10 weeks from the start of the experiment by determining the approximate comb areas covered with adult bee workers and sealed brood areas. These areas were determined using a grid frame (Jeffree, 1958). The measurements were initially taken in square inches and then transferred into square decimeters (Abou-Shaara et al., 2013).

2.5. Infestation rates with Varroa mites

The *Varroa* infestation rate was determined to be about 1% in the colonies at the beginning of the study. This rate was assessed again after 10 weeks to follow up any changes in the infestation rates. The method of powdered sugar was used to evaluate the infestation rate with *Varroa* mites (Dietemann et al., 2013).

2.6. Safety to bees

Two experiments were done to ensure the safety of the used plant extracts as feeding to bees. In the first experiment, some morphological characteristics of bee workers from each feeding group were measured and compared with the control group and reference values. The absence of huge variations than the control group or the reference values can indicate the safety of the used materials. Especially, these materials can reach to bee larvae during feeding and before their complete development. In the second experiment, survival of bee workers were assessed and compared with the control group. The similarities between the feeding groups and the control groups in survival rates can ensure the safety of these materials to bee workers.

2.6.1. Morphology of bee workers

Some morphological characteristics were used to test any potential negative effects of the added plant extracts on the normal development of bees especially these materials can reach to larvae during feeding. Morphological variations between bee workers from the same colony and from different colonies are normal. So, characteristics for hybrids of Carniolan bees measured by Abou-Shaara and Ahmed (2015) were used as reference values to judge the effects of the used materials, considering abnormal values (i.e. very low values) than the reference values as deleterious effects of plant extracts. Five characteristics (namely; head width, forewing length and width, hind wing length and width) related to body size and colony productivity (Waddington, 1989; Edriss et al., 2002) were measured as described by Ruttner et al. (1978). The measurements were taken using Scan Photo Technique (El-Aw et al., 2012) for twenty bee workers from brood combs of each colony (a total of 80 workers per group).

2.6.2. Effects on survival of bee workers

The survival of bee workers under laboratory conditions was evaluated using caged bees at room temperature of about 30 °C. Four small jars with perforated covers were assigned to each candy group (a total of 16 cages), and in each cage 25 bees from brood combs were placed (100 bees per candy type). The candy was available to the caged bees through the perforated covers, and the candy was replaced every two days (5 g at each time). So, the bees were able to absorb sugar syrup mixed with plant material from the fresh candy regularly. The survival of bees was recorded daily and up to 8 days. Then, the survival results were compared to detect any negative effects of the test candy types on bees.

2.7. Statistical analysis

Data of each experiment were subjected to normality tests using Shapiro-Wilk and Kolmogorov-Smirnov. Then, data with normal distribution were analyzed using parametric test (analysis of variance followed by Tukey HSD test) while data without normal distribution were analyzed using non-parametric test (Kruskal-Wallis test and pairwise comparisons using Mann-Whitney test). The survival of bees was analyzed using Kaplan-Meier test (Abou-Shaara, 2018b), calculating the estimated survival means and Mantel-Cox test for significant differences. The significance level was $P \leq 0.05$ and the analysis was done by SPSS (Version 16, Chicago, USA, 2007).

3. Results

3.1. Preference of bees for feeding types

The bees were able to consume from 56.1 to 100 g of the presented feeding within 72 h (Table 1). Mint candy was consumed more than the other candy types, follow by Chamomile candy, then cinnamon candy, and finally sugar candy (control). The difference between mint candy and Chamomile candy, cinnamon candy, and control was 3.14, 8.12, and 19.48 g, respectively. The comparison between the consumed amounts of the tested candy types showed the absence of significant differences (Kruskal-Wallis Test: Chi-Square = 2.43, df = 3, Sig. = 0.48). Two colonies were able to consume all amounts (100 g of each candy type) within 72 h while this period was not sufficient for the rest of the colonies to do so. The colonies differed significantly in their ability to consume all feeding types within 72 h (Kruskal-Wallis Test: Chi-Square = 13.06, df = 4, Sig. = 0.01).

3.2. Building of wax foundation

The percentages of drawn wax area on wax foundations are presented in Fig. 1. The percentages ranged from 52% (Chamomile) up to 95% (Cinnamon), and the lowest mean was to chamomile group (67.75%) while the highest mean was to cinnamon group (82.75%) with difference of 15%. However, there were no significant differences between treatments according to one way ANOVA (df between groups = 3, F = 1.36, P = 0.3 > 0.05).

