EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

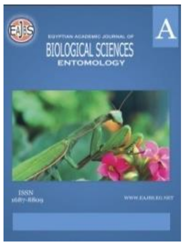
Vol. 15 No. 2 (2022)

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 15(2) pp.35-49 (2022)*
DOI: 10.21608/EAJBSA.2022.241766

Egypt. Acad. J. Biolog. Sci., 15(2):35-49(2022)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



The Novelty of *Azolla pinnata* As a Promising Alternative Feed for Honeybee, *Apis mellifera* (L.)

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ARTICLE INFO
Article History
Received:5/4/2022
Accepted:4/6/2022
Available:7/6/2022

Keywords:
Apis mellifera, Azolla, pollen substitute, hypopharyngeal glands, longevity.

ABSTRACT

Honeybees play a pivotal role in pollinating plants worldwide besides producing valuable products that are used commercially for several purposes. The main factor affecting the biological activities of honeybee colonies is the source of their food and components, especially the source of protein. Therefore, two experiments (in apiary and laboratory) were designed to use a novel protein source (*Azolla pinnata*) in an attempt to enhance the biological activities of honeybees. Nine colonies of honey bees were divided into three groups, each group received a different protein source (azolla, maize pollen grains, sugar syrup only as control). We found that although the consumption rate of pollen patty exceeds the azolla patty, all estimated biological activities were higher in the honeybee group fed on the azolla patty than in other groups. The estimated activities included the sealed brood area, Hypopharyngeal gland development (HPG) and its effect on royal jelly production, abdominal lipid content as well as the longevity of honey bee workers. Hence, the success of our idea of using Azolla as a promising source of protein in feed alternatives for honeybees has succeeded for the first time in the world, and it is considered a new starting point for many studies towards finding unconventional and environmentally safe feed alternatives.

INTRODUCTION

The honeybee *Apis mellifera* L. (Hymenoptera: Apidae) is one of the most important pollinators of various plant species around the world. Honeybees get their nutrition from nectar and pollen, nectar is the main source of carbohydrates providing energy for workers, while pollen is the main source of protein, amino acids, lipids, sterols, vitamins and minerals needed for brood rearing, maturation and development. (Wright *et al.,* 2018; Al-Kahtani *et al.,* 2020). Nutrition is an important unit for physiological processes and it plays a positive role in physiological processes for bee larvae and adults such as development, function and survival, resulting in a healthy colony (Wright *et al.*, 2018). A constant supply of pollen ensures the growth of colonies because it provides protein to adult bees and stimulates brood rearing, So, colonies with low nutrient reserves reduced brood rearing (DeGrandi-Hoffman *et al.*, 2008). During the scarce or unavailability of pollen, or if there is no good pollen substitute for the colonies, brood production often decreases or even stops altogether (Ahmad *et al.*, 2021)**,** also pollen deficiency during the rainy season can lead to the degradation or collapse of colonies (Neupane and Thapa, 2005). This protein lack in honeybee colonies affects the immune response, and it can also accelerate disease spread among nestmates and lead to increased levels of pathogens, reducing adult lifespan and survival. So, what started out as malnutrition can lead to losing the colony due to disease (DeGrandi-Hoffman *et al.*, 2010). In case of shortage flower resources as pollen and nectar, these led to lack of natural food and causing the weakness and loss of colonies during critical periods, so during pollen shortage periods, protein supplementation is the key management tool to maintain the strength and health of the bee colonies (Amro *et al.*, 2016).

Azolla (*Azolla pinnata*) is an aquatic floating fern that lives in a symbiotic relationship with *Anabaena azollae* a nitrogen-fixer blue-green alga, which causes an increase in its protein content to be 25-30% (Mathur *et al.* (2013). It is also found to contain 16.2% ash and 47.0% carbohydrate besides its high content of amino acids such as lysine, leucine, isoleucine and valine which exceed honeybee requirements of amino acids (Alalade and Iyayi, 2006). As well as, provitamins (such as β-carotene), chlorophyll, lipids and minerals like iron, calcium, magnesium, potassium, phosphorus, manganese, copper, sodium, and zinc in addition to appreciable amounts of vitamins A and B12 (El Naggar and El-Mesery, 2022). The nutritional value of azolla as a protein-rich and provitamins source for large animals and poultry has been recognized several years ago (Kumar and Chander, 2017). Due to its easy cultivation, high propagation, high productivity besides good nutritive value, it is considered to be an unconventional feed and cheap source of protein, which can be incorporated into the supplementary diets for honeybee colonies as well as farm animals and poultry (Bhatt *et al.*, 2020).

Protein quality and quantity in supplemental diets have a great effect on various activities of honeybee colonies such as brood-rearing (Ahmad et al., 2021), hypopharyngeal glands (HPGs) development (Hu et al., 2019) and its influence on royal jelly (RJ) production (Khan and Ghramh, 2022), abdominal lipid content (Corby-Harris, *et al.*, 2019) as well as longevity (Omar *et al.* 2017).

Believing in the importance of azolla as a rich source of protein, the objective of this study was chosen to adopt the idea of using *Azolla pinnata* as a novel source of protein in honeybee diets for the first time in the world and its influences on some honeybee activities.

MATERIALS AND METHODS

Two experiments, one at the apiary and one in the laboratory, were designed to examine the effect of diet components on honeybee activities.

The Apiary Experiments:

The apiary experiment was achieved on the honeybee *A. mellifera* L during the period from August to December 2021 in the apiary of the faculty of Agriculture, Benha university at Moshtohor, Egypt, (Elevation 52 ft., Latitude 30.3527° N, Longitude 31.2209° E).

Honeybee Colonies:

Nine colonies were used in this experiment and divided into three groups, each group containing three replicates: Group 1) fed on azolla patty, Group 2) fed on pollen patty, and Group 3) fed on the sugar syrup only as a control. Honey bee colonies were maintained according to standard beekeeping techniques.

Propagation of Azolla:

Azolla pinnata was obtained friendly from the soils, waters and environment Institute, Agricultural Research Center, Giza, Egypt. Azolla has been propagated at the apiary of the faculty of Agriculture, Benha university at Moshtohor, Egypt.

Three pits of uniform dimensions (2.0 x 0.8 x 0.3 m) were made under the shade of the trees, and covered with plastic sheets without any holes, then the edges were fixed with bricks. About 20 kg of fine clayey soil amended with 10-15 g monocalcium phosphate was spread in each pit, then tap water was transferred into the pit up to a height of 10 cm. The pits were left for two hours until the soil settled to the bottom and the impurities floating on the surface were removed. Finally, 500 g of fresh Azolla culture was inoculated over the surface and allowed to grow for 2 weeks before harvest (Bhatt et al., 2020). The harvested azolla was washed with tap water and dried in a drying oven at 60°C till constant weight then grinded and kept for further usage.

Pollen Grains:

Maize pollen grains were collected using mounted pollen traps in a commercial apiary at Moshtohor, Toukh, Al-Qalyoubia Governorate, Egypt, dried in a drying oven at 60°C then grinded upon until ready for use.

