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Impact of various artificial diets on the survival rate and food intake of Honeybees *Apis mellifera* (Hymenoptera, Apidae).

Amna Kidwai[1] and Barish E. James[2*]

Department of Zoology, Isabella Thoburn College, University of Lucknow, Lucknow 226001, Uttar Pradesh, India

ABSTRACT

The viability of the ecology and the beekeeping sector depend on a healthy honeybee population. Particularly, ata time when there is a shortage of pollen in the environment, the quality of the artificial diet has an impact on thecolony's growth and strength. This study illustrates that when pollen availability and variety are insufficient, supplemental meals or artificial diets may be used to improve the health and colony development of the bee. Theexperiment was designed to show the effectiveness of five protein-rich diets on honeybees. The *Apis mellifera* species was chosen as the subject, and the diets offered were sugar syrup, honey, jaggery, brewer's yeast, and jaggery + black gram weighing *16 ml* each offered in 2:1 diet to water ratios (w/v) consisting of 11gm solid and 5 ml liquid. The findings concluded that *Diet 1*, (containing sugar syrup) was more widely consumed by honeybees than the other diets offered over the course of a seven-day experiment. The data was then projected for statistical analysis and the test significance level was $P<0.001$.

KEYWORDS: Artificial diet, *Apis mellifera*, diet consumption, survival.

INTRODUCTION

The most significant eusocial insect, the honeybee, is directly useful to humans and plays a crucial role in preserving the natural ecology (Klein et al. 2007). Honeybees are known for the size of their colonies, the quantityof honey they produce and store, and their capacity to build wax colony nests that last for a very long period. The bee colonies require pollen and nectar to maintain heat in their nests, power foraging flights, and rear brood (G DeGrandi-Hoffman 2008). The main sources of nectar, pollen, and water are proteins, carbs, minerals, lipids, vitamins, and water and the main source of carbohydrates is nectar, and pollen provides all the other nutrients they need (De et al. 2010). The apiculture sector and agriculture as a whole are now very concerned about honeybee health. The quality of the diet has an impact on the health and strength of the colony, particularly for colonies getting ready for the winter and beginning population growth in the early spring (Kevan et al. 2010)

Weather conditions are unfavourable for bees at particular times of the year; therefore, nectar and pollen resourcesare limited during these times (dearth period). However, the capacity of honeybees to stay healthy is threatened by a variety of stressors that interact with one another, such as illnesses, parasitic mites, pesticides, and poor diets (Alaux et al. 2010). In this instance, the colony may be provided with a pollen substitute, which is food designedto totally replace pollen (Shehata and Nafea 2006; Shehata, 2016. The creation of artificial diets has placed a lot of emphasis on the pollen's protein content, which is essential for the colony. Honeybees are thought to be dependent on amino acids like arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine in particular (Taha, Al-Kahtani, 2019). Protein-rich substances found in soy, pea,yeast, casein, eggs, and microalgae are frequently used in artificial bee diets. It has been demonstrated that addinga small amount of pollen harvested by bees to some diets increases consumption and brood rearing (Ricigliano, Williams, and Oliver 2022).

In order to address the issue of floral scarcity, especially in stationary beekeeping, beekeepers and bee scientists have been interested in developing a pollen supplement or alternative artificial diet (El-Wahab 2016). The diet must have a variety of feed ingredients as alternative sources of nutrition comparable to their natural food sources and have the correct texture and consistency to be accepted by the animal in order to be of nutritional value for animals, including insects (Kevan et al. 2010). In order to ensure the survival and growth of bee colonies, a pollen substitute must be provided and this can be done by evaluating several indicators, including reproductive success, disease resistance, hive weight gain, nutritional intake, or the size of the worker broods.