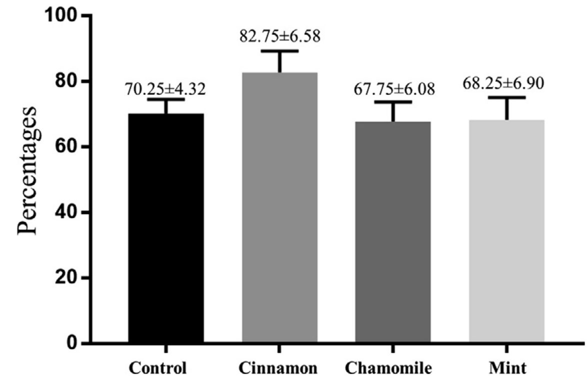

Fig. 1. The percentages of built wax area on wax foundations after 72 h for colonies fed with different feeding types. Means ± SE are presented for each candy type.

3.3. Colony development

The development was better in chamomile group than the other groups (Fig. 2). Chamomile group had the highest area of bees and sealed brood followed by cinnamon, mint, and finally control group, respectively. The variations between chamomile group and the other groups were significant (ANOVA for area of bees: df = 3, F = 14.19, p = 0.00 < 0.05 and ANOVA for sealed brood area: df = 3, F = 17.19, p = 0.00 < 0.05).

3.4. Infestation rates with Varroa mites

The *Varroa* infestation rates ranged from 0.66 (2 mites/300 bees) to 1.33% (4 mites/300 bees), 0 to 0.66% (2 mites/300 bees), 1 (3 mites/300bees) to 1.33% (4 mites/300 bees), and 0.33 (1 mites/300 bees) to 1.33% (4 mites/300 bees) for control, cinnamon, chamomile, and mint groups, respectively (Fig. 3). The variations between groups were significant (ANOVA: df = 3, F = 5.863, p = 0 .011 < 0.05). The highest infestation rate was in chamomile group followed by control group without significant variations while the lowest infestation rate was in cinnamon group followed by mint group without significant variations (Fig. 3). Notably, colonies fed with candy supplemented with cinnamon had the lowest infestation rates with a maximum infestation rate of 0.66% (2 *Varroa*/300 bees).

3.5. Safety to bees

3.5.1. Morphology of bee workers

No significant differences between groups (candy supplemented with plant extracts and control) were found in three characteristics: head width (Kruskal-Wallis Test: Chi-Square = 3.47, df = 3, Sig. = 0.32), forewing width (Kruskal-Wallis Test: Chi-Square = 3.60, df = 3, Sig. = 0.308) and hind wing length (Kruskal-Wallis Test: Chi-Square = 6.17, df = 3, Sig. = 0.10). While significant differences were observed in the other characteristics: forewing length (Kruskal-Wallis Test: Chi-Square = 17.22, df = 3,

Table 1
The consumed amounts (mean ± SE, g) of each feeding type after 72 h by the five bee colonies.

Colony	Cinnamon	Chamomile	Mint	Control	Mean (g) ± S.E
1	81.5	82.1	90.7	56.1	**77.60 ± 7.46**
2	67	91.3	98.4	70.5	**81.80 ± 7.70**
3	100	100	100	65.1	**91.27 ± 8.72**
4	100	100	100	100	**100 ± 0.00**
5	100	100	100	100	**100 ± 0.00**
Mean (g) ± S.E.	**89.70 ± 6.71**	**94.68 ± 3.56**	**97.82 ± 1.80**	**78.34 ± 9.13**	