Preparation and Chemical Analysis of Diets:

Two different diets patties have been prepared as follows (g/100g): (1) Azolla patty (20 azolla powder, 30 soybean flour, 20 honey, 30 sucrose powder); (2) Pollen patty (20 maize pollen, 30 soybean flour, 20 honey, 30 sucrose powder). Both two patties were amended with 5 ml glycerin/100g to improve pliability. Control colonies were fed on sucrose syrup (50%) only. Also, groups 1 &2 received sucrose syrup (50%). The two prepared diets were analyzed for total lipid, carbohydrates and protein contents according to the methods of AOAC (2005).

Determinations:

Consumption Rate: Throughout the experiment, diet consumption was recorded weekly by calculating the difference in diet weight before and after feeding (g/colony/week). Unconsumed diets were collected and weighed to calculate the consumption rate as follows (Ghazala and Nowar, 2013):

$$\text{Consumption rate (g)} = \frac{\text{DWb} - \text{DWa}}{\text{DWb}}$$

Where: DWb: diet weight before feeding,

DWa: diet weight after feeding

Sealed Brood Area: In all colonies, the areas of worker-sealed brood were measured every 13 days during the experiment using an empty Langstroth frame subdivided into square inches (Büchler et al., 2013; Nowar et al., 2018), it was placed over the sealed brood frames, and the area of the capped brood was measured.

Abdominal Lipid Content: Abdominal lipid content (both nutrient concentration and total nutrient content) was quantified using the ether washing protocol according to (Chakrabarti et al., 2020; Wilson-Rich et al., 2008) as follows: the abdomens were collected from 15 individual honey bees in the treated colonies and cut to remove guts. Each sample of 15 abdomens was dried at 45°C for 72 h in a drying oven to reach a stable dry mass, weighed and transfer in a clean glass tube. 4.5 ml of ethyl ether was added to each tube and gently shaken for 24 h on a microplate shaker to dissolve lipids. All tubes were let dry for the other 3 days and weighed again. The abdominal lipid content (mg/g) was calculated as follows:

$$\text{Abdominal lipid content (mg/g)} = \frac{A}{B} \text{x1000}$$

Where: A**:** Weigh the abdomens of bees after drying and before extraction,

B: Weigh the abdomens of bees after extraction and drying for 3 days.

Royal Jelly Production: All colonies in each group were orphaned (queens were removed from their colonies for 5 days) only once in April 2022 for estimation of royal jelly production using the dry grafting method as described by (Li, 2000). One frame containing 54 cup cells was grafted with worker larva at the age of 24-36 h from each colony, then all the grafted frames were distributed and placed in the middle of the colony for three days, then at the end of the third day of grafting the number of accepted cups in each colony was recorded and the royal jelly was collected, weighed and recorded as g/colony.

Laboratory Experiment: A laboratory experiment was conducted during the period from September to October 2021 to estimate some physiological parameters using the honeybee workers' rearing method as follows:

One sealed brood frame was taken from each fed colony, the adult bees were transferred from the brood frame to a mesh cage and placed in an incubator (32±0.5°C, 65-75 %RH), under full darkness. After brood hatching, 100 individuals of newly emerging bees (0-24hrs old) were transferred to small plastic cages (19×6.5×7.7cm) in groups and placed in an incubator (32±0.5°C, 65–75%RH), under full darkness. Each cage was provided with a 20ml-feeding syringe containing 50% sugar syrup, a 10ml syringe containing water, and a plastic pot containing azolla patty or pollen patty (the diets were renewed every 48h). Six replicates were used for each diet group. Then the following physiological parameters were estimated periodically.

HPG Development: Thirty bees were collected separately from three replicates of the previously described cages on the 5th, 7th, 10th, 12th and 15th days, the heads of all bees were removed and weighed to determine the development rate of the hypopharyngeal glands according to (Peng et al., 2012**)** and the weight gain milligrams per worker's head was calculated as follows:

$$\mathrm{W\,(g)} = \frac{Wa}{N}\,\mathrm{x1000} \qquad\qquad \mathrm{T\,(\%)} = \frac{Wx - W5th}{W5th}\,\mathrm{x100}$$

The total mean increase in the head weight

$$= \frac{\text{T in 7th, 10th, 12th and 15th day}}{4}$$

Where:

W: the weight of a single worker’s head (mg),

Wa: the weight of all bees’ heads (g),

N: number of weighted bees,

T: percentage increase in head weight,

W5th: the head weight on the 5thday,

Wx: the head weight on any day except the 5th day.

Longevity: After the first day, dead bees were removed daily from the floor of the cages described above and counted in the three replicates for each diet and control group to calculate the life span of honeybee workers. The life span of the adult honeybee was obtained by calculating the LT50 (the days required for 50% of the caged bees to die) using a computerized probit analysis, SPSS software version 20, 2002.

Data Analysis:

The effects of diets on food consumption, worker-sealed brood area, abdominal lipid content, royal jelly production and HPG development were evaluated using one-way ANOVA, SAS software.

RESULTS AND DISCUSSION

Chemical Composition of Prepared Diets:

Regarding the total protein content in prepared diets, results in (Fig. 1) showed that azolla patty contained 20.13% crude protein which accord with those obtained by (Shinde *et al.*, 2017; Joysowal *et al.*, 2018) that azolla contains crude protein around 20%. Results also indicated that the protein content in azolla patty was 13.8% more than its content in the pollen patty (17.5%) (Fig. 1). It could be attributed to the high protein content of azolla which ranges from 14.1 to 30% (Mathor *et al.*, 2013) and the correspondingly low protein content of maize pollen (about 18%) (Bujang *et al.*, 2021), as well as the high protein content in soybean flour that reached about 50% (Shurtleff and Aoyagi, 2013). It was also found that the protein content in azolla exceeds the minimum protein needs of bees by 20% (Brodschneider and Crailsheim, 2010), and this qualifies azolla as a good alternative protein source that can also be used to provide dietary nitrogen (El Naggar and El-Mesery, 2022). Furthermore, azolla increases its protein content through a unique symbiotic relationship with *Anabaena azollae* (a nitrogen-fixing blue-green alga) which fix atmospheric nitrogen into ammonia, then converted it into various amino acids. (Hossiny et al., 2008) found that azolla contains approximately all amino acids (remarkably lysine), pro-vitamins (β-carotene) and vitamin B12 (Leterme *et al.*, 2010). Additionally, it also provides macronutrients such as calcium, magnesium and potassium besides vitamins which suggested azolla as an unconventional feed with protein supplement for many animals (Joysowal *et al.*, 2018).

In concern to carbohydrates content, our results revealed that the pollen patty exceeds in its content of carbohydrates than azolla patty (Figure 1). This result was logical and predictable because the main component of maize pollen grains is carbohydrates (around 45%) (Bujang *et al.*, 2021), while azolla is considered a poor source of carbohydrates (about 35%) compared to pollen grains (El Naggar and El-Mesery, 2022). The high content of carbohydrates indicates that maize pollen grains could serve as a source of energy.

