Effect of Yeast and Sugar Solution Diets on Number of Adult Worker Honey Bees (*Apis Mellifera* L.) Apidae: Hymenoptera

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ABSTRACT:

Honey bees are social insects and have enormous importance as pollinators. In addition to their normal foraging on flowers, honey bees require essential nutritional elements as diet for their survival and reproduction. By providing different diets to the bees, the development of different life stages and production of honey can be increased. To determine the impact of food availability on the number of adult worker honey bees (*Apis mellifera* L.), an experimental trial was conducted with sixteen honey bees' boxes placed with four varying food levels and each food level (treatment) contained four replications. Honeybee colonies were placed in the fields and 20 cells from each frame were selected randomly to check the longevity of worker bees. Of all four treatments, the yeast diet (–708 ±59.84) yielded significant results for the number of adult worker bees following the sugar solution (–408.239 ± 26.22), water (–393.921 ±31.11), and natural foraging (–333.097), showing the maximum average mean values. The results are displayed in the form of graphs & tables and recommendations for the beekeepers based on the research findings. The model of analysis of variance was also significant as 5% significance level such as $F(3, 72) = 6.81; p < 0.05$. The finding of the study suggests that beekeepers can use the yeast- protein containing diet and sugar solution for the growth and development of adult worker bees particularly in the time of dearth of natural flora in the fields. However, it is suggested that the beekeepers should avoid the longer use of supplemental diets to the colonies since it can pose negative effects on the colony.

Keywords: Honey bees, longevity, supplemental diet, pollen substitute

INTRODUCTION:

Honey bees are social insects and have enormous importance as pollinators. In addition to their normal foraging on flowers, honey bees require essential nutritional elements as diet for their survival and reproduction (Hölldobler and Wilson, 2009). The bees perform foraging in order to collect pollen, nectar and water to accomplish their dietary requirements. The pollen is merely a diet containing protein and other indispensable amino acids regarding the improvement of the colony members (Huang, 2012). Keller et al., (2005a) described that the quantity of protein required by a single colony through a year can be acquired from 17-34 kg of pollen and the dearth of pollen sources in the colony grounds the diminution in brood creation and mortality and reduction in the longevity of adult worker bees (Toth and Robinson, 2005).

Honey bees are exceptionally reliant on the food stored in the colony, and adult bees might adapt the foraging approaches in accordance with respective requirement and supply of imperative nutrients (Schmickl and Crailsheim, 2004). In order to upsurge the ultimate body symphony, honey bees require various nutrients right after their emergence. Their basic diet is comprised of protein, enzymes, minerals, and lipids which are necessary for the bees' development (Haydak, 1970). In general, beekeepers provide a supplemental pollen mixed diet as an alternative to boost colony strength and to stimulate a colony's growth during pollen dearth periods. This pollen diet must support brood survival and support the longevity of adult workers (Winston et al., 1983). More recently in Pakistan, bee hives have begun suffering a long period of pollen dearth throughout the country. The pollen dearth period or short blooming period follows the hot summer in most of the regions where temperatures rise to around 45°C which necessitates supplemental feeding for the bees.

Different authors have reported on the effect of artificial diets on the longevity of worker honey bees (Di Pasquale et al., 2013; Paray et a;., 2021; Paiva et al., 2016; Kumar et al., 2013; DeGrandi-Hoffman e al., 2016). Free (1993), described that all these artificial diets confirmed that rich-protein diets (also having carbohydrates, vitamins, minerals and lipids in proper proportion) have a positive effect on worker bee longevity. All energy sources, including carbohydrates, are initially transformed into glucose and are energized through the Krebs cycle to produce Adenosinetriphosphate (ATP), the energy in almost all cells, in addition to carbon

dioxide (CO2) and water (H2O) as by-product (Huang, 2010). The adult worker bees are strongly reliant on colony food stores and they cannot stay alive for long periods without feeding because they do not have considerable carbohydrate, protein or lipid reserves in their body (Kunert & Crailsheim,1988). Since the bees have to perform many activities for the colony, workers need some enzymes like polysaccharides (starch) for flight metabolism at their foraging age (Hrassnigg & Crailsheim, 2005). It is concluded from the findings of the study that a supplemental diet may be helpful in the adult life span during pollen dearth periods. Our results corroborate with the research study by Mattila & Otis, (2006) that during the spring season, protein-rich diets provide nutritious food for honey bee colony in times of pollen shortage (Schmidt et al., 1995), or in those areas in which only poor-quality pollens are available (Somerville & Nicol, 2006).

