RESEARCH | Egyptian Journal of Agricultural Research | Plant Protection

Evaluation of royal jelly quality and queens production by using natural food supplements in honeybee colonies

Rasha Shawkat Shoukry Sakla *, *Samah N. El-shafeiy*
Address:
Plant Protection Research Institute, Agriculture Research Center, Dokki, Giza, Egypt.
*****Corresponding author: **Rasha S. Sakla** rasha.s.sakla@gmail.com
Received:28-05-2022; **Accepted**:18-7-2022;**Published:11**-08-2022 DOI: 10.21608/ejar.2022.141398.1237

ABSTRACT
The queen's production is one of the important and sensitive processes in beekeeping and certainly depended on royal jelly quality which plays an effective role in queen difference and vitality. This work suggested a novel plant feed supplements to improve royal jelly quality and enhance the vitality of newly emerged queens. Equal aliquots of Mahlab seed kernel powder (*Prunus mahaleb* L.)and date palm pollen (*Phoenix dactylifera* L.) were provided into colonies in a sugar patty formula for seven days before queen grafting and along for 21 days of the experiment compared to a traditional pollen patty and control sugar syrup. The supplemented patty recorded higher consumption rate of (85.44%) compared to the pollen patty (45.54%). Moreover, the new supplements showed the highest rate of queen acceptance and emergence percent compared to colonies fed on the pollen patty and syrup only. Regarding the new virgin queens' parameters, plant supplements lead to an increase in body and ovary weights to (0.2250 and 0.00562 g respectively) over those of traditional pollen patty (0.1826 and 0.00495 g) and control sugar syrup (0.1439 ±0.00414 g) likewise, an increase in the ovarioles number was detected. Moreover,a protective effect on DNA in ovary cells was recorded by agarose gel fragmentation analysis with the plant supplement group compared to the other groups. The SDS-PAGE analysis of royal jelly showed an improvement in protein quality and quantity with supplemented feeding patty. So, we can recommend the tested plant supplements to enhance royal jelly and queens' production qualities.
Keywords: *Apis mellifera*, supplements, queen production, royal jelly, DNA fragmentation.

INTRODUCTION
The queen honeybee is the most valuable member in the colonies and the only one in the hive able to produce female offspring furthermore, their chemical pheromones give identity to each colony and regulate the social behaviour of the hive.Queens production is one of the most important pillars in beekeeping.Intuitively, good beekeeping requires re-queening of colonies every two years or annually as possible(Trhlin and Rajchard, 2011). The process of re-queening is very necessary to replaceunproductive, injured or lost one to ensure colony survival (Akyol, *et al*., 2008; Seeley, 2010). Moreover, queens are reared as necessary to improve colony traits and the stock (Rangel *et al*., 2013;Hailu and Tadesse, 2016). Consequently, beekeepers strive to have queens with good characteristics as fecundity, vigorously and gentleness besides disease resistance and absence of absconding. (Amiri *et al*., 2017).

In this race, few researchers aforesaid that queen vitality and characters were affected when the nutritional sources were different under the hypothesis of change in royal jelly components (Hartfelder *et al*., 2015). On other hand, several supplemental diet recipes were formulated all over the world may help for maintaining colony strength and development especially during the pollen dearth period (Paray *et al*., 2021). Elaidy (2010) recommended vitamins and gluten patties for their positive effects on virgin queens' bodyweight, abdominal and ovary diameter, as well as ovarioles number. In the same trend, (Gamal Eldin *et al*., 2018) illustrated the positive effects of clover pollen supplements on queen weight, length as well as ovaries weight and ovarioles number. Also, (Strachecka *et al*., 2014; and Strachecka *et al*., 2015) confirmed that natural bioactive substances such as caffeine, curcumin, piperidine, and coenzyme Q_{10} which is known as ubiquinone, ubidecarenone contribute to increase bee health. Furthermore, some plant extracts such as polypore mushrooms extract can work with pollen substitutes diets and showed a better effect (Stamets *et al*., 2018; Amro *et al*., 2020). Hence, the role of nutritional supplements and substitutes on colony health is unignorable. On the other hand, various studies have inspected the royal jelly SDS-PAGE major proteins profilingas (Tamura *et al*., 2009; Liu *et al*., 2014; Furusawa *et al*., 2016).

Mahlab seed kernel (*P. mahaleb*) a traditional spice in Arab countries used in the food industry (Özçelik *et al.,* 2012) as well as in folk medicine in many countries. (Mariod *et al*., 2010; Güven *et al.,* 2022). The date palm pollen (*P.dactylifera*), the male reproductive cells of palm flowers, is commonly used in the Middle East as an effective natural food supplement because it is rich in bioactive compounds that played a crucial role as a strong antioxidant and anti-breast-cancer (Kroyer and Hegedus, 2001; Bishr and Desoukey, 2012; Vladimir *et al.*, 2012) and increase tissues resistance to pathogens and toxins (Taghian *et al*., 2017).

Both Mahlab seed kernel and date palm pollen are known for their nutritional-physiological implications as health-promoting factors and dietary supplements worldwide. In view of that RJ is responsible for the unique qualities of queens such as longevity, high fertility, excellent learning, and good memory as well as it is vital rolein caste differentiation and

development (Sabatini *et al*., 2009; Tamura *et al.,* 2009; Li *et al*., 2010). This work aims to rate changes in royal jelly quality represented in SDS-PAGE of major proteins profiling in addition to some morphological and biochemical parameters of new virgin queens; after feeding with new natural supplemented-patty (mahlab seed kernel and date palm pollen).