Related to the lipid content, it was clear that the lipid content was at close values in the two prepared diets, but the pollen patty has more lipids than azolla patty (Fig. 1). This result was true although the azolla exceeds maize pollen grains in its crude fats content and was found to be around 3 and 1 %, respectively (El Naggar and El-Mesery, 2022; Bujang *et al.*, 2021). In solidarity with our results, (He and MacGregor, 2008) reported that maize pollen possessed a plausible lipid content (unsaturated and saturated fatty acids), and is directly linked to honey bee health (Alaux *et al.*, 2010). Notably, the nutritional value of pollen may be more precisely defined by its amino acid components rather than by its total protein contents, because its nutritional value is decreased when there are insufficient amounts of essential amino acids present (Al-Kahtani *et al.*, 2020).

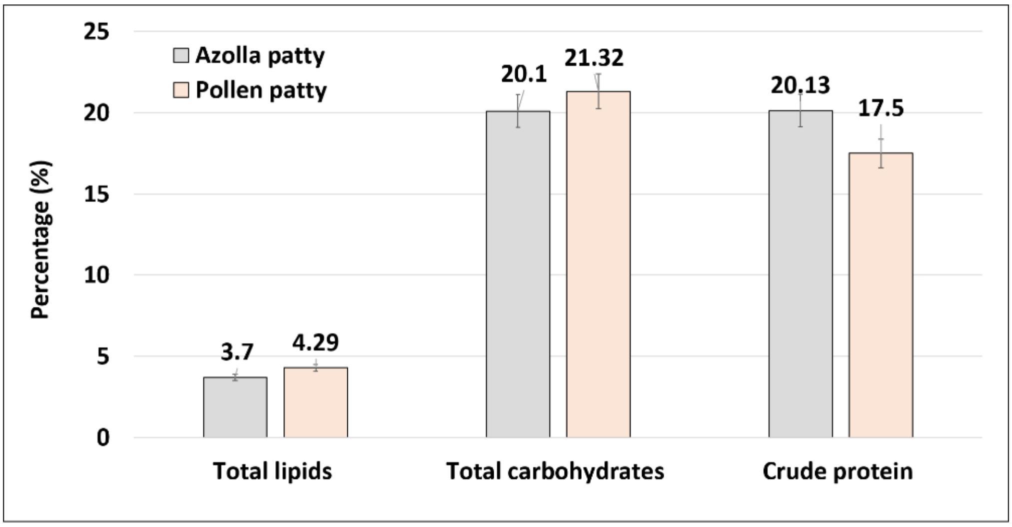


Fig. 1. Differences between two prepared diets in chemical composition.

Diet Consumption:

The differences between the two prepared diet consumption were recorded weekly as shown in (Fig. 2a). Generally, results indicated that the pollen patty was preferred for honeybees than azolla patty. In colonies fed on the pollen patty, the highest weekly amount of diet consumption (97 g/colony) was recorded during the fifteenth week while the lowest rate (29 g/colony) was recorded in the second week. On the other hand, the consumption rate of azolla patty was very similar during the first six weeks, then decreased sharply and re-increased again to record the highest consumption rate during the tenth week (55 g/colony) and then decreased to record the lowest consumption rate during the twelfth week (22 g/colony). During August (the 2nd to the 5th week), the natural source of pollen was available (the corn season) so the consumption rate was decreased.

Furthermore, the pollen patty was consumed at a higher rate than azolla patty with a mean of 65.35 g/colony and 37.26 g/colony, respectively (Fig. 2b). The higher consumption rate of pollen patty might be due to its low content of crude protein, where the rate of consumption is affected by the protein content of the diet. Our results were confirmed by (Paray *et al.*, 2021) findings that diets containing high carbohydrates content are preferred by honeybees and consumed easily and in larger quantities, in contrast to diets low in carbohydrate content, which found that bees did not prefer and consume them in small quantities.

The diet consumption by honey bees is considered a complex process and controlled by multiple factors as discussed by (Rodney and Purdy, 2020), it is known that the nutritional needs differed within various castes and also according to the life stages; there is seasonal variability in energy requirements; colony size, health, and genetics affect foraging. Also, loading and air temperature were vital factors effect on diet consumption rate (Human *et al.*, 2013). While Avni *et al.* (2009) found that the surface area of pollen patties fed to honeybee colonies has a noticeable effect on their consumption.

Additionally, (Ghosh and Jung, 2022) found that the fatty acids content (especially α-linolenic acid) in pollen patty influences honeybee feed consumption and longevity through its critical role as an energy store that supplies cells with energy during metabolic processes. Thus, they also recommended modifying the nutritional content of the diet by increasing the amino acids and decreasing the fatty acids content which agreed with our idea that the azolla patty (high protein content diet) was more useful to honeybee activities than pollen patty (high lipid content diet).

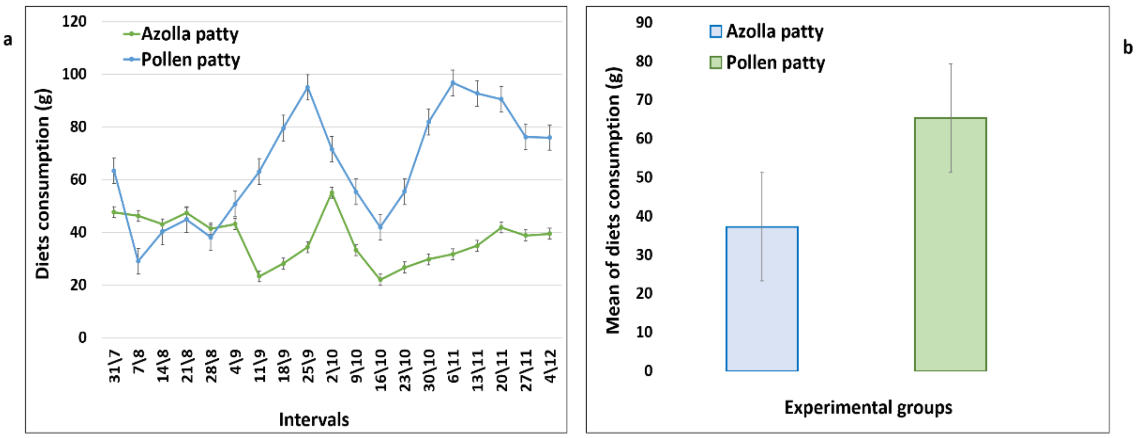


Fig. 2. Diets consumption, a) weekly consumption, b) mean of consumption.

Sealed Brood Area:

The area of sealed brood was observed at a high rate during the first three weeks and then gradually decreased to maintain a nearly constant level during the following weeks of the study (Fig. 3a). This trend was true in both colonies fed on azolla or pollen patties as well as control colonies.