On the other hand, there are several plant substances that can be used to improve the health of bee colonies, suchas mint, cinnamon, and chamomile. These substances can help in the management of bee diseases such as Varroa mites and Nosema (Author, El-Wahab, and El-Wahab 2012). Therefore, in addition to these criteria, nectar or its

substitutes, such as carbohydrates, are necessary for the growth of bee larvae (Rortais et al. 2005). Bee workers provide food for the larvae; therefore, plant extracts can reach the larvae during feeding and may have an impact on the morphology of the adult bee. There hasn't been enough research done on this issue. (Al-Ghamdi, Abou- Shaara, and Ansari 2021)

Bees in winter may endure colder temperatures if they are given an adequate energy source like an artificial diet.The goal of this study was to evaluate different commercially available bee meals in liquid formulations by monitoring consumption and their effects on some biological activities of honeybee colonies. The trial lasted seven days during the first week of January 2023. Five different diets were taken, with ten boxes for each diet. Each box contained 20 worker bees (*Apis mellifera)* in number. Later, the bees' food consumption and survival rate were noted.

MATERIALS AND METHODS

The experiment was carried out for seven days at 20± 5℃ and 55± 5% relative humidity. Beehives with two brood frames were purchased from the beekeeper. The newly hatched adult honeybees were moved into each thermocol box made specifically for the experiment and they were then given five different artificial diets that were made by combining food items and water in a 2:1 ratio (w/v = weight to volume) comprising 11gm of solid and 5ml liquid.

Diet 1 = Granulated sugar: Water (2:1 w/v)

Diet 2 = Jaggery: Water (2:1 w/v)

Diet 3 = Honey: Water (2:1 w/v)

Diet 4 = Black gram *(Vigna mungo):* Jaggery: water (2:1:1 w/v)

Diet 5 = Brewer's yeast *(Saccharomyces cerevisiae)*: Water (2:1 w/v)

Five pollen substitutes or artificial diets that were provided to the honeybees were: For this study, a variety of foodstuffs with high protein content were selected. To store the diet mixture, shallow, round, fibrous plastic bowls were used. For each diet, a total of 10 boxes (23cm × 30cm × 33cm) were made from thick thermocol sheets; two of the boxes were joined together (46cm × 30cm × 33cm) and partitioned with another sheet of thermocol, which was then piled on top of one another. Before placing honeybees inside the boxes, bowls full of artificial diets were placed inside each one. The boxes were then left for seven days at room temperature and were examined every day. Care was taken to ensure that the diets were not overly diluted to prevent bees from becoming trapped or damp while feeding. The boxes had three sides that were completely sealed, while one side had a mesh for proper ventilation.

Over the course of seven days, the survival rate was monitored every 24 hours. The measurements were made based on the overall number of bees that survived after consuming the diets, and in the end, the weight of the consumed diets was assessed. The percentage of surviving bees was compared between feeding options in order to identify the best feeding option with regard to enhancing honey bees' survival rate under cold temperature conditions.

Bees feeding on sugar syrup

Boxes designed for the experiment

Bearding in *Apis mellifera*

STATISTICAL ANALYSIS

The total amount of diet consumed by honeybees was measured across the treatments. The analysis of variance (ANOVA) was used to calculate the findings as (Mean ± Standard Error) using IBM SPSS Statistics 29.0 software.The graphs created from the collected data were made using the GraphPad Prism 9 software for Microsoft. The significance value of the test was P<0.001.