Basically the proteins make up 66–74% of dry matter of adult workers (Hrassnigg and Crailsheim, 2005) and due to anabolism of protein in the first few days of a bee's life, the protein content increases (DeGroot, 1953) and, somewhat decreases in the older bees. Hence, they ultimately experience an extended lifespan. The pollen complements which contain 20% or more of soybean flour or brewer's yeast are easily edible to the bees and have great nutritive, developmental, and reproductive results (Mattila & Otis, 2006a).

When insufficient amount of pollen is present in the field, commercially manufactured honey bee food can supplement the accessible pollen and present a practical technique for sustaining regarding brood rearing in the colonies (Loidl and Crailsheim, 2001; Arien et al., 2018). Beekeepers nourish the colonies to encourage brood nurturing in the late wintry weather and near the spring beginning or to relieve dietary pressure through the dearth or lack of the natural pollen (Nabors, 2016).

Standifer et al. (1977) makes a distinction among pollen supplements (non-natural protein diets comprised with some pollen) and pollen substitutes (non-natural protein diets with no pollen but slight protein levels derived from soybeans, brewer's yeast, etc). Honey bees acquire inorganic diet essentials principally from pollen; and, as said by Imdorf et al. (1998), bees nurtured through pollen deficiency with supplemental include comparable amounts of nearly all minerals as compared to the bees nurtured through the constructive foraging atmosphere. The constructive foraging atmosphere recommends other imperative mineral resources such as nectar

and water. Water is obligatory for bee endurance; hence, bees must gather water at a high cost in some circumstances as widely discussed by the (Nicolson, 2009).

Honey bee health encompass quite tricky metabolic system therefore, it is intricate to presuppose the health factors and deciders and ultimately the longevity and colony strength. Hence, it is quite practicable to anticipate that the yeast diet may strengthen the survival and number of adults in the colony. This study was aimed at determining the effect of yeast diet on the number of adult of *A. mellifera*.

MATERIALS AND METHODS:

The present research was carried out at College of Agriculture, University of Sargodha with 32.08° North latitude, 72.67° East longitude and 193 meters sea elevation. Four balanced (equalized: containing same food resources) Langstroth hives each with eight frames per colony were established and placed on the ~0.4 m high wooden stands in order to keep away from the contaminants attack.

Healthy colonies (containing queens, brood cells, workers, adults) were used in the experiment. The bee colonies were represented to the similar food resources like accessibility of pollen, nectar etc in the colony and in and out activities of the foragers stabled prior to the experimentation. Since the distance from the hove and preference of food affect the foraging and eventually the food supply, so all the experimental bee colonies were placed at the same distance from the potential and preferred food resource (sunflower field). All the colonies (treatment-wise) were placed in the cardinal direction such as east, west, north, and south. The bee colonies were shifted to the experimental site one day before starting of experiment in order to make the bees familiar to the surrounding (Basari et al., 2018).

Four treatments with four replications of each (total of sixteen bee colonies) were employed in this experiment. All the colonies were provided with the following diets: T1 – sugar syrup (1000g water + 250g sugar) (Ahmad et al., 2021), T2 - Brewers' yeast (1000g water and 50g Brewers' yeast), T3 – water (1000 water lone), and (4) T4 – without any diet; on natural foraging as a Control treatment. The polythene bags with specifications of 3 × 15 × 15cm were used to provide the diets by inserting them beside the frames in the colonies. The food was replaced in each polythene/ plastic bag regularly twice a week at same time intervals.

Five frames from each colony were randomly selected and marked for observation (for counting number of adults). Twenty indiscriminately chosen cells from each of the frame were

marked and examined for all the colonies (total of 100 cells per colony). Through the observation period, the counting of adult worker bees were started three days after administering the diet to each colony at the same time (09:00 and 16:00 hrs) twice a week over a two and a half month period. This data was allowable to us to calculate the number of adult bees on various diets. The effect of different diets on adult workers was assessed by means of analysis of variance (ANOVA) was computed through "R" and further the means were also calculated through the post hoc test LSD.

