



# Effects of Different Pollen Substitute Diets on the Survival Rate and Diet Preference of Foraging Honey Bees (*Apis mellifera* L.)

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

Honey bee is an eusocial insect from class Insecta. The potential for thriving honey bee colonies hinges on the availability of an effective pollen substitute, particularly during periods of scarcity when natural pollen supply is limited. Numerous beekeepers provide their bees with various pollen substitutes to ensure they receive adequate nutrition during periods of insufficient pollen quantity or quality. For Methodology transparent boxes were made and each artificial diet were kept in each box for each ten replicas of Honey Bees. The experiment was based on Artificial diets as pollen substitutes. For experiment Honey Bee, *Apis mellifera* was taken and feed with five different diets and data was analysed with test significance level is 0.001.

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## 1. INTRODUCTION

Pollen serves as the main provider of essential nutrients like protein, lipids, vitamins, and minerals, crucial for the growth and reproductive processes of bees (Hamed A. Ghramh, 2023). Both sufficient quantity and quality of pollen are necessary to promote the health of honey bee colonies (Brodschneider and Crailsheimn, 2010) [1,2].

Pollen serves as the primary protein source for brood rearing, overall colony development, and the longevity of adult workers [3]. The annual pollen requirement for honey bee colonies fluctuates depending on factors such as colony location, available floral sources, and colony strength [4]. Since bee-attractive flora may not be consistently available throughout the year, efforts have been made to sustain colony strength by providing pollen substitutes and supplements [5]. These interventions aim to bolster brood survival rates and prolong the adult lifespan [6] (Haydak 1935), Doull (1980a, 1980b, 1980c), Safari et al. (2004).

Throughout history, honeybees have emerged as among the select insects directly benefiting mankind. Revered for their role, both the bees themselves and the honey they produce hold significant mentions in various religious epics. Descriptions extolling the value and necessity of honey can be found in texts such as the Rigveda, Upanishads, and other religious writings, [7], Kaushik (2012).

Naturally, it's logical to assume that honeybees would have a preference for consuming their natural diet over a substitute [8]. Nevertheless, some researchers have observed instances where bees consumed even more of the substitute diet than their natural one when both options were available to them.

Mishra et al. (1979) utilized brewer's yeast, soybean flour, and skimmed milk powder to create moist patties as a pollen substitute diet for honey bees. They conducted numerous experiments on colonies of *Apis mellifera* [9]. The results indicated that the artificial diet positively influenced various colony parameters. However, it's worth noting that satisfactory honey production was also reported as an outcome of the treatment.

Doull (1980a, 1980b, 1980c) suggested a diet composition comprising krayeast and skimmed

milk powder for honey bee colonies. When colonies were fed this pollen supplement, researchers noted higher longevity, increased brood area, and enhanced foraging activity compared to control colonies [10-12]. Moreover, experimental colonies exhibited a significant rise in honey production.

Artificial feeding, including the provision of nectar and pollen substitutes, has been explored and developed as a strategy to sustain egg laying, brood rearing, and foraging activities. These efforts aim to maintain a sufficient population of bees within the colony [13,14,15].

A separate study, researchers combined six protein-rich ingredients—defatted soybean flour, chickpea flour, maize flour, wheat germ, pea flour, and dried brewer's yeast—in different ratios with sugar powder, bee honey, and water to formulate ten diets [16]. These diets were found to enhance various biological activities, including diet consumption, sealed worker brood area, and pollen and honey store area (Younis M.S., 2019) [17,18].

Until now, research has primarily focused on commonly foraged feed elements like pollen and nectar, rather than considering atypically foraged materials that bees may consume during periods of drought. The objectives of the present study were twofold: firstly, to evaluate the preference of honey bees for different diets, and secondly, to assess honey bee preferences for various diet supplements positioned at different distances from the colonies (Hamed A. Ghramh, 2023) [19,20].