OBSERVATIONS

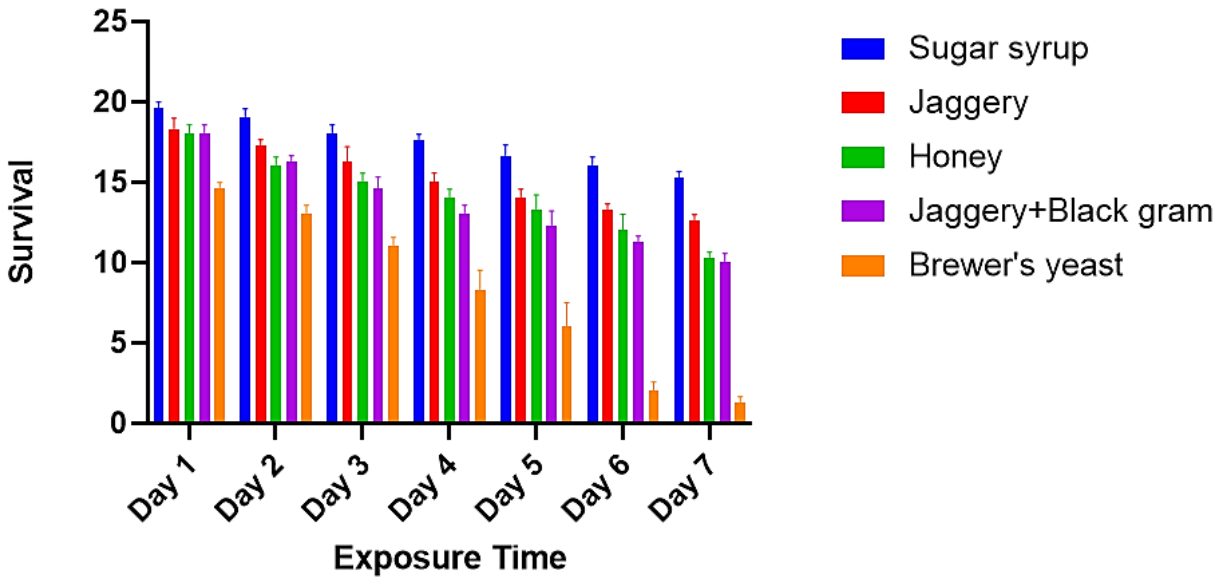
TABLE 1

Survival of *Apis mellifera* on different artificial diets

S. no.	Diets	N	Survival (Mean ± *SE*)						
			DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7
1	Sugar Syrup	20	19.66±0.33	19.00±0.57	18.00±0.57	17.66±0.33	16.66 ± 0.66	16.00 ± 0.57	15.33 ± 0.33
2	Jaggery	20	18.33±0.66	17.33±0.33	16.33±0.88	15.00 ± 0.57	14.00 ± 0.57	13.33 ± 0.33	12.66 ± 0.33
3	Honey	20	18.00±0.57	16.00±0.57	15.00±0.57	14.00 ± 0.57	13.33 ± 0.88	12.00 ± 1.00	10.33 ± 0.33
4	Jaggery + Black gram	20	18.00±0.57	16.33±0.33	14.66±0.66	13.00 ± 0.57	12.33 ± 0.88	11.33 ± 0.33	10.00 ± 0.57
5	Brewer's yeast	20	14.66±0.33	13.00±0.57	11.00±0.57	8.33 ± 1.20	6.00±1.52	2.00 ± 0.57	1.33 ± 0.33

*N = Number of honeybees
*Three out of ten replications are considered
*Test Significance level P<0.001

GRAPH 1



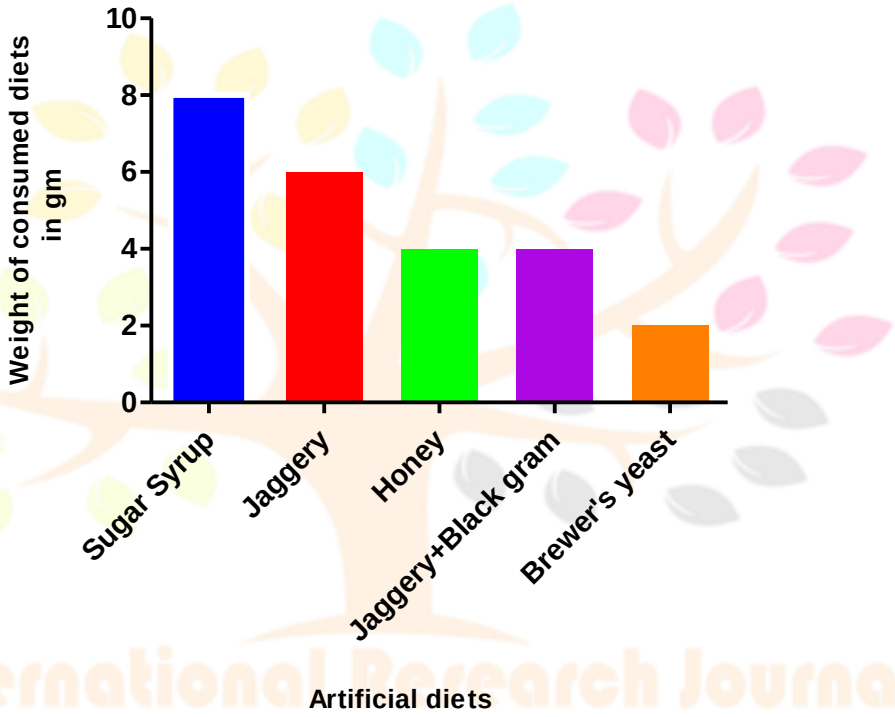
Mean survival of *Apis mellifera* treated with different artificial diets against days