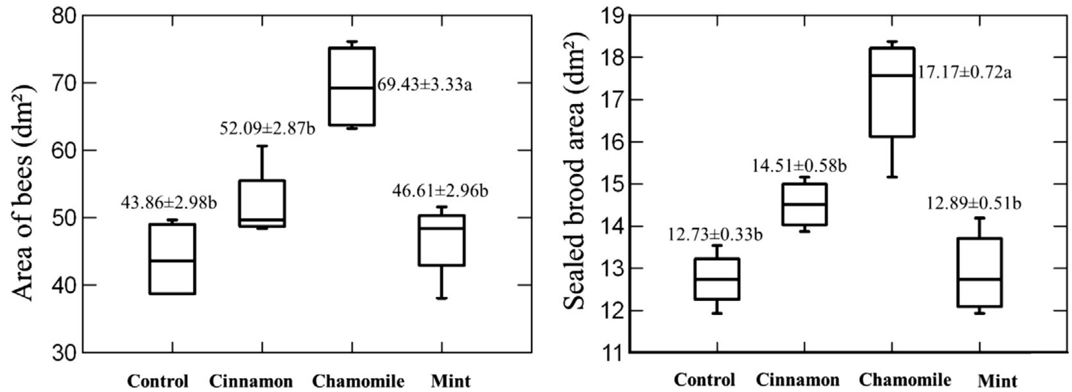

Fig. 2. Development of bee colonies fed with different candy types. The significant differences between candy types for area of adult bees (dm^2) and sealed brood are (dm^2) are indicated using different letters after means based on Tukey test.

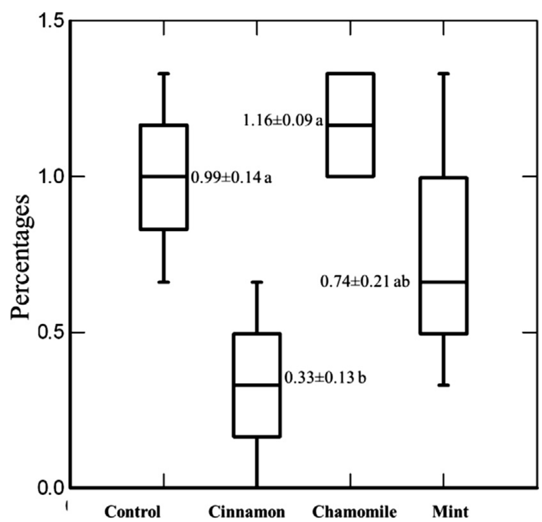

Fig. 3. Percentages of *Varroa* infestation in feeding groups. The significant differences between candy types are indicated using different letters after means based on Tukey test.

Sig. = 0.001), and hind wing width (Kruskal-Wallis Test: Chi-Square = 22.21, df = 3, Sig. = 0.00). The pairwise comparisons between the control group and treatment groups showed the significant variations in forewing length and hind wing width due to cinnamon and chamomile groups according to Mann-Whitney Test (Table 2). Also, very few variations were detected between control group and treatment groups generally.

The measured values in feeding groups differed than the reference values by 0.28–0.3, 0.01–0.05, and 0.14–0.12 mm for head width, forewing length, and forewing width, respectively (Table 3). Head width was higher in feeding groups than the reference value unlike forewing length and width. The measured values in feeding groups differed than the reference values by 0.01–0.03 and 0.01–0.02 mm for hind wing length and hind wing width, respectively (Table 3). The overall variations between the measured characteristics except head width for bee workers in feeding groups and reference group ranged only from 0.01 to 0.14 mm, suggesting lacking of deleterious effects of plant extracts on bees. The reference value was less than the feeding groups in head width.

3.5.2. Effects on survival of bee workers

The number of dead bees during the 8 days was very low for the studied feeding groups and was not more than 4 bees in a single day (Fig. 4). Only 4, 6, 11, and 13 bees out of 100 bees (25 bees in each replicate) died during the 8 days in the control group, chamomile group, mint group, and cinnamon group, respectively. The survival of bees in the test groups with plant materials showed no significant differences than the control group (Mantel-Cox test: df = 3, Chi-Square = 6.984, P = 0.072 > 0.05). The survival means (mean ± SE) were estimated to be 7.98 ± 0.02, 7.91 ± 0.06, 7.76 ± 0.10, and 7.74 ± 0.09 days for control group, chamomile group, mint group, and cinnamon group, respectively.

4. Discussion

4.1. Preference of bees for feeding types

This experiment showed the attractiveness of bees to all the tested candy types. In fact, the bees attracted to all candy types but consumed more mint candy than the other types within 72 h. The mint odor may be played a role in the attraction and consumption of mint candy by bees. It was clear that bees preferred the odor of mint over cinnamon and chamomile. The bees showed also preference to chamomile than cinnamon. The odors of these candy types perhaps affected the choice of candy by bees. All candy types were completely consumed by bees as shown from the weekly inspection of colonies. This means that the period of 72 h and the availability of all candy types for bee colonies at the same time was a challenge. The data showed the absence of significant differences between the consumed amounts of the tested candy types. This proves the ability of bees to consume all candy types; hence their suitability as feeding to bee colonies. All candy types

Table 2
Significance values according to pairwise comparisons between control group and treatment groups using Mann-Whitney Test. Significant variations are highlighted in bold.