MATERIAL AND METHODS

Supplementary materials:

1. Mahlab seed kernel (*P. mahaleb*) was obtained as commercial products from local market.
2. Date palm pollen (*P.dactylifera*) powder for medical use,was purchasedas commercial products from IMTENAN company.

Preparation of nutritional supplemented patties:

Patties were made by grinding the components using an electric grinder, mixed till homogeneity and provided with suitable amount of honey and powdered sugar. The ingredients were as follows:

Diet (A): Patty was prepared as a new formula of (15g pollen grains + 10g date Palm pollen +10g Mahlab seed kernel powder + pepper traces + 10g honey + 5g powdered sugar).

Diet (B): Patty was prepared as a traditional pollen supplement diet that obtained by mixing (15g pollen grains + 10g honey + 25g powdered sugar)

Diet (C): Ordinary sugar syrup (1:1 sugar: water).

Field Experiment design:

The experiment apiary is owned by the honeybee Research department, plant protection Research Institute, Agric. Res. Centre which located in Zagazig city. All experiments were carried out in late winter for a period of 21 days prior to floral pollen being available in the field.

A total of 9 healthy (Italian hybrid bee, *Apis mellifera* Ligustica) colonies, headed by sister queens were used in the experiment. All colonies were fed with sugar syrup (1:1 sugar: water) throughout the experiment. Colonies were divided into three experimental groups three colonies each as follows:

Group(a): A colonywas fed weekly with 100 g of diet (A) and a half-litre of sugar syrup at 5 days interval.

Group (b): A colony was fed weekly with 100 g of diet (B) and a half-litre of sugar syrup at 5 days interval.

Group (c): A colony was fed only a half-litre of sugar syrup at 5 days interval as control group.

Patties were weighed as 100g each for a colony and wrapped with wax paper to avoid dryness, taken care to make cuts on both sides when put in brood nests inside the experimental colonies. Patties replaced with new one after one week or after completely consumed.

Estimation of feed consumption rate:

The patties were provided to colonies (100g / colony) at one-week intervals and the unconsumed portions were weighed after feeding to estimate consumption rate. Where, the food consumption percent (%) was calculated as the difference between the fresh weight of the patty and the weight after a week, then divided by fresh weight.

Grafting technique:

Grafting process was started after a week of the apiary feeding with the supplements. The experimental colonies were dequeened, and the brood frames were removed for 24 hrs before the grafting process. Only two honey frames were holed in each nucleus colony. A grafting frame with empty plastic cups was added to each experimental colony to be habituation, varnished and prepared by honeybee workers. Each grafting frame had three wooden bars with 15 plastic cups fixed to each bar. One strong colony was used as a donor of larvae to all experimental groups to minimize the genetic variation. One-day-old larvae were carefully transferred to plastic cups using grafting metal needle. The grafting frames were quickly returned to their experimental hives avoiding exposure to direct sunlight or wind.

Sampling:

Post three days of grafting process, queen cells acceptance of the three groupswasrecorded by counting the cells containing the larvae and royal jelly. Five plastic cells from each group colonieswere collected, and their growing larvae were removed. The royal jelly was then collected using micro spatula, weighed and kept at -14°C until analysis.

Two days before emergence, sealed queen cells were counted, removed from grafting frames and fixed into honeycombs in their colonies. The queen cells were caged individually by half ball cages till emergence. Emerged queens were counted for emergence percentages calculation.

Laboratory assays:

The study included the effect of the tested nutritional supplement on some morphometric and biochemical parameters of the newly emerged queens. For weight determination 30 newly emerged virgin queens from each treatment were weighed using an electronic balance with precision of (0.0001 mg). Collected royal jelly during queens rearing from each experimental group was analysed.

Internal morphometric measurements of virgin queens:

Virgin queens were anesthetized at -20 ºC and then fixed onto a dissection plate. The dorsal midline was carefully cut using fine scissors and the tergal parts were removed with the aid of a binocular microscope. For clear observation of the

ovaries the internal parts were dissected in a saline solution (0.09% NaCl). The two ovaries were carefully isolated for the following measurements:

- Ovaries were weighed using sensitive electronic balance to the nearest 0.0001 mg after being passed on filter paper to absorb the saline solution.

- The ovarioles numbers of the right ovary were counted. The right ovary was carefully separated and mounted on a glass slide by a drop of xylene (Jackson *et al*., 2011). For ease and clarity of counting, a drop of Puri's medium (10 ml distilled water + 5 ml glycerin + 3 ml glacial acetic acid + 7 g chloral hydrate) was placed onto the ovary for about two minutes to separate the ovarioles. The ovary was then well washed with water. The terminal filament of the ovary was cut off and divided longitudinally into small bundles to avoid the miscounting as possible. The counting was carried out under stereoscopic binocular using minute dissecting needles.