It was also clear through the comparison between two patties that the maximum sealed brood area of workers was observed in the colonies that fed with azolla patty followed by those fed with pollen patty (1718.33 and 1564.33 inch^2, respectively), while the least brood area (1297.66 inch^2) was in the control colonies that feed on sugar syrup only over the 13 days intervals (Fig. 3b). It could be attributed to the high content of azolla patty in crude protein (25-30%), amino acids, minerals, chlorophyll, vitamins, and lipids (Basak *et al.*, 2002) which are essential for brood rearing (Ahmad *et al.*, 2021). As well, azolla components were sufficient to provide honeybees with their nutritional requirements as (Amro *et al.*, 2016) demonstrated that the required protein for ideal growth of honeybee workers found to be 20-23%, so azolla patty contains protein exceeds their requirements and this translated in high activities such as brood area. The previous study confirms our results that honeybees consumed pollen patty more easily than azolla patty but azolla patty gave high brood rearing, (Mostafa, 2000) found that although honeybees prefer diets with high carbohydrates content these diets did not give high brood cell number. Whereas a diet with 16.58% protein, encouraged brood rearing in honey bees more than the other diets.

As illustrated in (Fig. 3 a, b), it was clear that the sealed brood area was increased in colonies fed on patties than in un-fed colonies (control group). (Lamontagne-Drolet *et al.*, 2019; Islam et al., 2020) reported similar results that the surface of sealed brood area was increased after the consumption of various supplements and pollen substitutes by honey bees. Also, (Abd El-Wahab *et al.*, 2016) revealed that the sealed brood area was increased in supplementarily fed bee colonies compared to un-fed ones. Moreover, (Sihag and Gupta, 2013) reported that although supplemental diets are not equally effective in stimulating various activities of honeybee colonies, they are a good stimulator of population density compared to colonies that only feed on the sugar syrup.

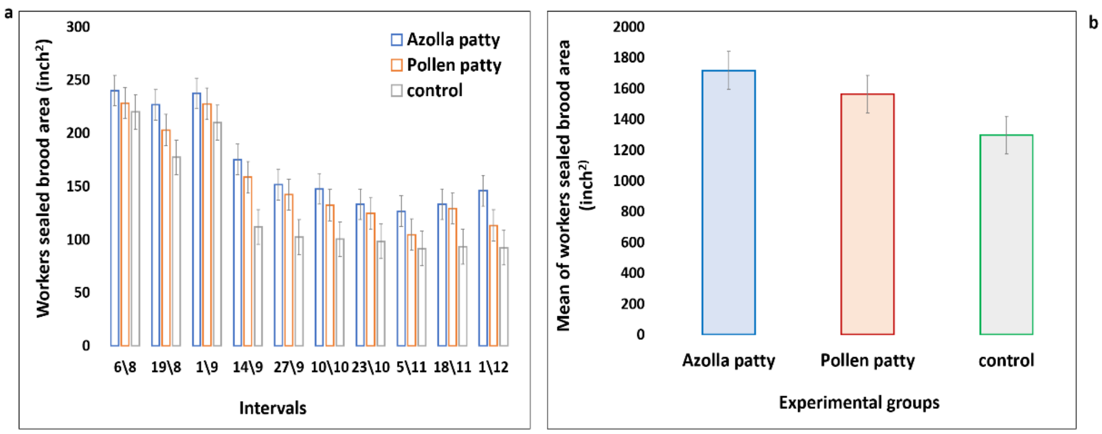


Fig. 3. Effect of diets on brood rearing area in honeybee colonies a) weakly area, b) mean area.

Royal Jelly (RJ) Production:

The mean weight of RJ per colony (g) and per queen cell (mg) between trial bee colonies are shown in (Fig. 4 a, b). Results indicated that the production of RJ was higher in colonies fed with protein diets than in un-fed colonies (control). The highest production of RJ was (10.527g per colony) in the colonies fed with azolla patty followed by those fed with pollen patty (8.912 g per colony), whereas the lower production of RJ (2.844g per colony) was obtained in the control group (Fig. 4 a). As well as when estimating the amounts of RJ per queen cell, the data were in the same trend as illustrated by (Fig. 4 b), that the maximum of RJ production per cell was higher in the colonies fed with azolla patty (319 mg/cell cup) follows with those fed with pollen patty (297.07 mg/cell cup), whereas the lowest production of RJ per cell cup was in the control group (237mg per cell cup). Our explanation of these results is that the honey bee colonies that were fed with azolla patty had taken their sufficient nutritional needs, especially vitamins, which led to the development of the glands secreting royal jelly and thus increasing their RJ production. In a similar trend, (Ahmad *et al.*, 2021) highlighted the importance of the supplementation of the honeybee diet with vitamin c which led to a significant increase in all proportion of biological parameters in the control group fed on sugar syrup only. Also, (Sahinler and Kaftanoglu, 2005) indicated that providing honey bee colonies with sucrose syrup with vitamin E gave the highest effect on RJ production while pollen substitute and sucrose syrup came later. Also, Chhuneja and Gill (2012) reported that the addition of vitamin B6 to honeybee diets was important and had a significant impact on increasing RJ productivity.

Concerning the distinctive effect of azolla patty on RJ production, we can highlight the high micro-and macro-nutrient content of azolla (El Naggar and El-Mesery, 2022) which led to an increase in all honeybee activities. A similar trend was observed by (Brodschneider and Crailsheim 2010) that the diet quality and its impacts on royal jelly production depends on its protein content, the bioavailability of proteins, amino acid composition of proteins, lipids and vitamins and the poor-quality diets leads to an incomplete development of hypopharyngeal glands (HPG), which in turn reduces royal jelly production. Moreover, (Omar *et al.*, 2017) represented that the honeybees fed only on sucrose syrup (50%) had the poorest developed HPG and lowest royal jelly production, as well for optimal development of the HPG, newly emerged bees must feed on protein food. Further, (Khan and Ghramh, 2022) discussed the biotic and abiotic variables that affect RJ production and found that the nutritional source was a very pivotal factor, they also reported that the quantity and structure of essential RJ components (amino acids, carbohydrates, and vitamins) are significantly altered in bees fed on sugar syrup only.

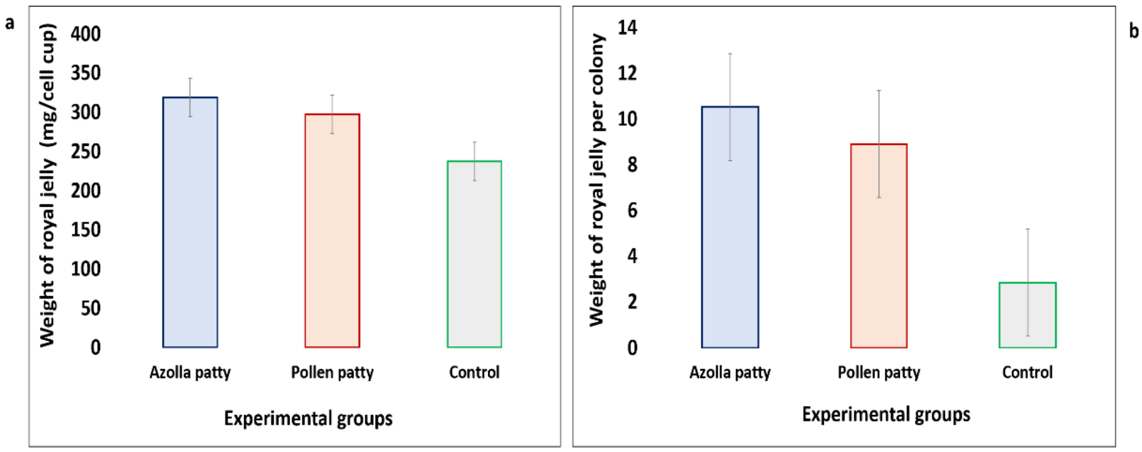


Fig. 4. Effect of diets on royal jelly production a) weight/queen cell, b) weight/colony.