TABLE 2

Consumption of diets by *Apis mellifera*

S.no.	Diets	Diets consumed (weight in grams) (16gm)
1	Sugar Syrup	8 ml
2	Jaggery	6 ml
3	Honey	4 ml
4	Jaggery +Black gram	4 ml
5	Brewer's yeast	2 ml

GRAPH 2



Mean weight (g) of artificial diet consumed by (*Apis mellifera*) in each treatment

RESULTS

Statistically significant differences were seen between the varied diet consumption over the course of the observation period. It was recorded that Diet 1 greatly increased the survival rate of honeybees, followed by Diet 2 (Jaggery), Diet 3 (Honey), Diet 4 (Jaggery + Black gram), and Diet 5 (Brewer's yeast). In contrast, Diet 2 (Honey) and 4 (Jaggery+Black gram) were consumed in nearly similar proportions. As shown in Table 1 and Graph 1, the first diet which is sugar syrup has the maximum (Mean ± SE) value as compared to the other diets. The F value for various diets changed significantly for each day of observation, with the highest value occurring on Day 7 (F=178.00) and the lowest value occurring on Day 1 (F=12.79) followed by Day 3 (F=15.12), Day 5 (F=16.76), Day 2 (19.73), Day 4 (F= 22.87) and Day 6 (F= 74.47). The results were made by analyzing the test of variance (ANOVA).

Moreover, it is observed through Table 2 and Graph 2 depicting the weight of diets consumed in ml. The bees fed on a significantly higher amount of Diet 1(8ml of 16ml) in comparison to the remaining four diets. The second most consumed diet was Diet 2 (6ml of 16ml) followed by Diet 3 and Diet 4 showing almost similar consumption (4ml of 16ml), and lastly Diet 5 (2ml of 16ml). Therefore,it can be represented as Diet 1>Diet 2>Diet 3=Diet 4>Diet 5.

DISCUSSION

In this study, we evaluated how different artificial foods affected the health and survival rate of honey bees. According to the findings of the experimental study, honeybees preferred and consumed sugar syrup, which was 8gm of a 16gm diet more than jaggery (6ml of a 16ml diet) and other diets. (Abou-Shaara 2017) studied that worker honeybees (*Apis mellifera L.*) demonstrated the highest survival rate when fed sugar syrup in comparison

to other diets under cold temperatures. It was examined that bees survived more in non-acidified sugar syrup compared to the bees

fed with acidified sugar solution as acidity in dietary products may affect intestinal epithelial integrity and alter the composition and abundance of the gut microbiota in the study of possible side effects of sugar supplementary nutrition on honey bee health. (Frizzera et al. 2020; Abou-Shaara 2017). Bailey discovered that 2:1 acid-hydrolyzed carbohydrates in particular are poisonous to bees, however, the exact mechanism of action is still somewhat unknown. Bailey also ruled out the idea that the documented toxicity could have been caused by hydroxymethylfurfural (HMF) and/or its breakdown products, laevulinic acid, and formic acid, at the concentrations seen in syrups (0.04–0.2%). (Frizzera et al. 2020).