Royal jelly SDS- PAGE:

Protein content was estimated using Biuret reagent according to Gornall *et al.*, 1949. Samples of RJs were dissolved in 50µl sample buffer (62.5mM Tris-HCl (pH 6.8), 10% (v/v) glycerol, 2% (w/v) SDS, and 50mM mercaptoethanol), vortexed, and then boiled for 3min at 95◦C according to the standard (Laemmli, 1970) method. After adding the tracking dye bromophenol blue, 10 μl of the protein was loaded onto 10 % acrylamide SDS-PAGE gels, in (omniPAGE Mini Vertical Protein Electrophoresis System, Cleaver Scientific Ltd, United Kingdom) and run with a constant current of 25 mA/gel for 70 min. using running buffer consisting of (0.025M Tris, 0.192M glycine, & 0.2% (w/v) SDS). Thegel was stained using coomassie blue R-250 dye solution overnight with vigorous shaking then washed with methanol: acetic acid solution (4:1) for distaining to visualize protein bands.

DNA fragmentation analysis of ovaries:

DNA fragmentation assay described by (Bortner *et al*., 1995) was followed. About 50 µg of ovaries collected from newly emerged queens were put in one ml microfuge tube. 600µl of extraction buffer (1.5 M NaCl, 100 mM Tris-Cl (pH 8.8), 50 mM EDTA, 5% CTAB) was added, tissues were crushed in the buffer till dispersed and 2 µl of proteinase-K solution (10 mg/ml) was added. Tubes were incubated at 56 °C overnight with occasional vigorous mixing.

After incubation, the DNA solution was washed with a mixture of 600µl phenol, chloroform and isoamyl alcohol (25: 24: 1) then samples were inverted several times and centrifuged for 5 min at 6000 rpm. The 400-500 µl upper aqueous layer of each sample was transferred carefully into a new tube, containing 900 µl of water and 100 µl of exchange buffer (CTAB 5%, 0.4M NaCl). The mixture sited two min. at room temperature then spin at 6000 for 15 min.

The supernatant was discarded, 300 ml of 1.2 M NaCl was added, shacked gently followed by the addition of 750 µl of absolute ethanol, inverted to mix and let to set at -20 °C overnight for DNA precipitation. Then centrifuged at 10000 rpm for 20 min. and pellets were then washed with 250µl ethanol 70% and spined again. The supernatants were discarded, pellets were sited to air for drying and 50 µl of Tries- EDTA buffer was added then let to set at 37 °C overnight till complete dissolving. Samples were electrophoresed on 1.2% agarose gel at a low voltage (50 volts) which improves the resolution of DNA fragments, gel was stained using ethidium bromide. The analysis of the agarose gel image was determined using Gel Analyzer Software (version 19.1) by GelAnalyzer.com. http://www.gelanalyzer.com/?i=1.

Statistical analysis: Statistical analysis

Data were analyzed using CoState software 2005 (version .9) and analysis of variance (ANOVA) was implied. One-way ANOVA followed by Tukey- HSD test was used to check the difference between the groups.

RESULTS

Feed consumption rate:

Data presented in Table (1) showed the consumption rate of the tested pollen supplement diets A&B. Colonies fed on diet A consumed significantly more amount of 299.04 ± 1.9214g diet during 21days with 85.44 ± 0.7686% consumption than diet B where colonies consumed only 159.35±1.2619g diet/21 days with 45.54 ± 0.50477% consumption percent (P<0.01)**.**

Table 1. Feed consumption rate of the tested diets during 21 days feeding.

Treatment	**Daily colony intake (g/colony)**	**Totalfeed consumption in 21 days (g/colony)**	**feed consumption (%)**
Diet A	14.24^{a} ± 0.1281	299.04^{a} ± 1.9214	85.44^{a} ± 0.7686
Diet B	7.59^{b} ± 0.0841	159.35^{b} ±1.2619	45.54^{b} ± 0.50477
$LSD_{0.01}$	1.2221	25.6627	7.3330
P	0.0000 ***	0.0000 ***	0.0000 ***

Data expressed as (Mean ±SE). (***) means highly significant at F=627.56.

(Diet A: Mahlab and date palm pollen supplemented diet, Diet B: Traditional pollen diet).

Queen cell acceptance rate and percentage of emergence

The effect of tested diets on queen cell acceptance rate and emergence percentages is described in Table (2). The percentage of queen cell acceptance was significantly high in group colonies that received diet A (p=0.0001). Acceptance rates were 85 ± 0.9718%, 64 ± 0.8432 & 54 ±1.4298 % in groups received diet A, B & control, respectively. Concerning the percentages of emerged queens, results in Table (2) cleared that bee colonies provided with diet A induced the maximum queens emergence percent (88.678 ± 0.9531%) followed by diet B (80.023 ± 0.9520%). There was clearly significant difference (p=0.0002) between the two tested diets and the control (61.380 ±1.7427%).

Table 2. Queen acceptance rate and emergence percent in colonies fed on the tested diets.

Treatment	Acceptance rate (%)	Percentage of emergence (%)
Diet A	85^{a} ± 0.9718	88.678^{a} ± 0.9531
Diet B	64^{b} ± 0.8432	80.023^{a} ± 0.9520
Control	54^{b} ±1.4298	61.380^{b} ±1.7427
$LSD_{0.01}$	13.7607	15.75809
P	0.0001 ***	0.0002 ***
F	20.2972	12.0318

Data expressed as (Mean ±SE), (***) means highly significant.
(**Diet A:** Mahlab and date palm pollen supplemented diet**, Diet B:** Traditional pollen diet).