Researchers worldwide have created numerous alternative meal formulas for bees, considering factors like the nutritional value of honey and pollen, the palatability, digestibility, and cost-effectiveness of ingredients. This effort aims to maintain stable colony characteristics, optimizing their performance during the upcoming nectar flow season. Nonetheless, there's still a need for a universally acknowledged, well-balanced diet standard for commercial beekeeping [21].

## 2. MATERIALS AND METHODS

For an experiment Methodology followed is Mishra et al. [7] and Kumar and Agarwal, [13].

For an experiment two hives of European Honey Bee, *Apis mellifera* was taken with new colony

approximately two hundred bees in each hive. A fundamental necessity was attended to cleaning brood, removing pupa of wax moth, removing ants from outside chamber of brood, applying of ant repellents, cleaning of area around where brood was kept, Greece was applied on legs of stand on which brood chamber was kept.

Two hives were purchased comprising frames kept apart from each other which include honeybee cells, developing queen chamber, pollen and nectar inside cells. Each brood measures 20.1 cm in length, 16.0 cm breadth and 9.8 cm height. Opening of brood in front from where bees enter is restricted to few centimetres to prevent any foreigner to enter inside.

The experiment was conducted in month of March for seven days. Boxes of transparent plastic container measuring 16.0 cm length, 9.0 cm breadth and 8.0 cm height were taken. Boxes were holed for aeration. Boxes of each diet were separate in each box total count of ten bees were kept along with replicas of each diet.

For an experiment five pollen substitute diets were formulated which include:

1. SUGAR SYRUP = (DIET 1)
2. JAGGERY = (DIET 2)
3. FOXNUT = (DIET 3)
4. ALMONDS = (DIET 4)
5. DRY DATES = (DIET 5)

## 2.1 Diet Formulation

- **Dry Dates Diets** -were grinded into a fine powder in a Mixer grinder to make a fine powder.
- **Sugar syrup Diet**- sugar was grinded into a powder and mixed in a water to make sugar syrup.

- **Jaggery Diet** -was mixed in a one tablespoon of water for bees to consume haphazard free.
- **Foxnuts Diet** - first grinded into a fine powder then they are mixed with half tablespoon honey.
- **Almond Diet**- were grinded into a fine powder in a Mixer grinder to make a fine powder.

Each prepared diet with measurements in tablespoon were kept in each box and its replica.

Diet was kept in a very small shallow container that can fit into box perfectly consuming less space. In Sugar syrup and Jaggery diet container dry twig was inserted from holes for bees to easily come out from shallow container easily in case not able to come out.

## 2.2 Collecting Foraging Bees for Experiment

With the help of transparent holed containers foraging bees were collected from both hives (hive A and hive B). After they were collected from the entrance opening of hive they were immediately kept in a fridge (Fridge used from Department of Zoology). Bees were kept in a fridge to narcotize them with the help of low temperature maintained inside fridge. Low temperature will narcotise bees it become easy to distribute them in different sample diet boxes.

All preparations were done accurately before performing an experiment bee were distributed in sample boxes and their replicas. During a span of seven their Mortality rate was monitored and at end of experiment their diet preference and on which diet more bees survived during span of seven days. At end of experiment weight of boxes were measured along with bees.



Fig. 1. Distribution of different Diets

**Table 1. Diet measurement and formulation**

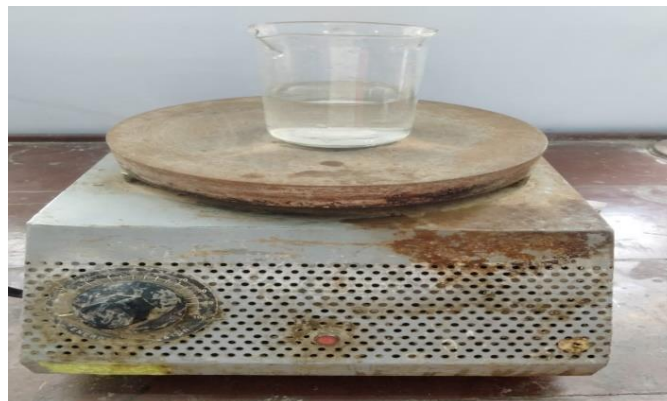
S.No.	Diets	Diet Formulation
1.	Dry Dates	1/4 <sup>TH</sup> tbsp DRYDATES + ½ tbsp HONEY
2.	Foxnut	1/4 <sup>TH</sup> tbsp FOXNUTS + ½ tbsp HONEY
3.	Almonds	1//4 <sup>TH</sup> tbsp ALMONDS + ½ tbsp HONEY
4.	Sugar syrup	½ tbsp SUGAR + WATER
5.	Jaggery	1/4 <sup>th</sup> tbsp JAGGERY + ½ HONEY