RESULTS:

Number of adult worker bees with varying diets

The results revealed that the adult population of honey bees varies with the varying diets. Each diet has its own proper composition with unique qualities, which strengthens the colony by physiological, biological, and chemical processes after entering into each bee's body however; all diets may not be equally effective in terms of invigorating these activities (Shaheta, 2016). By feeding on yeast, the population of adult worker bees increased and remained stable with showing some slight population decrease throughout because since the yeast could have sufficient nutritional requirements for the growth and reproduction of the bees (Abd El-Wahab et al., 2016). Average number of adult bees was found 490.842 ± 43.12 with maximum average as 629.5 ±125.45 and minimum as 419 ± 24.79. The yeast mixed solution demonstrated the most significant contribution in the development, growth and longevity of adult worker bees comparatively.

Worker bees take on their nectar and pollen collection or brood-care approach according to their particular need and supply of carbohydrate and proteins (Abou-Sahaara, 2014; Khan et al., 2021). By providing sugar solution to the bees, population of the adult workers on average mostly increased with some showing slight population decrease. Because it fulfills their protein and carbohydrate requirement up to normal level and strengthen the colony. The average number of adult bees on sugar solution was 408.239 ± 26.22. However over this diet, maximum average was 708 ±59.84 and minimum as 284.25 ± 12.75. After the high impact of the yeast mixed diet, the sugar solution diet measured a significant difference between these diet parameters in the development of the colony.

Over water feeding, there was a gradual and continuous increase in the adult workers' population and it demonstrated no harmful effects on overall colony population. Average number of adult bees found was 393.921 ±31.11. Maximum average was 467.75 ±29.69 and minimum as 205.8 ± 15.85. On natural foraging, there was comparatively a slow increase in the population of adult bees because pollen is the only natural source of protein in honey bees and it depends upon floral availability and on the accessibility of bees. Average adult bees were found 333.097 with maximum average as 702.95 and minimum as 186.65. In this study, the natural foraging gave the strength to the worker bees in the colony and encouraged behavior comparatively to the other parameters to the longevity of the adult worker bees.

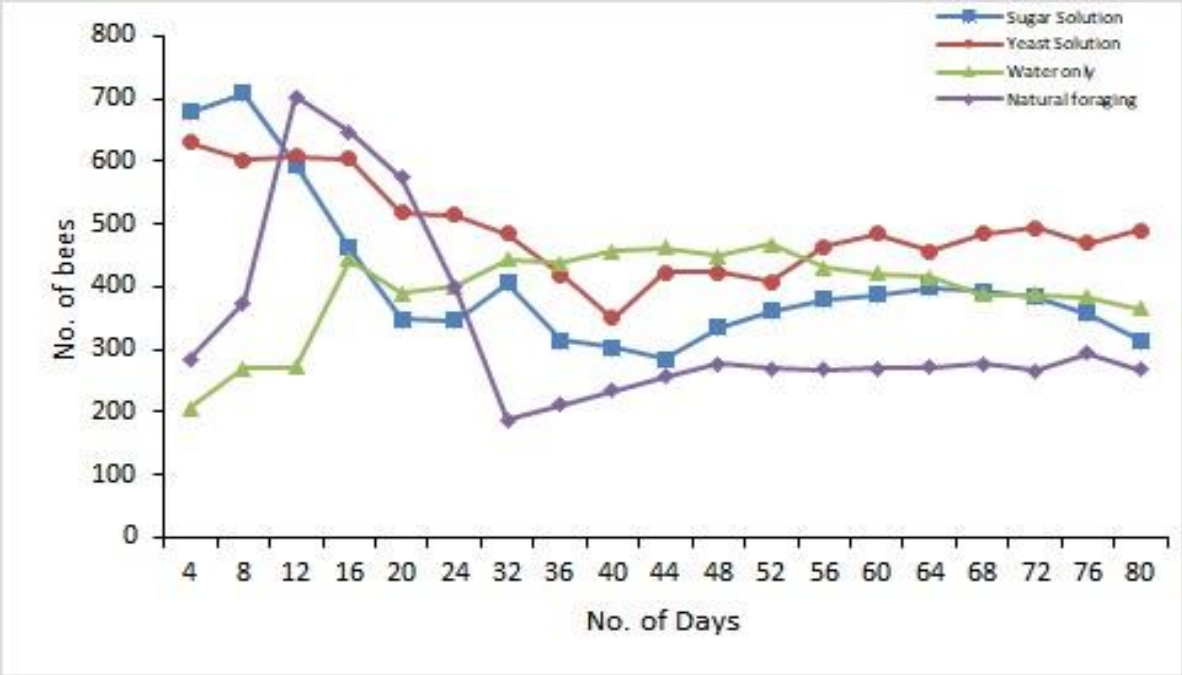

Fig 1. Effect of varying artificial diets on the number of adult worker honeybees *Apis mellifera.*

Effect of diets on adult population:

ANOVA was conducted to evaluate the effect of different diets on the adult population of honey bees. The result of ANOVA showed that the effect of diet was significant with P-value 0.000416, F value 6.813982 and f tabulated value as 2.731807.