HPGs Development:

The hypopharyngeal gland is one of various young worker bees' glands responsible for royal jelly secretion besides mandibular, most cerebral, and thoracic (Hu *et al.*, 2019). Regarding the effect of two diets on HPGs development, data in Table (1) showed that the weight of the worker honeybee head gradually increased from the age of the 5th day until the 12th day and then decreased on the 15th day and this was seen in all treatments. This result was predictable because the HPGs in the newly emerged bee workers are inactive and the secretory vesicle develops by the age of the 3rd day and also it increases and decreases with age (Deseyn and Billen, 2005). Also, (Corby-Harris et al., 2019) demonstrated that the HPGs are small in newly emerged workers but grow extraordinarily in the first 15 days of life.

Data presented in (Table 1) also showed that the overall mean increase in the head weight of the newly hatched beefed with the azolla patty was (1.58 mg/bee) which was higher than the pollen patty (0.85 mg/bee) and the control group (0.63 mg/bee), respectively. Good feeding has been reported to positively correlate with gland development (Corby-Harris et al. 2016). Thus, the growth and development of the HPG can be considered important criteria that can be used to evaluate the suitability of natural pollen diets or protein supplements fed to young bees. Furthermore, the contribution of a suitable protein in supplementary diets for honeybees, as well as its quality activates their hypopharyngeal glands (Mostafa, 2000). Compatible with our findings, (Corby-Harris *et al.*, 2016) found that HPG size was influenced by diet components and recorded smaller glands in bees fed on pollen as the sole source of lipids and protein compared to bees fed on a diet containing more than one source for nutrients. Results represented the mean of honey bee worker head weight under the influence of both azolla and pollen patties during the experimental period as evidence of HPG development. The mean weight of workers' heads fed on any of both diets was higher than un-fed bees. Als, data showed that bees fed on azolla patty had the highest average head weighing (0.790 mg/bee), which might be attributed to the high protein, vitamins and minerals content in azolla patty (Bhatt *et al.*, 2020; Anitha *et al.*, 2016).

On the other hand, the pollen patty introduced to bees caused an increase in worker head weight by 25.88% compared to bees fed only sugar syrup. The mean weight of the worker's head that fed on pollen patty and sugar syrup was only recorded (0.425mg/bee) and (0.315mg/bee), respectively. This might be due to the fact that bees have taken in their nutritional requirements through protein substitutes, which in turn led to an increase in the head weight of workers and this is considered evidence of the development of HPGs. Compatible with our results, (De Grandi-Hoffman *et al.* 2010) found that bees fed sugar syrup alone had lower protein content and poorly hypopharyngeal glands while it was more developed in the other tested diets.

Table 1. Honey bee worker head weight (W) and percentage of this increase (T) under the influence of two diets during successive intervals as evidence of HPG development.

Days	**Diets**					
	Azolla patty		**Pollen patty**		**Control**	
	W (mg)	T (%)	W (mg)	T (%)	W (mg)	T (%)
5th	$7.59^{c}\pm0.062$	--	$9.38^{e}\pm0.007$	--	$8.23^{c}\pm0.012$	--
7th	$8.09^{b}\pm0.062$	6.59	$9.61^{d}\pm0.007$	2.45	$9.03^{a}\pm0.012$	9.72
10th	$8.67^{aa}\pm0.062$	14.23	$10.00^{a}\pm0.007$	6.61	$8.66^{b}\pm0.012$	5.22
12th	$8.56^{aa}\pm0.062$	12.78	$9.89^{b}\pm0.007$	5.44	$7.63^{dd}\pm0.012$	-7.29
15th	$8.51^{a}\pm0.062$	12.12	$9.72^{c}\pm0.007$	3.62	$7.61^{d}\pm0.012$	-7.53
Overall	8.284	11.43	9.72	4.53	8.072	0.12

Abdominal Lipid Content:

Fats and fatty acids are the basis for the formation of phospholipids, which are a component of cell membranes, as well as enter the synthesis of hormones and serve as a store of energy in the form of their fatty bodies (Hulbert *et al.*, 1999). Estimating the amount of fat in honey bee workers after being fed with the tested patties, we found that the highest value of fat was in the workers provided with azolla patty (234.93 mg/g). The pollen substitute ranked second in terms of the amount of fat in bees with a value of 134.46 mg/g, while the control treatment ranked last with a value of 115.08 mg/g (**Fig. 5**). (Chan *et al.*, 2011) reported that the abundance of proteins in supplementary diets has a role in lipid metabolism, which explains our results that although pollen patty had higher lipid content than azolla patty, honeybee feeding on azolla patty caused an increase in abdominal lipid content compared to other experimental groups, meaning that the patty with higher protein content was better for increasing the abdominal lipid content.

Many factors influenced the abdominal lipid content in the honeybee, in this regard (Uyeda and Repa, 2006) suggested that several carbohydrate enzymes such as pyruvate carboxylase, cytosolic malate dehydrogenase, and ATP-citrate lyase are connected to lipid metabolism as well in the vertebrate. Furthermore, lipid synthesis was associated with honeybee worker age as mentioned by (Toth and Robinson, 2005) that the fat body loses lipids and volume before the bees start to forage indicating a decrease of biosynthetic activity in this tissue. A previous study by (Corby-Harris, *et al.*, 2019) demonstrated that feeding honeybees to poor nutrition sources was associated with lower abdominal fat, and at the same time failed to note any relationship between consumption rate and fat content. in worker head and fat. body, which differed from our findings that there is an inverse relationship between the consumption of pancakes and abdominal fat content.