**Fig. 2. Collection of worker bees from hive entrance**



**Fig. 3. Narcotization of live bees by freezing**



**Fig. 4. Boiling of granulated sugar for sugar syrup**

### 3. RESULTS

The experiment involved artificially feeding worker bees aged 17-20 days. Ten groups of worker bees were placed in each feeding box. After conducting the experiment for seven consecutive days, we collected and analysed the mean values of mortality and diet preference using prism software. Over the course of seven days, the worker bees consumed different pollen substitute diets, including sugar syrup, jaggery, foxnut, almonds, and dry dates. The results of the experiment demonstrate that a diet consisting primarily of dry dates resulted in a lower mortality rate than a diet primarily made up of jaggery. Among the diets tested, bees showed a preference for dry dates as their top choice, followed by almonds as diet 3, Foxnut as diet 2, Sugar Syrup as diet 4, and Jaggery as diet 5. The observed difference between these diets was highly significant with a  $p$ -value of less than 0.001 ( $p < 0.001$ ). Therefore, based on the observational study conducted, we reject the null hypothesis.

The ANOVA test perform for the statistical analysis and Graphs were made with help of GRAPH PRISM 10.2.0 software.

### 4. DISCUSSION

Honey bee hives are provided with artificial nourishment to compensate for the insufficient availability of nutritional pollen sources in their surroundings. This supplementary feeding is aimed at boosting the production of brood and the expansion of colony populations, particularly in preparation for pollination services, during which beekeepers receive compensation according to the size of the colonies [22].

Results of diet in an experiment concluded as five different diets were given the more successful one in this experiment is Dry dates Diet. As Dry dates have nutritional value when given to bees mixed with honey as a best pollen substitute for them.

Previous studies have reported similar results, where bees consumed artificial protein-rich foods instead of naturally available pollens (Saffari et al., 2004); [23]. For instance, diets enriched with soybean have been used as substitutes for pollen for the brood of *A. mellifera*, incorporating soybean, wheat, maize, and gram flours. For an experiment mainly diets that are rich in protein were taken along with blend of honey.

**Table 2. Survival rate of *Apis mellifera* on different pollen substitute (Artificial diets)**

#### SURVIVAL (MEAN±STANDARD ERROR)

Sl.no.	Diets	N	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1.	Dry dates	10	2.66±0.57	1.66±0.57	1.33±0.57	1.66±0.57	1.33±0.57	1.30±0.57	1.33±0.57
2.	Fox nuts	10	2.56±0.55	3.33±1.55	3.32±0.57	3.35±0.57	3.32±0.57	3.30±0.54	3.32±0.57
3.	Almonds	10	3.54±0.56	3.56±0.57	3.66±0.57	3.67±0.57	3.54±0.56	3.54±0.56	3.44±0.57
4.	Sugar syrup	10	4.33±0.57	4.54±0.57	4.33±0.56	4.75±0.55	4.52±0.57	4.66±0.54	4.54±0.57
5.	Jaggery	10	5.66±0.57	5.33±0.57	5.67±0.57	5.66±0.57	4.55±0.57	5.66±0.57	5.66±0.57