Control	Characteristics (mm)	Cinnamon	Chamomile	Mint
Control	**Head width**	0.602 > 0.05	0.174 > 0.05	0.968 > 0.05
	Forewing length	0.350 > 0.05	**0.000 < 0.05**	0.756 > 0.05
	Forewing width	0.382 > 0.05	0.345 > 0.05	0.950 > 0.05
	Hind wing length	0.424 > 0.05	0.092 > 0.05	0.795 > 0.05
	Hind wing width	**0.000 < 0.05**	**0.001 < 0.05**	0.369 > 0.05

Table 3
Means ± SE (mm) of head width, forewing length and width, and hind wing length and width for bee workers from four feeding groups beside reference values (Abou-Shaara and Ahmed, 2015).

Feeding	Head width	Forewing length	Forewing width	Hind wing length	Hind wing width
Cinnamon	3.50 ± 0.01	8.65 ± 0.013	2.90 ± 0.007	6.05 ± 0.005	1.77 ± 0.006
Chamomile	3.52 ± 0.01	8.69 ± 0.012	2.92 ± 0.007	6.07 ± 0.005	1.76 ± 0.005
Mint	3.51 ± 0.01	8.63 ± 0.012	2.91 ± 0.006	6.06 ± 0.005	1.74 ± 0.006
Control	3.50 ± 0.01	8.64 ± 0.011	2.91 ± 0.007	6.06 ± 0.005	1.74 ± 0.005
Reference	3.22 ± 0.09	8.68 ± 0.04	3.04 ± 0.02	6.08 ± 0.05	1.75 ± 0.03

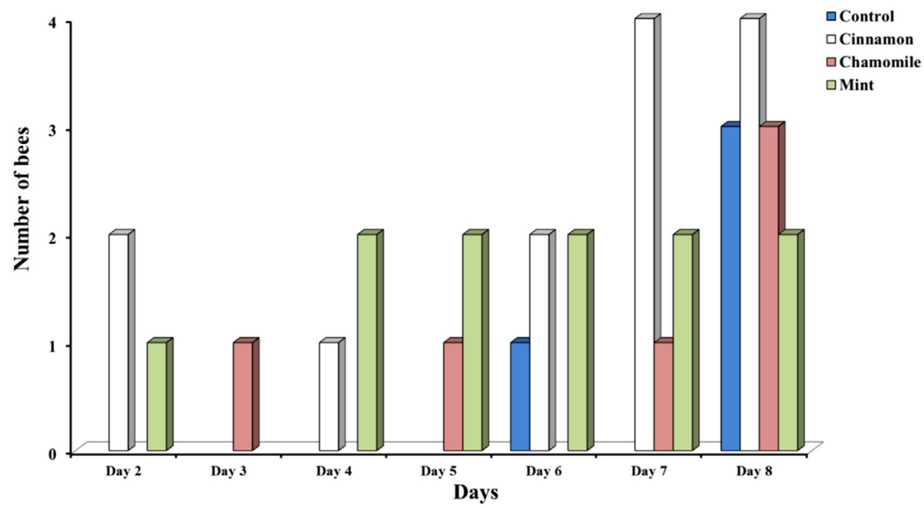

Fig. 4. Number of dead bees during the experiment in the four feeding groups (100 bees in each group).

contained sucrose, the most perfect feeding for bees as alternative to nectar with high sweetness (Hough and Phadnis, 1976; Barker, 1977; Abou-Shaara, 2017). So, the attractiveness and consumption was expected. This is supported by a previous study as bees attracted to different sugar feeding types in a choice experiment (Abou-Shaara, 2017). The plant extracts showed effects on the attractiveness due to their odors without impacting the suitability of sugar candy as feeding for bees.

4.2. Building of wax foundation

Building wax foundations require energy especially the consumption of nectar or its alternatives (Hepburn et al., 2014). The ability of bees to build wax combs is influenced by the type of sugar feeding (Carrillo et al., 2015). Colonies fed with cinnamon candy showed the highest ability to build wax foundations over the other tested candy types. This reflected the ability of this candy type to promote secretion of beeswax by workers more than the other candy types. The results also showed the absence of significant differences between colonies in the percentages of built wax area based on the overall means. This can be understood by the role of sugar candy in providing bees with energy especially all types of candy contained sucrose. However, the role of plant extracts especially cinnamon should not be neglected and beekeepers may use this type of feeding when building wax combs within short period of time is required.