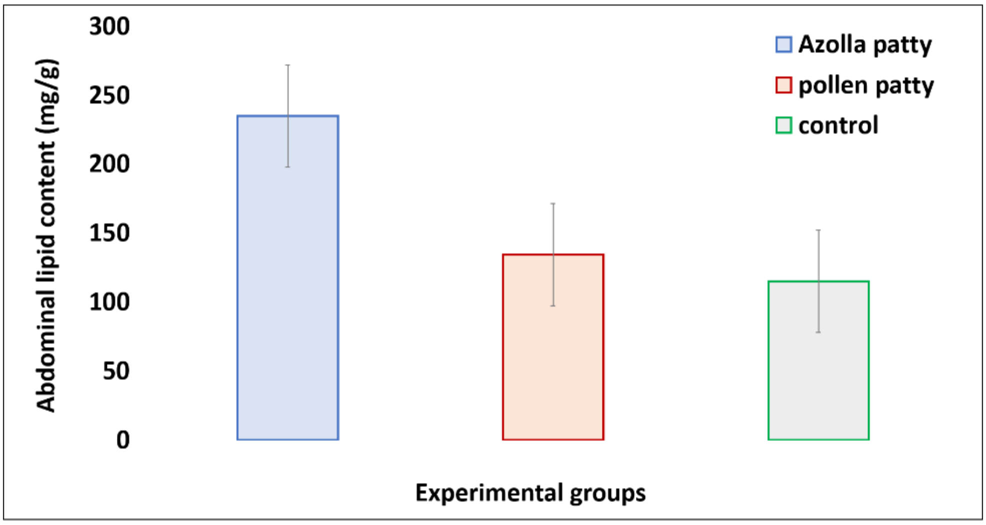
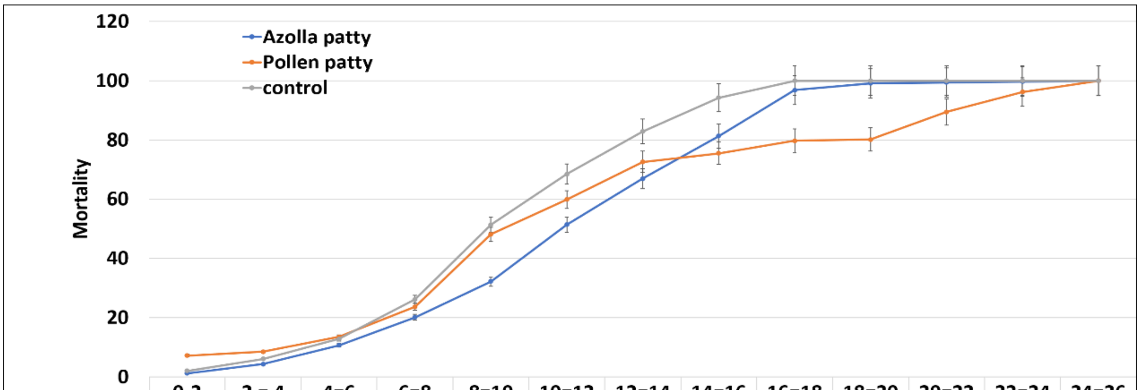


Fig. 5. Effect of diets on the abdominal lipid content in honeybee worker.

Longevity of Newly Emerging Honeybee Workers as Affected by Two Diets:

Dead honey bee workers were counted, and the mortality rate was calculated after feeding on the tested diets. Data illustrated in (Fig. 6) showed that the lowest mortality rates were obtained after feeding honey bee workers on azolla patty and all bees died after 24-26 days. Pollen patty treatment also had good results, similar to a lesser degree than the azolla patty, where the bees died after 24-26 days as well, while the longevity of the workers was less in the control treatment by a large difference compared to the rest of the treatments where the bees died after 16-18 days. By calculating LT50, we found that it was 9.72 days in the case of the azolla patty, and it was lower in the case of the pollen substitute by 9.07 days, but in the control group, the death rate was 8.83 days. The longevity of the honey bee worker is affected by several factors, the most important of which is nutrition source which explains our results that the azolla protein may be of higher quality and may not even require processing (digestion). Previous studies were indicated that honey bee workers live longer when fed on pollen substitute than when fed on sucrose syrup only (Almeida Dias et al., 2018), while (Al-Qarni, 2006; Manning, 2018) found that honeybee workers usually live longer when fed with pollen than pollen substitutes. On contrary, (Omar *et al.*, 2017; Almeida Dias *et al.*, 2018) showed that the longevity of workers is sometimes unaffected when provided with a high-quality pollen substitute. However, no significant differences in mortality between protein-fed bees were observed within the rather short period of 18 days, but bees without a pollen diet had significantly higher mortality, which supports the importance of a protein diet for bee survival (Omar *et al.*, 2017).



	0-2	2 = 4	4=6	6=8	8=10	10=12	12=14	14=16	16=18	18=20	20=22	22=24	24=26
Azolla patty	1.15	4.31	10.63	20.11	32.18	51.43	66.95	81.32	96.83	99.13	99.42	99.71	100
Pollen patty	7.17	8.44	13.5	23.63	48.1	59.91	72.57	75.52	79.74	80.17	89.45	96.2	100
control	2.01	6.04	12.75	26.17	51.34	68.46	82.89	94.29	100	100	100	100	100

Days

Fig. 6. Longevity of honeybee workers as affected by the two tested diets.

Conclusion

According to our findings in this study, we recommend the use of azolla (*Azolla pinata*) as a food additive for honey bee colonies with other protein sources such as soybean flour to extend their longevity and thus increase the colony population which ultimately leads to a remarkable and impressive increase in all biological activities. We also suggest the addition of some compounds to increase the consumption rate and its elaboration by honeybee workers.

REFERENCES

Abd El-Wahab, T.; Ghania, A. and Zidan E. (2016) Assessment a new pollen supplement diet for honey bee colonies and their effects on some biological activities. *International Journal of Agriculture*, 12(1):55-62.

Ahmad, S.; Khan, K.A.; Khan, S.A.; Ghramh, H.A. and Gul, A. (2021) Comparative assessment of various supplementary diets on commercial honey bee (*Apis mellifera*) health and colony performance. *PLoS ONE*, 16(10):e0258430.

Alalade, O. A., and Iyayi, E. A. (2006). Chemical composition and the feeding value of Azolla (*Azolla pinnata*) meal for egg-type chicks. *International Journal of Poultry* Science, 5 (2):137-141.

Alaux, C.; Ducloz, F.; Crauser, D.; Le Conte, Y. (2010) Diet effects on honeybee immunocompetence. *Biological Letters*, 6(4):562-5.

Al-Kahtani, S.N.; Taha, E.K.; Khan, K.A.; Ansari, M.J.; Farag, S.A.; Shawer, D.M.; et al. (2020). Effect of harvest season on the nutritional value of bee pollen protein. *PLoS ONE*, 15(12): e0241393.

Almeida-Dias, J.M., Morais, M.M., Francoy, T.M., Pereira, R.A., Turcatto, A.P., and De Jong, D. (2018). Fermentation of a pollen substitute diet with beebread microorganisms increases diet consumption and hemolymph protein levels of honey bees (Hymenoptera, Apidae). *Sociobiology*, 65:760-765.

AlQarni, A.S. (2006). Influence of some protein diets on the longevity and some physiological conditions of honeybee *Apis mellifera* L. workers. *Journal of Biological Science*, 6:734-737.

Amro, A.; Omar, M. and Al-Ghamdi, A. (2016). Influence of different proteinaceous diets on consumption, brood rearing, and honey bee quality parameters under isolation conditions. *Turkish Journal of Veterinary and Animal Science*, 40(4):468-75

Anitha, K.C.; Rajeshwari, Y.B.; Prasanna, S.B. and Shree, J.S. (2016). Nutritive evaluation of Azolla as livestock feed. *Journal of Experimental Biology and Agricultural Sciences*, *4*(6):670-674.

AOAC. 2005. Association of Official Analytical Chemists. Official Methods of Analysis. 18^{th} Ed., Maryland, USA.