**Fig. 5. Dead bees**

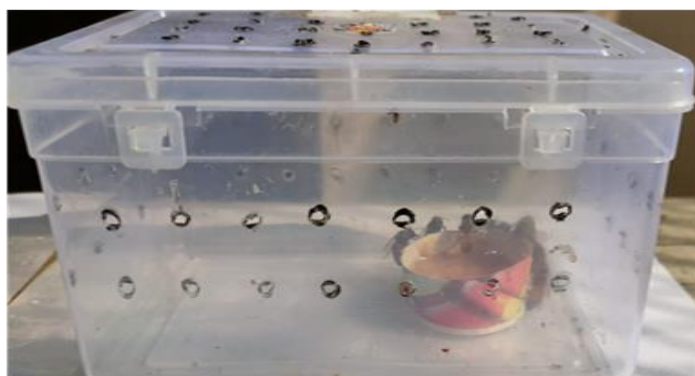


Fig. 6. Feeding of pollen substitute diet by *Apis mellifera*

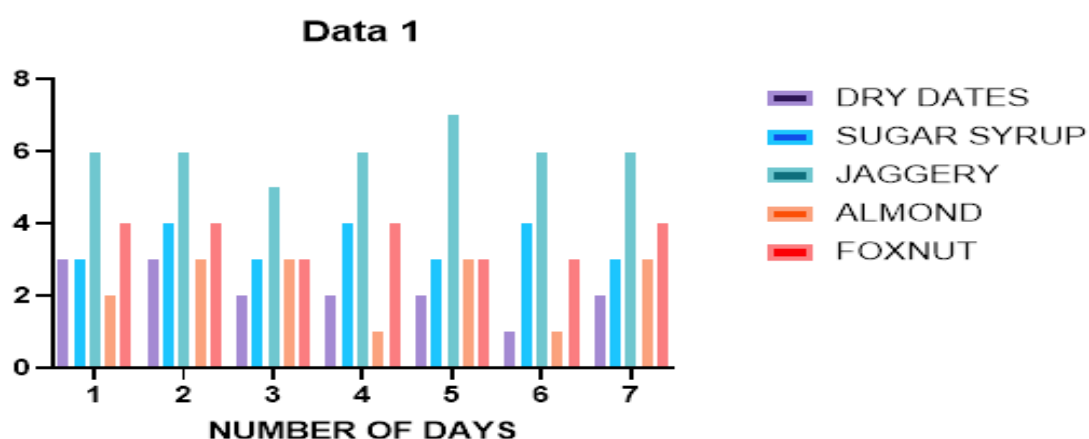


Fig. 7. Graph showing mean mortality rate of *Apis mellifera* after consumption of different artificial diets

Similarly, numerous studies have observed a significant increase in worker sealed brood when bee colonies are fed with pollen substitutes enriched with nutrients for expanding the worker brood area (Saffari et al., 2010); [24,25]. Several reports support these findings, indicating that the provision of supplementary dietary substances results in a greater number of frames per colony compared to control colonies, leading to higher honey production [26], (Dodologlu et al., 2004; De Grandi Hoffman et al., 2008); [27,24].

The process of creating and assessing new artificial diets entails examining how sensitive honey bees are to protein deficiency using nutritional indicators. Over recent decades, a series of studies have deepened our comprehension of alternative diets for humans, animals, and honey bees, and their effects on health. Factors such as high protein levels, digestibility, and nutrient utilization in the body played pivotal roles in determining the most optimal artificial diet [28].

Diets have been administered both in summer and winter seasons, adjusted according to the severity of lean periods in various countries [29,30], (Kumari and Kumar, 2020). Nevertheless, reports indicate that feeding diets to bees during lean periods, regardless of geographical location, has a positive impact on colony parameters.

## 5. CONCLUSION

In conclusion, the use of artificial diets enriched with proteinaceous substances as pollen substitutes for honey bees has shown promising results in various studies. These diets have led to significant improvements in worker brood development and colony productivity. By providing supplementary dietary substances, beekeepers can potentially enhance honey production and overall colony health. Various supplement diets were used in an experiment which can be used to increase population. Artificial Diets could be help to reduce migration for beekeepers in the dearth period.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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