Morphological parameters of the newly emerged queen:

.Data in Table (3) cleared that the new emerged virgin queens fed on diet A had significantly (P=0.000) higher body weights (0.2250 ± 0.0032 g) than those of queens supplemented with diet B (0.1826 ± 0.00295 g) or control (0.1439 ±0.00226 g). Data reveals no significant differences between the tested diet B and control. The average weight of queen ovary (g) and ovarioles number/ovary in control, diet A and diet B – fed colonies are given in Table (3). Analysis of variance showed a highly significant difference (P=0.0000) in ovaries weight with diet A (0.00562 ± 0.00007 g) in comparable to control (0.00414 ± 0.00007g) meanwhile, no significant difference was observed between diet B and both diet A and control groups. Similarly, the ovarioles number was significantly higher in colonies that provided with diet A than those of diet B and control (P=0.0000). The mean number of ovarioles/ ovary was 98.4 ±0.85531, 85.3 ±0.52079 and 73.6 ±0.79888 for diet A, diet B and control, respectively.

Table 3. Morphological parameters of the newly emerged queen in colonies fed on the tested diets.

Treatment	Body weight of emerged queens (g)	Ovaries weight (g)	Number of ovarioles/ovary
Diet A	0.2250^{a} ± 0.0032	0.00562^{a} ± 0.00007	98.4^{a} ±0.85531
Diet B	0.1826^{b} ± 0.00295	0.00495^{ab}±0.00004	85.3^{b} ±0.52079
Control	0.1439^{b} ±0.00226	0.00414^{b} ± 0.00007	73.6^{c} ±0.79888
$LSD_{0.01}$	0.03555	7.7875	9.1642
P	0.0000 ***	0.0000 ***	0.0000 ***
F	19.986	13.9046	28.1395

Data expressed as (Mean ±SE), (***) means highly significant.
(Diet A: Mahlab and date palm pollen supplemented diet, Diet B: Traditional pollen diet)

Effect of feeding by pollen and plant supplemented diets on major royal jelly proteins:

Data in Figure (1) showed SDS-PAGE Electrophoresis gel of royal jelly collected from queen cups reared in colonies feed on plant supplemented patty, pollen patty and control colonies. Three major protein bands were detected approximately at 66, 63, 55 kDa. The plant supplemented treatment in (lane 1) showed highly intensity and broad bands of the three detected protein bands followed by the pollen treatments in lane 2 compared to the control sample in lane3 which showed the lowest concentration and intensity protein bands. Moreover, there are two minor bands detected at 28, and 5 kDa only in the supplemented samples while absent in the control.

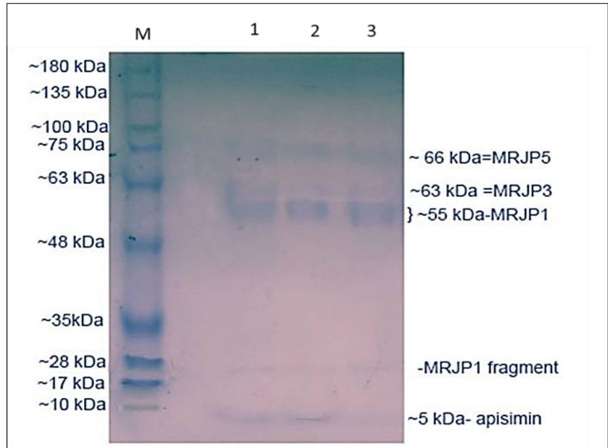

Fig 2 . SDS-PAGE gel electrophoresis analysis of royal jelly collected from supplemented and control colonies against standard molecular weighted protein marker (M). RJ from plant supplemented colonies represented in (lane 1), RJ from pollen colonies (lane 2) and (lane 3) represent control colonies.

Effect of feeding by pollen and plant supplemented diets on genomic DNA fragmentation in virgin queen ovaries:
The degree of genomic DNA damage or repair was measured by viewing the DNA laddering on agarose gel electrophoresis for both treated queens' ovaries against untreated ones. Fig (2). The gel visualizing revealed that feeding on supplemented diet A caused no DNA fragmentation in ovaries cells (lane 1) revealing a protective effect for chromatin materials. Otherwise, pollen patty (diet B) was found to be less protective where it caused a teeny DNA fragmentation appeared as low migration at the start line (lane 2) whereas, control samples (Lane3) expressed a noticed fragmentation.

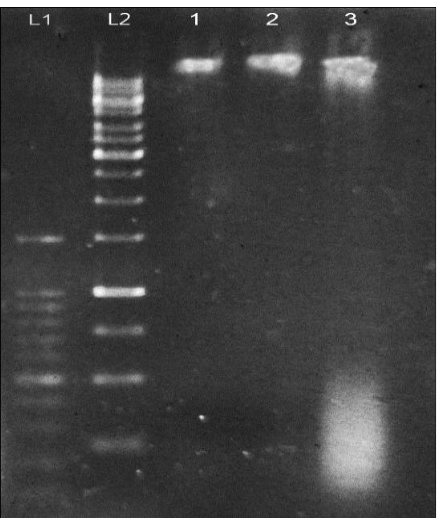

Fig. 2. Analysis of DNA fragmentation of Genomic DNA isolated from ovaries of newly emerged virgin queens supplemented with tested diets; analyzed by 2% agarose gel electrophoresis followed by ethidium bromide staining. Lanes show results as: **(L1)** 100 bp ladder**. (L2),** 1000 bp ladder**. Lane 1:** DNA of sample from supplemented (diet A) colonies**. Lane 2:** DNA of sample from supplemented(diet B) colonies. **Lane 3**:Queen ovaries sample from control colonies.