Avni, D.; Dag, A. and Shafir, S. (2009). The effect of surface area of pollen patties fed to honey bee (*Apis mellifera*) colonies on their consumption, brood production and honey yields. *Journal of Apiculture Research*, 48(1):23-28.

Basak, B.; Pramanik, M.A.H.; Rahman, M.S.; Tarafdar, S.U. and Roy, B.C. (2002). Azolla (*Azolla pinnata*) as a feed ingredient in broiler ration. *International Journal of Poultry Science*, 1(1):29-34.

Bhatt, N.; Chandra, R.; Kumar, S.; Singh, K.; Singh, N.P.; Rajneesh; Singh, A.K. and Chauhan, S.P.S. (2020). Nutritive analysis of *Azolla pinnata* and its cultivation during winter season. *International Journal of Current Microbiology and Applied Sciences*, 9(3):2012-2018.

Brodschneider, R. and Crailsheim, K. (2010). Nutrition and health in honey bees. *Apidologie*, *41*(3):278-294.

Büchler, R.; Andonov, S.; Bienefeld, K.; Costa, C.; Hatjina, F.; Kezic, N.; Kryger, P.; Spivak, M.; Uzunov, A. and Wilde, J. (2013). Standard methods for rearing and selection of *Apis mellifera* queens. *Journal of Apicultural Research*, *52*(1):1-30.

Chakrabarti, P.; Lucas, H.M. and Sagili, R.R. (2020). Evaluating effects of a critical micronutrient (24-methylenecholesterol) on honey bee physiology. *Annals of the Entomological Society of America*, *113*(3):176-182.

Chan, Q.W.T.; Mutti, N.S.; Foster, L.J.; Kocher, S.D.; Amdam, G.V.; et al. (2011). The Worker honeybee fat body proteome is extensively remodeled preceding a major life-history transition. *PLoS ONE*, 6(9): e24794.

Chhuneja, P.K. and Gill, A.K. (2012). Effect of number of grafts, material of cell cups and kind of feed to *Apis melifera* L innaeus cell builder colonies on royal jelly production during summer under Punjab conditions. *Journal of Insect Science (Ludhiana)*, 25(3):299-303.

Corby-Harris, V.; Meador, C.A.; Snyder, L.A.; Schwan, M.R.; Maes, P.; Jones, B.M.;

Walton, A. and Anderson, K.E. (2016). Transcriptional, translational, and physiological signatures of undernourished honey bees (*Apis mellifera*) suggest a role for hormonal factors in hypopharyngeal gland degradation. *Journal of Insect Physiology*, 85:65-75.

Corby-Harris, V.; Snyder, L. and Meador, C. (2019). Fat body lipolysis connects poor nutrition to hypopharyngeal gland degradation in *Apis mellifera*. *Journal of Insect Physiology*, 116:1-9.

De Grandi-Hoffman, G.; Chen, Y.; Huang, E. and Huang, M. H. (2010). The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). *Journal of Insect Physiology*, 56:1184-1191.

DeGrandi-Hoffman, G.; Chen, Y.; Huang, E. and Huang, M. H. (2010). The effect of diet on protein concentration, hypopharyngeal gland development and virus load in worker honey bees (*Apis mellifera* L.). *Journal of Insect Physiology*, 56(9):1184-1191.

DeGrandi-Hoffman, G.; Corby-Harris, V.; Carroll, M.; Toth, A.L.; Gage, S.; Watkins, E.; et al. (2021). The Importance of time and place: nutrient composition and utilization of seasonal pollens by European honey bees (*Apis mellifera* L.). *Insects*, 12(3):235.

DeGrandi-Hoffman, G.; Wardell, G.; Ahumada-Segura, F.; Rinderer, T.; Danka, R. and Pettis, J. (2008). Comparisons of pollen substitute diets for honey bees: consumption rates by colonies and effects on brood and adult populations. *Journal of Apicultural Research*, 47(4):265-270.

Deseyn, J. and Billen, J. (2005). Age-dependent morphology and ultrastructure of the hypopharyngeal gland of *Apis mellifera* workers (Hymenoptera, Apidae). *Apidologie*, 36:49-57.

El Naggar, S. and El-Mesery, H. (2022). *Azolla pinnata* as unconventional feeds for ruminant feeding. *Bulletin of the National Research Centre*, 46(66):1-5.

Ghazala, N.E. and Nowar, E.E. (2013). Effect of Brewer's yeast and soya bean cake on brood rearing, pollen gathering and honey yield in honey bee colonies. *Annals of Agriculture Science at Moshtohor*, 51(3):285-292.

Ghosh, S. and Jung, C. (2022). Temporal changes of nutrient composition from pollen patty to bee bread with special emphasis on amino and fatty acids composition. *Journal of Asia-Pacific Entomology*, 25:101873

He, F.J. and MacGregor, G.A. (2008). Beneficial effects of potassium on human health. *Physiologia Plantarum*, 133(4):725-735.

Hossiny, H.; Setoudeh, M.; Rokni, H.; Dehghanzadeh, H. and Cheraghcheshm, M. (2008) Using of silage azolla in Guilan male calves' nutrition. In: Proceedings of 3rd National Congress of Recycling and Reuse of Renewable Organic Resources in Agriculture Islamic Azad University, Khorasgan branch (Isfshan) Agricultural faculty, Waste and waste water Research center.

Hu, H.; Bezabih, G.; Feng, M.; Wei, Q.; Zhang, X.; Wu, F.; *et al.* (2019) In-depth proteome of the Hypopharyngeal glands of honeybee workers reveals highly activated protein and energy metabolism in priming the secretion of royal jelly. *Molecular and Cellular Proteomics*, 18(4):606-21.

Hulbert, A.J.; Turner, N.; Storlien, L.H. and Else, P.L. (1999) Dietary fats and membrane function: implications for metabolism and disease. *Biological reviews of the Cambridge Philosophical Society*, 80:155-169.

Human, H.; Brodschneider, R.; Dietemann, V.; Dively, G.; Ellis, J.D.; *et al.* (2013). Miscellaneous standard methods for *Apis mellifera* research. *Journal of*

Apiculture Research, 52(4):1-53.

Islam, N.; Mahmood, R.; Sarwar, G.; Ahmad, S. and Abid, S. (2020). Development of pollen substitute diets for Apis mellifera ligustica colonies and their impact on brood development and honey production. *Pakistan Journal of Agricultural Research*, 33(2):381-388.

Joysowal, M.; Aziz, A.; Mondal, A.; Singh, S.M.; Boda, S.S.; Chirwatkar, B. and Chhaba, B. (2018) Effect of azolla (*Azolla pinnata*) feed on the growth of broiler chicken. *Journal of Entomology and Zoology Studies*, 6(3):391-393.

Khan, K.A. and Ghramh, H.A. (2022). Evaluation of queen cell acceptance and royal jelly production between hygienic and non-hygienic honey bee (*Apis mellifera*) colonies. *PLoS ONE*, 17(3): e0266145.