After sugar syrup, the survival rate and diet consumption by honeybees were highest in jaggery. The pH of jaggery, which is produced from sugarcane juice, ranges from 5.8 to 6.4. About 70–85 g of sucrose, 7–10 g of reducing sugars (glucose and fructose), 0.50–0.750 g of fat, 0.35–0.45 g of protein, and 0.5–1.0 gm of ash is included in 100 gm of jaggery (Rao and Singh 2022). Since honey bees are commonly observed being lured to jaggery in local shops, it was used as a test product. Bees consumed it after sugar syrup, which generated favorable results, and it can be utilized as artificial food in cold climates. Another test product used in this experiment was a mixture of jaggery and black gram. As the studies show that proteinaceous diets are considered favorable for supplement feeding, therefore, it was studied that the amounts of protein and carbohydrate in whole black gram seeds varied from 24.5 to 28.4% and 54.1 to 56.5% respectively (Kamani and Meera 2021). Over seven days, it was observed that honeybees consumed a meager amount of the mixture as an artificial diet, contributing to their low survival rate. A startlingly high mortality rate in honey bees kept in cages may be caused by the detrimental effects of nitrogen-rich meals (protein or amino acids) on survival (Pirk et al. 2010). Nitrogen-rich diets may increase oxidative stress by overproducing reactive oxygen species (ROS), however, the underlying mechanism for this is unclear. Diets with a protein-to-carbohydrate ratio of 1:5 significantly lower honey bee survivability in cages (Canché-Collí et al. 2021). Despite the fact that the survival rate was not ideal in some diets, it might still be utilized as a supplemental or artificial diet in extremely low temperatures to keep the bee colony from going extinct. It is vitally important that more research be done in this area.

After sugar syrup and jaggery, the survival rate of honeybees was seen more in liquid honey, this was confirmed by the study of the effects of various sugar-feeding choices on the survival and tolerance of honey bees (*Apis mellifera L.*) where it was concluded that bees survived and consumed more amount of liquid honey after sugar syrup (Abou-Shaara, 2017). Approximately 80 percent of honey is sugar and 18 percent to 19 percent is water. It also contains traces of pollen, essential oils, tannins, salts, minerals, and other substances (Supplemental feeding of Honey bee colonies, n.d.). It is often considered ideal by beekeepers for supplement feeding although it is not thought to be the best food to feed bees for a variety of reasons: Honey can spread a variety of microbe-borne illnesses, including American and European foulbrood, especially since American foulbrood is fatal to bee colonies. As honey ages, the amount of the naturally occurring acid hydroxymethylfurfural (HMF) increases and it has also been observed that sucrose, as opposed to honey, extends the life of adult bees (D. Somerville, 2014). Additionally, acid-hydrolyzed carbohydrates can be hazardous to bees, and other foods, such as heated or old honey, are less ideal for feeding bees (Feliciano-Cardona et al. 2020; Prakash Agrawal et al, 2013.; Gloria DeGrandi-Hoffman et al. 2016). High fructose corn syrup and honey were not shown to have any advantages over sucrose syrup when feeding animals. Bees that were given grape syrup had a shorter lifespan and developed diarrhea (Barker and Lehner, 1978). Liquid honey's high viscosity and propensity to disperse disease among colonies are issues (Abou- Shaara 2017).

Numerous strategies exist for nutrition to improve important aspects of health and immunological function. For instance, dietary protein supports the growth of fatty body tissue by supplying the necessary amino acids for the synthesis of vitellogenin, phenol oxidase, and glucose oxidase (Canché-Collí et al. 2021). The yeast biomass plays a significant role in the pharmaceutical and food industries since it is known as a rich natural source of protein, including enzymes, carbohydrates, peptides, vitamin B, all amino acids, numerous trace minerals, and small amounts of lipids and sodium (Jach et al. 2022). In this study, honeybees treated with brewer's yeast showed least survival rate and diet consumption however, this does not correlate with the study of Canché-Collí (Canché-Collí et al. 2021) where it was contended that the usage of yeasts naturally found in bee colonies' food reserves does not harm bee health. According to recent research, adding brewers' yeast to pollen substitutes can keep bees (A. m. carnica) alive in cages longer and increase survival rates to levels close to those seen in bees fed with bee bread (Škerl and Gregorc 2014).

CONCLUSION

In conclusion, it is evident that sugar syrup can function as an optimal artificial diet for honeybees during periods of low temperature and food scarcity. In order to create a healthy diet to maintain the health of bee colonies and boost their likelihood of survival during times of food scarcity, certain supplemental diets can be made utilizing sugar syrup as one of the ingredients. The findings also demonstrated that being the second most popular diet, jaggery also served as the ideal artificial diet for bees. Honeybees are one of the most eusocial and economically advantageous insects, thus it is necessary to watch out for their health and survival, especially in low temperatures, which can be lethal for honeybees if not taken care of. It is vitally important that more research be done in this area.

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