DISCUSSION

The absence or rarity of pollen may influence colonies strength and their production (Keller *et al*., 2005). Therefore, Beekeepers can provide colonies with pollen supplements or substitutes to compensate pollen shortage, and these supplements must be palatable and nutritious to bees (Mattila and Otis, 2006). Our results cleared that the patty of diet A was clearly consumed nearly twice as much as diet B which may result in an increase in brood and honey production because of higher consumption rate. In the same context, (DeGrandi,Hoffman *et al*., 2008) stated that the amount of reared brood is influenced by the nutritional quality of the diets depending on protein and carbohydrate contents and perhaps, on the accessibility and digestibility of the nutrients.

On the other hand, many previous studies reported the positive correlation between supplemental feeding during queen rearing and larval acceptance rate (Gamal Eldin *et al*., 2018; and Cengiz *et al*., 2019) where, it improves the acceptance rate of grafted larvae (Gençer *et al*., 2000) and fitness traits of the resulted queens (Eremia *et al*., 2014). Feeding of the queen rearing colonies with high protein diet content (diet A) maybe led to an increase in the acceptance and emerged rate. The

same trend of results obtained with (Sagili *et al*., 2018; and Cengiz *et al*., 2019) adding pollen or vitamins mixture to sugar syrup increases the larval acceptance rate.

Traits of queen as weight at emergence, ovarian weight and ovarioles number are among the most important criteria for queen quality and success (Carreck *et al*., 2013). In the current study, the maximum mean weight of newly emerged queen, ovaries weight and ovarioles numbers were noticed in diet (A) containing Palm pollen and Mahlab seed compared to the other diets. This might be owed to the high protein content in date palm pollen as stated by (Fawzey *et al*., 2008) who recorded the highest total protein content in palm pollen when compared to other pollen types maize, clover and faba bean pollen. On the other hand, Mahlab kernel is also a considerable source of protein(28%) beside14% carbohydrates (Farag *et al*., 2021). In addition, (Pernal and Currie, 2000) found that ovaries development and hypopharyngeal glands responsible for the secretion of royal jelly are directly correlated with protein consumption and subsequently, plays a major role queen development. In the same context, (Abd Al-Fattah, 1996), got twice the amount of royal jelly from colonies provided with pollen supplement more than those obtained from unfed colonies which had a positive effect on the weight of newly emerged queens.

Various studies have inspected the RJ major proteins profiling. Depending on these studies, the band detected at~55 kDa refers to the reported major protein, named Apalbumin 1 or MRJP1 also known as Apisin which is an authentic protein of honey and pollen pellet. It induces the physiological changes that result in the differentiation of larvae into queen bees, shortens the developmental time and increases both body and ovary size (Simuth, 2001). So, we can find that the increase in this protein reflects raising the quality of royal jelly in general.

Hence, data cleared that the band at ~ 63 kDa refers to MRJP3 according to the profiling by (Schmitzova *et al*., 1998). On other hand, (Kohno *et al*., 2004) investigated the health aspects of MRJP3, likewise, (Tamura *et al*., 2009; Okamoto *et al*., 2003) reported MRJP3 influences immune responses. Regarding MRJP5 which was detected at~ 66 (kDa figure 1) is expressed at different levels in all stages of honeybees (Liu *et al*., 2014). It also provides nutritive components such as essential amino acids to RJ (Scarselli *et al*., 2005). We also detect a ~ 5kDa band on SDS-PAGE that appeared only in supplemented treatments, it refers to Apisimin; an important peptide in the apisin; a major oligomer of MRJP1 unique to royal jelly (Furusawa *et al*., 2016).

Thus, our finding suggests that the supplemented patty that increases MRJP1, MRJP3 and MRJP5 improves the royal jelly quality and so enhance the immunity of queens and the entire colony as well. Late studies on insect ovarian DNA fragmentation (Sauman and Berry, 2002) detected a DNA fragmentation in developing follicles of *Manduca sexta* ovary resulting from Cytochalasin-D treatment. Also, (El-Gendy and Sabry, 2021) recorded DNA damage in ovaries and testes tissues of *Spodoptera littoralis* mothers treated with *Ruta angustifolia* and *Moringa oleifera* oils. In opposite, many studies searched about the protective role of plant materials and compounds against DNA damages where, (Mansour *et al*., 2008) recorded the protective effect of N-acetylcysteine against DNA damage and hepatic toxicity in rats. The antioxidant and protective effects of *Hygrophila schulli* seeds on oxidative damage of DNA were reported by (Islam *et al*., 2022). In the same context, palm pollen may play a protective role for chromatin materials of queen ovaries due to its high protein and high flavonoid and phenolic contents (Taghian *et al.,* 2017) whereas, control samples (Lane3) expressed a noticed fragmentation which may due to poor protein feeding.

CONCLUSION

Diet A is a best formulation for honeybee colonies feeding during queen production. It awarded the best results regarding consumption rate, queen cell production, positive impact on queen morphometric parameters and royal jelly quality besides its protective effect for DNA chromatin materials of queen ovaries which can enhance colony immunity and development. So, the plant supplemented feed (diet A) is considered as an excellent diet and can be recommended as being used by beekeepers during queen production and dearth period.