Kumar, G. and Chander, H. (2017). Study on the potential of *Azolla pinnata* as livestock feed supplement for climate change adaptation and Mitigation. *Asian Journal of Advanced Basic Sciences*, 5:65-68.

Lamontagne-Drolet, M.; Samson-Robert, O.; Giovenazzo, P. and Fournier, V. (2019). The impacts of two protein supplements on commercial honey bee (*Apis mellifera* L.) colonies. *Journal of Apiculture Research*, 58(5):800-813.

Leterme, P.; Londoño, A.M.; Ordoñez, D.C.; Rosales, A.; Estrada, F.; Bindelle, J. and Buldgen, A. (2010). Nutritional value and intake of aquatic ferns (*Azolla filiculoides* Lam. and *Salvinia molesta* Mitchell.) in sows. *Animal Feed Science and Technology*, *155*(1):55-64.

Li, J. (2000). Technology for royal jelly production. *American Bee Journal*, 140(6):469-472.

Manning, R. (2018). Artificial feeding of honeybees based on an understanding of nutritional principles. *Animal Production Science*. 58:689-703.

Mathur, G.N.; Sharma, R. and Choudhary, P.C. (2013). Use of azolla (*Azolla pinnata*) as cattle feed supplement. *Journal of Krishi Vigyan*, 2(1):73-75.

Mostafa, A.M. (2000). Influence of some supplementary feeding on physiological characters and productivity of honey bees. *PhD, Assiut University, Assiut, Egypt*.

Neupane, K.R. and Thapa, R.B. (2005). Pollen collection and brood production by honeybees (*Apis mellifera* L.) under Chitwan condition of Nepal. *Journal of the Institute of Agriculture and Animal Science*, 26:143-148.

Nowar, E.E.; Khattab, M.M.; Omar, R.E. and Mashaal, T.F. (2018). Evaluation of some natural components for controlling *Varroa mites* in honeybee colonies. *Middle East Journal of Agriculture Science*, *7*(2), 264-268.

Omar, E.; Abd-Ella, A.A.; Khodairy, M.M.; Moosbeckhofer, R.; Crailsheim, K. and Brodschneider, R. (2017). Influence of different pollen diets on the development of hypopharyngeal glands and size of acid gland sacs in caged honey bees (*Apis mellifera*). *Apidologie*, 48:425-436.

Paray, B.A.; Kumari, I.; Hajam, Y.A.; Sharma, B.; Kumar, R.; Albeshr, M.F.; et al. (2021). Honeybee nutrition and pollen substitutes: A review. *Saudi Journal of Biological Sciences*, 28(1):1167.

Peng, Y.; D'Antuono, M. and Manning, R. (2012). Effects of pollen and artificial diets on the hypopharyngeal glands of newly hatched bees (*Apis mellifera* L.). *Journal of Apicultural Research*, 51(1):53-62.

Rodney, S. and Purdy, J. (2020). Dietary requirements of individual nectar foragers, and colony-level pollen and nectar consumption: a review to support pesticide exposure assessment for honey bees. *Apidologie*, 51(2):163-79.

Sahinler, N. and Kaftanoglu, O. (2005). The effect of season and honeybee (*Apis melifera* L.) genotype on acceptance rates and royal jelly production. *Turkish Journal of* *veterinary and animal sciences*, 29(2):499-503.

Shinde, P.N.; Prasade, N.N., Kumar, S.; Desai, B.G.; Dhekale, J.S.; Dandekar, V.S. et al. (2017). Effect of supplementary feeding of azolla (*Azolla pinnata*) on carcass quality and biochemical parameters of broilers. *International Journal of Chemical Studies*, 5(4):854-857.

Shurtleff, W. and Aoyagi. A. (2013). History of soy flour, grits and flakes (510 CE to 2013): Extensively Annotated Bibliography and Sourcebook. Lafayette, California: Soyinfo Center, 2053 pages.

Sihag, R.C. and Gupta, M. (2013). Testing the effects of some pollen substitute diets on colony build up and economics of beekeeping with *Apis mellifera* L. *Journal of Entomology*, 10(3):120-35.

Toth, A.L. and Robinson, G.E. (2005). Worker nutrition and division of labour in honeybees. *Animal Behaviour*, 69:427-435.

Uyeda, K. and Repa, J.J. (2006). Carbohydrate response element binding protein, ChREBP, a transcription factor coupling hepatic glucose utilization and lipid synthesis. *Cell Metabolism*, 4:107-110.

Wilson-Rich, N.; Dres, S.T. and Starks, P.T. (2008). The ontogeny of immunity: development of innate immune strength in the honey bee (Apis mellifera). *Journal of Insect Physiology*, 54(10-11):1392-1399.

Wright, G.A.; Nicolson, S.W. and Shafir, S. (2018). Nutritional physiology and ecology of honeybees. *Annual Review of Entomology*, 63:327-344.

ARABIC SUMMARY

الأزولا (*Azolla pinnata*) كبديل غذائى حديث وواعد لنحل العسل (.L) *Apis mellifera*

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يلعب نحل العسل دورًا محوريًا في تلقيح النباتات في جميع أنحاء العالم إلى جانب إنتاج منتجات قيمة تُستخدم تجاريًا لعدة أغراض. ويعتبر مصدر الغذاء ومكوناته هو العامل الرئيسي الذي يؤثر على الأنشطة البيولوجية لطوائف نحل العسل، وخاصة مصدر البروتين. لذلك ، تم تصميم تجربتين (في المنحل والمعمل) لتقييم إستخدام الأزولا (*Azolla pinnata*) كمصدر بروتينى جديد فى بدائل التغذية لنحل العسل ومدى تأثيرها على أنشطته البيولوجية. وفيها تم إستخدام تسع طوائف من نحل العسل وتقسيمها إلى ثلاث مجموعات ، تغذت كل مجموعة على عجينة تحتوى على مصدر بروتينى مختلف (أزولا ، حبوب لقاح الذرة، محلول سكرى فقط ككنترول). ووجد أنه على الرغم من أن معدل إستهلاك عجينة حبوب اللقاح تجاوز عجينة الأزولا ، إلا أن جميع الأنشطة البيولوجية المقدرة كانت أعلى في مجموعة نحل العسل التي تغذت على عجينة الأزولا مقارنة بالمجموعات الأخرى. تضمنت الأنشطة المقدرة مساحة الحضنة المقفولة ، وتطور غدة الغذاء الملكى (HPG) وتأثيرها على إنتاج غذاء ملكات النحل، ومحتوى دهون البطن وكذلك طول عمر شغالات نحل العسل. ومن هنا، فإن نجاح فكرتنا في إستخدام الأزولا كمصدر واعد للبروتين في بدائل التغذية لنحل العسل قد نجحت ولأول مرة في العالم وتعتبر نقطة إنطلاق جديدة للعديد من الأبحاث نحو إيجاد بدائل تغذية غير تقليدية وآمنة بيئيًا.

الكلمات الدالة: نحل العسل ، الأزولا ، بديل حبوب اللقاح ، غدة الغذاء الملكى ، طول عمر الشغالة.