Table 1. Summary of the model

Groups	*Count*	*Sum*	*Average*	*Variance*
Sugar Solution	19	7756.55	408.239	14667.05
Yeast Solution	19	9326	490.842	5721.307
Water only	19	7484.5	393.921	5189.669
Natural foraging	19	6328.85	333.097	21500.27

Analysis of Variance

Table 2. Summary of ANOVA model

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	240593	3	80197.67	6.81	0.000416	2.731807
Within Groups	847409.4	2	11769.58			
Total	1088002	5				

LSD Analysis

After finding the results significant by applying ANOVA, the post hock test LSD was used to compare the difference between all the diet categories provided to the bees. The result of LSD test showed that the yeast solution plays a more significant role as compared to other diets in the development of the number of adults with the highest average mean value 490.84 and standard error of 75.64 (significant letter "**a**"). Subsequently, the sugar solution was found to be the next best diet application with average mean 408.24 and standard error as 121.11 (significant letter "**b**"). The diet containing water only was also found good in raising adult bees with average mean 393.92, and standard error of 72.03936 (significant letter "**bc**"). Finally, natural foraging shows with average mean 333.098, and standard error of 146.63 (significant letter "**c**").

All the diets were found significantly important and results revealed that the provision of yeast containing diet, sugar solution and water to the honey bee colonies can help in the development of adult honey bees. The natural foraging on the accessible and sufficient flora and fauna is also imperative and play significant role in the development of adult bees.

Table 3. LSD values

Mean	CV	MS error	LSD
406.525	26.68659	11769.58	70.16603

Graphical representation of the comparison of different diet levels

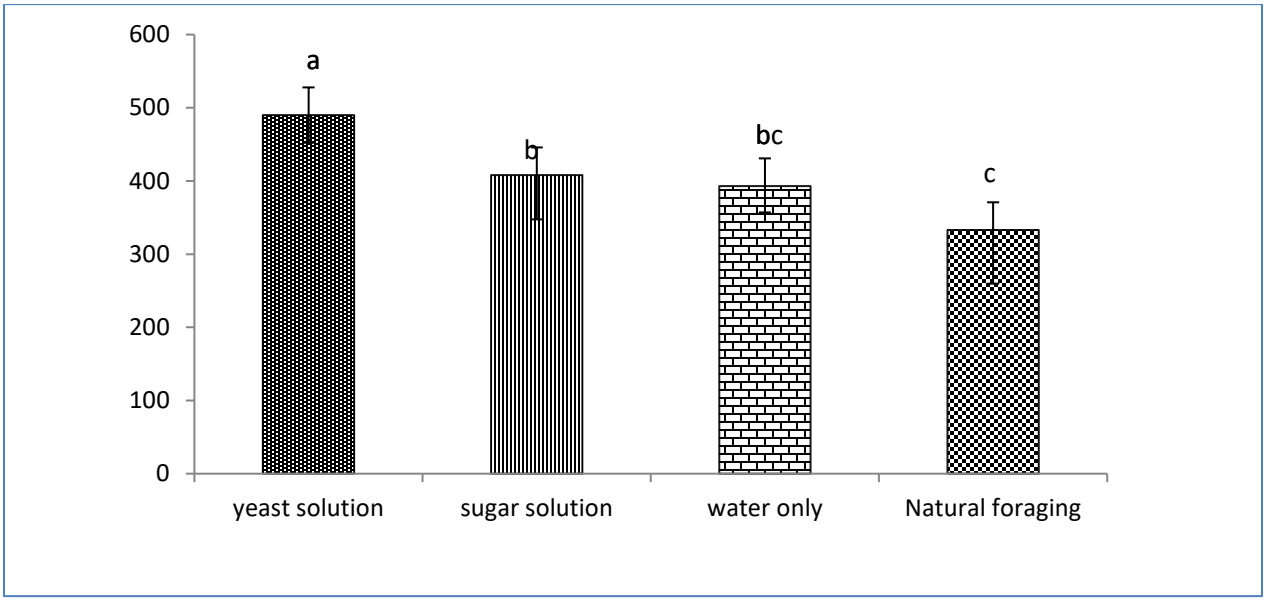

Fig 2. Comparison of different diet levels affecting the number of adult worker honeybees *Apis mellifera.*