REFERENCES

Al-Fattah, M. A. A. (1996). Factors increasing the acceptance of transplanted larvae in artificial queen cells and royal jelly production of the honeybee colonies. *Journal of Agricultural Science*, *Mansoura University*, *21*, 4555-4563.

Akyol, E., Yeninar, H., Korkmaz, A., and Çakmak, I. (2008). An observation study on the effects of queen age on some characteristics of honey bee colonies. *Italian Journal of Animal Science*, *7*(1), 19-25.

Amiri, E., Strand, M. K., Rueppell, O., and Tarpy, D. R. (2017). Queen quality and the impact of honey bee diseases on queen health: potential for interactions between two major threats to colony health. *Insects*, *8*(2), 48.

Amro, A., Younis, M., and Ghania, A. (2020). Physiological effects of some pollen substitutes diets on caged honey bee workers (*Apis mellifera* L.). *International Journal of Environment*, *9*(1), 87-99.

Bishr, M., and Desoukey, S. Y. (2012). Comparative study of the nutritional value of four types of egyptian palm pollens. *Journal of Pharmacy and Nutrition Sciences*, *2*(2), 50-56.

Bortner, C. D., Oldenburg, N. B., and Cidlowski, J. A. (1995). The role of DNA fragmentation in apoptosis. *Trends in Cell Biology*, *5*(1), 21-26.

Carreck, N. L., Andree, M., Brent, C. S., Cox-Foster, D., Dade, H. A., Ellis, J. D., and Van Englesdorp, D. (2013). Standard methods for Apis mellifera anatomy and dissection. *Journal of Apicultural Research*, *52*(4), 1-40.

Cengiz, M., Yazici, K., and Arslan, S. (2019). The effect of the supplemental feeding of queen rearing colonies on the reproductive characteristics of queen bees (*Apis mellifera* L.) Reared from egg and different old of larvae. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 25, 849-855.

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.

Elaidy, W. K., Ebeid, A. A., Fathy, H. M., and Salama, S. Z. A. (2010). Effect of some food additives on workers and queens of honey bee (*Apis mellifera* L.). *Journal of Plant Protection and Pathology*, 1(12), 1013-1021.

El-Gendy, R. M., and Sabry, H. M. (2021). Bioactivity of *Moringa oleifera* and *Ruta angustifolia* Oils on *Spodopteralittoralis* (Boisd.) Moths' Vitality. *Journal of Plant Protection and Pathology*, *12*(3), 251-257.

Eremia, N., Zagareanu, A., and Modvală, S. (2014). Use of additive in bee feeding at queens' growing. In *Scientific Papers Series D. Animal Science* (Vol. 57, pp. 189-195).

Farag, M. A., Khattab, A. R., Shamma, S., and Afifi, S. M. (2021). Profiling of Primary Metabolites and Volatile Determinants in Mahlab Cherry (*Prunus mahaleb* L.) Seeds in the Context of Its Different Varieties and Roasting as Analyzed Using Chemometric Tools. *Foods (Basel, Switzerland)*, 10 (4), 728.

Fawzey, A. M., Nafea, E. A., and El Mohandes, S. S. (2008). Effect of feeding four pollen types on some honeybee characters (*Apis mellifera* L.). *Journal of Plant Protection and Pathology*, *33*(4), 2845-2850.

Furusawa, T., Arai, Y., Kato, K., and Ichihara, K. (2016). Quantitative analysis of Apisin, a major protein unique to royal jelly. *Evidence-Based Complementary and Alternative Medicine*, *2016*.

Gamal Eldin, N. K., Ebeid, A. A., Sallam, A. M., and Basuny, N. K. (2018). Effect of pollen supplements and substitutes on honeybee queen ovaries and worker hypopharyngeal glands. *Journal of Plant Protection and Pathology*, *9*(2), 83-91.

Gençer, H. V., Shah, S. Q., and Firatli, Ç. (2000). Effects of supplemental feeding of queen rearing colonies and larval age on the acceptance of grafted larvae and queen traits. *Pakistan Journal of Biological Sciences* 3: 1319-1322.

Gornall, A. Q., Bradawill, C. J., and David, M. N. (1949). Determination of serum proteins by means of biuret reaction. *Journal of Biological Chemistry,* 177: 751- 766.

Güven, Z., Doğan, Z. E. Y. N. E. P., Saraçoğlu, İ. C. L. A. L., Picot, L., Nagatsu, A., and Başaran, A. R. İ. F. (2022). Food plant with antioxidant, tyrosinase inhibitory and antimelanoma activity: *Prunus mahaleb* L. *Food Bioscience*, *48*: 1-11.

Hailu, T. G., and Tadesse, A. (2016). Queen rearing and colony multiplication for promoting beekeeping in Tigray, Ethiopia. *Elixir Ent*, *92*, 39257-39259.

Hartfelder, K., Guidugli-Lazzarini, K. R., Cervoni, M. S., Santos, D. E., and Humann, F. C. (2015). Old threads make new tapestry—rewiring of signalling pathways underlies caste phenotypic plasticity in the honeybee, *Apis mellifera* L. In *advances in insect physiology* (Vol. 48, pp. 1-36). Academic Press.

Islam, M. S., Parvin, M. S., and Islam, M. E. (2022). The protective and antioxidant effects of *Hygrophila schulli* seeds on oxidative damage of DNA and RBC cellular membrane. *Heliyon*, *8*(1), e08767.