4.3. Colony development

In fact, the development of bee colonies is impacted by sugar feeding (Bodla et al., 2009). The results showed the high ability of using chamomile in improving the strength of bee colonies. In fact, this can be understood by the role of chamomile in controlling pathogens that infect the abdomen of bees; therefore, improving the health of adult bees and colony development. This role of chamomile or similar plants was proved in previous studies including studies on *Nosema* (Michalczyk et al., 2016; Abou-Shaara, 2018a). Each of cinnamon and mint showed less positive effects on colony development than chamomile. This suggests that bee feeding can be supplemented with chamomile extract when enhancing the strength of colonies is needed.

4.4. Infestation rates with Varroa mites

Varroa mites are known to have a good ability to infest honey bee colonies and can be transmitted from colony to another during the foraging activity of bees (Peck et al., 2016). Therefore, the presence of *Varroa* mites in bee colonies from all the treatment groups was expected. The results showed that chamomile candy and control group had the highest infestation rates. This suggests that the extract of chamomile supplied as feeding to bees is not perfect in controlling *Varroa* mites. Such extract showed promising results towards the control of *Nosema* that infest bee gut (Abou-Shaara, 2018a). The results clearly showed the low infestation rates in cinnamon candy group. This suggests a potential use of this feeding type to combat *Varroa*. Accordingly, Goswami and Khan (2013) found efficacy of cinnamon oil against *Varroa* mites. Additionally, cinnamon oil in combination with other treatments showed a good efficacy against these mites (Kotwal, and Abrol, 2013). Also, mint candy showed some effectiveness in controlling *Varroa* but with low rates than cinnamon. Indeed, mint oil showed some efficacy against *Varroa* mites (Castagnino and Orsi, 2012). This suggests that some volatile compounds of cinnamon and mint can help in controlling *Varroa*. The exact mode of action of these materials on bee physiology and *Varroa* mites may worth further studies.

4.5. Safety to bees

4.5.1. Morphology of bee workers

The measurements showed the high similarities between the control group and the other groups with few exceptions in forewing length and hind wing width for cinnamon and chamomile groups. This supports the absence of harmful effects of the used plant extracts on bees after feeding on the tested candy types. These variations between treatments are normal, and such variations can be attributed to many factors including genetic characteristics and hybridization between bees (Garnery et al., 1998; Arias et al. 2006; Marghitas et al. 2008). The comparisons between results of the tested candy groups including the control group and the reference values showed few variations up to 0.14 mm only. Also, head width was higher in the feeding groups than the reference value. This confirms that the used plant extracts had no noticeable deleterious effects on bee morphology.

4.5.2. Effects on survival of bee workers

The tested candy types showed insignificant variations than the control group. This indicates that plant extracts added to sugar candy had no deleterious effects on the survival of bees. The death of bee workers over the experimental period was very low, supporting the absence of negative effects of the plant extracts on bee feeding and survival. Chamomile candy occupied the second rank after sugar candy in values of survival means followed by cinnamon candy and mint candy with a difference of 0.24 days only. In a previous study under laboratory conditions, caged bees infected with Nosema and fed with chamomile extract had better survival rates than infected bees without any treatments (Abou-Shaara, 2018a). This supports the role of chamomile in improving survival of caged bees.

5. Conclusion

The study showed the effects of some plant extracts on performance and some parameters of bee colonies. Cinnamon extract was promising in accelerating wax comb building and reducing *Varroa* infestation rates. Further studies on its role in impacting *Varroa* mites are required. Chamomile was the best in enhancing the development of bee colonies. The studied extracts can be arranged from the first rank to the last rank as chamomile, cinnamon, and finally mint according to their effects on the development of bee colonies. No deleterious effects of these plant extracts were observed either on bee survival or morphology, suggesting the safety of these extracts. This study highlights the importance of adding plant extracts in bee feeding.

Authors contribution

A.A.-G. and H. A.-S. proposed and designed the study, H. A.-S. performed the experiments, A. A.-G., H. A.-S. and M. J. A. analyzed the data, wrote and revised the manuscript. All authors read and approved the final submitted manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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