DISCUSSION:

Here we studied that effect of yeast as supplementary diet along with sugar solution and water on the number of adult worker bees. The result revealed that yeast as supplementary diet has significant effect of the number of adults. Since the preferred flora by the bees is not accessible throughout the year so offering supplemental diet to the bees is promising for colony strengthening in terms of number of adult worker bees (2006). The sugar solution has a maximum effect on the adult bees' longevity since they consume it more as compared to rest of the diets after yeast. According to our research, the yeast solution has more significant effect as

compared to the other diet parameters and control in the adult life span by showing average mean of 490.84 ± 43.12. Secondly, the sugar solution effect was more encouraging as compared to the others and control by showing average mean as 408.24 ± 26.22. Lastly, the water only diet shows relatively soothing effect as compared to control with average mean as 393.92 ± 31.11.

Our findings revealed that supplemental feeding gives additional support to colonies but, the real necessities and optimal levels required by honey bees remain relatively unexplored. The bees experienced negative effects in foraging and other in/out-colony activities when proteins mixed supplemental diets are used frequently or for an extended period of time. The growth and development of bees, according to many scientists, can benefit from supplemental feeding (yeast, pollen mixed diets, sugar solutions etc). Because yeasts have a protein content of around 50% and typically endow with additional balanced set of amino acids, they are more appealing to bees for their expansion and improvement. Vandenberg and Shimanuki (1987) reported in their study that the applying of yeast-mixed diet could also significantly improve the body weight of adult honey bees.

CONCLUSION

Yeast has been found to be essential for the growth of adult honey bees. The ideal location for Langstroth single deep, equalized, and unpolluted bee colonies is close to equivalent food sources in a shaded area with premium flower-patterned convenience for the bees to engage in field foraging. Colonies need to be placed away from fields that have been sprayed or treated chemically. Brewer's yeast, a sugar and water solution, and non-floral proteins must be used in the colonies at the recommended concentrations. In the colonies next to the frames, a suitable artificial diet should be provided for the honey bee adults, and it should be changed every day or every other day with a brand-new polythene bag. It should be kept in mind that extra queen and other cells in the colonies must be immediately removed and the colonies are disinfectant of diseases and pests.

ACKNOWLEDGEMENT

We thank Ejaz Ashraf for helping data analysis and Jamie Ellis, Gerbson Mendonca for reviewing manuscript thoroughly and providing technical write-up advice.

CONFLICT OF INTEREST

The authors declared no conflict of interest

REFERENCES:

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 Sciences, 6,* 734–737.

DeGroot, A. P. (1953). Protein and amino acid requirements of the honeybee, Apis mellifera L. *Physiologia Comparata et Oecologia 3,* 1–83.

Free, J. B. (1993) *Insect pollination of crops* (pp. 684). New York: Academic press.

Huang, Z. (2012). Pollen nutrition affects honey bee stress resistance. *Terrestrial Arthropod Reviews*, *5*(2), 175-189.

Hrassnigg, & Crailsheim, K. (2005). Differences in drone and worker physiology in honeybees (Apis mellifera L.). *Apidologie 36,* 255–277.

Imdorf A., Rickli M., Kilchenmann V., Bogdanov S., Wille H. (1998). Nitrogen and mineral constituents of honey bee worker brood during pollen shortage, Apidologie 29, 315–325.

Kunert, & Crailsheim, K. (1988). Seasonal changes in carbohydrate, lipid and protein content in emerging worker honeybees and their mortality. *Journal of Apicultural Research, 27,* 13 21.

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

Mattila, H. R., & Otis, G. W. (2006a). The effects of pollen availability during larval development on the behavior and physiology of spring-reared honey bee. *Apidologie 37*, 533–546.

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

Nabors, R. (2000). The effects of spring feeding pollen substitute to colonies of Apis mellifera. *American Bee Journal*, *140*(4), 322-323.