Jackson, J. T., Tarpy, D. R., and Fahrbach, S. E. (2011). Histological estimates of ovariole number in honey bee queens, *Apis mellifera*, reveal lack of correlation with other queen quality measures. *Journal of Insect Science*, *11*(1), 82.

Keller, I., Fluri, P., and Imdorf, A. (2005). Pollen nutrition and colony development in honey bees: part 1. *Bee world*, *86*(1), 3-10.

Kohno, K., Okamoto, I., Sano, O., Arai, N., Iwaki, K., Ikeda, M., and Kurimoto, M. (2004). Royal jelly inhibits the production of proinflammatory cytokines by activated macrophages. *Bioscience, Biotechnology, and Biochemistry*, *68*(1), 138-145.

Kroyer, G., and Hegedus, N. (2001). Evaluation of bioactive properties of pollen extracts as functional dietary food supplement. *Innovative Food Science and Emerging Technologies*, *2*(3), 171-174.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227* (5259), 680-685.

Li, J., Feng, M., Begna, D., Fang, Y., and Zheng, A. (2010). Proteome comparison of hypopharyngeal gland development between Italian and royal jelly producing worker honeybees (*Apis mellifera* L.). *Journal of Proteome Research*, *9* (12), 6578-6594.

Liu, H., Wang, Z. L., Tian, L. Q., Qin, Q. H., Wu, X. B., Yan, W. Y., and Zeng, Z. J. (2014). Transcriptome differences in the hypopharyngeal gland between Western Honeybees (*Apis mellifera*) and Eastern Honeybees (Apis cerana). *BMC genomics*, *15*(1), 1-12.

Mansour, H. H., Hafez, H. F., Fahmy, N. M., and Hanafi, N. (2008). Protective effect of N-acetylcysteine against radiation induced DNA damage and hepatic toxicity in rats. *Biochemical Pharmacology*, *75*(3), 773-780.

Mariod, A. A., Ibrahim, R. M., Ismail, M., and Ismail, N. (2010). Antioxidant activities of phenolic rich fractions (PRFs) obtained from black mahlab (*Monechma ciliatum*) and white mahlab (*Prunus mahaleb*) seedcakes. *Food Chemistry*, *118*(1), 120-127.

Mattila, H. R., and Otis, G. W. (2006). Influence of pollen diet in spring on development of honey bee (Hymenoptera: Apidae) colonies. *Journal of Economic Entomology*, *99* (3), 604-613.

Okamoto, I., Taniguchi, Y., Kunikata, T., Kohno, K., Iwaki, K., Ikeda, M., and Kurimoto, M. (2003). Major royal jelly protein 3 modulates immune responses in vitro and in vivo. *Life Sciences*, *73*(16), 2029-2045.

Özçelik, B. E. R. R. I. N., Koca, U., Kaya, D. A., and Şekeroğlu, N. A. Z. I. M. (2012). Evaluation of the in vitro bioactivities of mahaleb cherry (Prunus mahaleb L.). *Romanian Biotechnological Letters*, *17*(6), 7863-7872.

Paray, B. A., Kumari, I., Hajam, Y. A., Sharma, B., Kumar, R., Albeshr, M. F., and Khan, J. M. (2021). Honeybee nutrition and pollen substitutes: A review. *Saudi Journal of Biological Sciences*, *28*(1), 1167-1176.

Pernal, S. F., and Currie, R. W. (2000). Pollen quality of fresh and 1-year-old single pollen diets for worker honey bees (*Apis mellifera* L.). *Apidologie*, *31*(3), 387-409.

Rangel, J., Keller, J. J., and Tarpy, D. R. (2013). The effects of honey bee (*Apis mellifera* L.) queen reproductive potential on colony growth. *Insectes Sociaux*, *60* (1), 65-73.

Sabatini, A. G., Marcazzan, G. L., Caboni, M. F., Bogdanov, S., and Almeida-Muradian, L. B. D. (2009). Quality and standardisation of royal jelly. *Journal of ApiProduct and ApiMedical Science*, *1*(1), 1-6.

Sagili, R. R., Metz, B. N., Lucas, H. M., Chakrabarti, P., and Breece, C. R. (2018). Honey bees consider larval nutritional status rather than genetic relatedness when selecting larvae for emergency queen rearing. *Scientific Reports*,8 (1), 1-11.

Sauman, I., and Berry, S. J. (2002). Cytochalasin-D treatment triggers premature apoptosis of insect ovarian follicle and nurse cells. *International Journal of Developmental Biology*, *37*(3), 441-450.

Scarselli, R., Donadio, E., Giuffrida, M. G., Fortunato, D., Conti, A., Balestreri, E., and Felicioli, A. (2005). Towards royal jelly proteome. *Proteomics*, *5*(3), 769-776.

Schmitzova, J., Klaudiny, J., Albert, Š., Schröder, W., Schreckengost, W., Hanes, J., and Šimúth, J. (1998). A family of major royal jelly proteins of the honeybee *Apis mellifera* L. *Cellular and Molecular Life Sciences CMLS*, *54*(9), 1020-1030.

Seeley, T. D. (2010). Honeybee democracy. In *Honeybee Democracy*. Princeton University Press.

Simuth, J. (2001). Some properties of the main protein of honeybee (*Apis mellifera*) royal jelly. *Apidologie,* 32, 69–80.

Stamets, P. E., Naeger, N. L., Evans, J. D., Han, J. O., Hopkins, B. K., Lopez, D., and Sheppard, W. S. (2018). Extracts of polypore mushroom mycelia reduce viruses in honey bees. *Scientific Reports*, *8*(1), 1-6.

Strachecka, A. J., Olszewski, K., and Paleolog, J. (2015). Curcumin stimulates biochemical mechanisms of Apis mellifera resistance and extends the apian life-span. *Journal of Apicultural Science*, *59*(1), 129-141.

Strachecka, A., Olszewski, K., Paleolog, J., Borsuk, G., Bajda, M., Krauze, M., and Chobotow, J. (2014). Coenzyme Q10 treatments influence the lifespan and key biochemical resistance systems in the honeybee, Apis mellifera. *Archives of Insect Biochemistry and Physiology*, *86*(3), 165-179.

Taghian, R. A., Abd El-Ati, M. N., Allam, F. M., and Mahmoud, G. B. (2017). Effect of Date Palm Pollen and Bee Pollen as Growth Promoters on the Performance of Saidi Rams. *Assiut Journal of Agricultural Science*, *48*, 86-98.

Tamura, S., Kono, T., Harada, C., Yamaguchi, K., and Moriyama, T. (2009). Estimation and characterisation of major royal jelly proteins obtained from the honeybee *Apis mellifera*. *Food Chemistry*, 114(4), 1491-1497.

Trhlin, M., and Rajchard, J. (2011). Chemical communication in the honeybee (*Apis mellifera* L.): a review. *Veterinary Medicine* 56(6), 265-73.

Vladimir-Knežević, S., Blažeković, B., Bival Štefan, M., and Babac, M. (2012). *Plant polyphenols as antioxidants influencing the human health*. IntechOpen.

Copyright: © 2022 by the authors. Licensee EJAR, EKB, Egypt. EJAR offers immediate open access to its material on the grounds that making research accessible freely to the public facilitates a more global knowledge exchange. Users can read, download, copy, distribute, print or share a link to the complete text of the application under Creative Commons BY-NC-SA International License.

تقييم جودة الغذاء الملكي وانتاج الملكات في خلايا نحل العسل باستخدام مكملات غذائية طبيعية

رشا شوكت شكرى*، سماح نور الشافى

معهد بحوث وقاية النباتات، مركز البحوث الزراعيه، الدقى، جيزه، مصر

*بريد المؤلف المراسل: rasha.s.sakla@gmail.com

يعتبر إنتاج الملكات هو أحد العمليات الهامة في تربية نحل العسل حيث يعتمد على جودة الغذاء الملكى الذي يلعب دورًا هاما في تمايز الملكة وحيويتها. وفي هذا الاطار ، تم اقتراح مكملات غذائية نباتية جديدة لتحسين جودة الغذاء الملكى وبالتالي تعزيز حيوية وجودة الملكات. تم امداد الخلايا المختبرة بأجزاء متساوية من مسحوق نواة بذور المحلب (Prunus Mahaleb L.) وحبوب لقاح النخيل (*Phoenix dactylifera* L) كمكمل بروتينى نباتى لحبوب اللقاح لمدة 7 أيام قبل عملية تطعيم الملكات وعلى امتداد 21 يومًا من التجربة مقارنة بالخلايا التى تغذت على مكمل حبوب اللقاح التقليدي وايضا بالتى تغذت على المحلول السكرى فقط ككنترول. وقد سجل المكمل النباتى لحبوب اللقاح معدل استهلاك مرتفع (85.44٪) بإجمالي (299.04 جم / مستعمرة / 21 يوم) مقارنة مع معدل استهلاك أقل لحبوب اللقاح (45.54٪) حوالي (159.35 جم/ مستعمرة / 21 يوم) ، علاوة على ذلك ، أظهرت المكملات الجديدة أعلى نسبة معدل قبول للملكات وايضا اعلي نسبة خروج في الخلايا المختبرة مقابل الخلايا التي تغذت على حبوب اللقاح والمحلول السكرى. وفيما يتعلق بمقاييس الملكات حديثة الخروج، أظهرت المكملات النباتية زيادة فى أوزان كل من الجسم والمبيض مسجلة (0.2250 و 0.00562 جم على التوالي) مقارنة بتلك المقاسة مع مكمل حبوب اللقاح (0.1826 و 0.00495 جم) والكنترول (0.1439 و 0.00414 جم). وبالمثل ذادت عدد فروع المبايض. و بالمزيد من التدقيق ، تم تسجيل تأثير وقائي على الحمض النووي في خلايا المبايض عن طريق تحليل التجزئة علي جيل الاجاروز مع كل من المكمل النباتي ومكمل حبوب االقاح مقارنة بعينات الكنترول . أيضًا ، أظهر تحليل التفريد الكهربي لبروتينات غذاء الملكات تحسنًا في جودة البروتين وكميته مع تغذية المكمل النباتي. لذلك، يمكن التوصية بإستخدام المكملات النباتية المختبرة لتعزيز جودة إنتاج الغذاء الملكى والملكات.

الكلمات المفتاحية: نحل العسل ، مكملات غذائية، انتاج الملكات، الغذاء الملكى، تحليل التجزئة للحمض النووى.