Nicolson S.W. (2009) Water homeostasis in bees, with the emphasis on sociality, Journal of Expimental Biology. 212, 429– 434.

Schmickl T., Crailsheim K. (2004). Inner nest homeostasisin a changing environment with specialemphasis on honey bee brood nursing and pollensupply, Apidologie 35, 249 263.

Standifer L.N., Moeller F.E., Kauffeld N.M., Herbert E.W., Shimanuki, H. (1977). Supplemental feeding of honey bee colonies, USDA Agr. Inform. Bull. No. 413, 8 p.

Schmidt, Schmidt, Wang, & Xu. (1995). Feeding preference of young worker honey bees (Hymenoptera: Apidae) fed rape, sesame, and sunflower pollen. *Journal of Economic Entomology, 88*, 1591– 1595.

Somerville, D. C., & Nicol, H. I. (2006). Crude protein and amino acid composition of honey bee-collected pollen pellets from south-east Australia and a note on laboratory disparity. *Australian Journal of Experimental Agriculture, 46*, 141–149.

Toth, A. L., & Robinson, G. E. (2005). Worker nutrition and division of labour in honeybees. *Animal behaviour*, *69*(2), 427-435.

Hölldobler B, Wilson EO. (2009). The superorganism: the beauty, elegance, and strangeness of insect societies. New York: Norton.

Loidl A, Crailsheim KJJoCPB. (2001). Free fatty acids digested from pollen and triolein in the honeybee (Apis mellifera carnica Pollmann) midgut. J Comp Physiol B. 171(4):313–9.

Arien Y, Dag A, Shafir S. Omega-6: 3 ratio more than absolute lipid level in diet affects associative learning in honey bees. Front Psychol. 2018;9:1001.

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

Huang, Z. (2010). Honey bee nutrition. *American Bee Journal*, *150*(8), 773-776.

Di Pasquale G, Salignon M, Le Conte Y, Belzunces LP, Decourtye A, Kretzschmar A. (2013). Influence of pollen nutrition on honey bee health: do pollen quality and diversity matter? *PLoS One*. 8(8):e72016.

Paray BA, Kumari I, Hajam YA, Sharma B, Kumar R, Albeshr MF. (2021). Honeybee nutrition and pollen substitutes: A review. *Saudi J Biol Sci*. 28(1):1167.

Paiva JPLM Paiva HM, Esposito E Morais MM. (2016). On the effects of artificial feeding on bee colony dynamics: a mathematical model. *PLoS One*. 11(11):e0167054.

Kumar R, Mishra R, Agrawal O. (2013). A Study on Consumption of some artificial diet formulations by Apis mellifera colonies maintained at Panchkula & Gwalior. *J Entomol Res*. 37(2):123–7.

DeGrandi-Hoffman G, Chen Y, Rivera R, Carroll M, Chambers M, Hidalgo G. (2016). Honey bee colonies provided with natural forage have lower pathogen loads and higher overwinter survival than those fed protein supplements. *Apidologie*. 47(2):186–96.

Shehata I. (2016). Evaluation of Carniolan and Italian honey bee colonies fed on artificial diets in dearth and flowering periods under Nasr city conditions. *Int J Environ*. 5(2):19–25.

Abd El-Wahab T, Ghania A, Zidan E. (Shaheta, 2016). Assessment a new pollen supplement diet for honey bee colonies and their effects on some biological activities. *Int J Agric*. 12(1):55–62.

Abou-Shaara H. (2014). The foraging behaviour of honey bees, Apis mellifera: a review. *Vet Med (Praha)*. 59(1).

Khan KA, Ghramh HA, Ahmad Z, El-Niweiri MA, Mohammed MEA. (2021). Honey bee (Apis *mellifera*) preference towards micronutrients and their impact on bee colonies. *Saudi J. Biol Sci*. 28(6):3362–6.

Safari A, Kevan PG, Atkinson JL. (2006). Feed-Bee: A new bee feed is added to the menu. *Bee Culture*. 134(1):47–8.

Basari, N., Ramli, S. N., & Mohd Khairi, N. A. S. (2018). Food reward and distance influence the foraging pattern of stingless bee, Heterotrigona itama. *Insects*, *9*(4), 138.

Vandenberg, J. and H. Shimanuki. Technique for rearing worker honeybees in the laboratory. *Journal of Apicultural Research*26(2):90-97 (1987)