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REVIEW ARTICLE

Artificial diet: a novel technique for honey bee sustenance - a review

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Abstract

Colony maintenance during honey dearth period is a tricky and cumbersome task for bee keepers worldwide. The development of artificial diet has improved remarkably to confront the challenges of dearth period like reduced foraging activities, decreased brood development, susceptible to diseases due to lack of immunity and so on. The complexity of artificial diets grew over time as many studies demonstrated the vital significance of balanced nutritional profile of honey bees such as carbohydrates, proteins, vitamins, lipids, phytochemicals and phytosterols. Recent advancements have incorporated microbes such as cyanobacteria, yeast and other natural plant extracts to artificial diet to support colony growth, to enhance disease resistance and to maintain a healthy gut microbiome. Commercial diets are pre-formulated blends enriched with all nutrients including probiotics which can improve digestion, nutrient absorption and bolsters the bee's immune systems and to overcome the difficulties faced by honey bees during dearth period. By combining a well-balanced diet with all essential nutrients, beekeepers can better support their colonies with enhanced resilience through which year-round productivity and overall well-being of honey bees can be maintained. This article gives an insight into artificial sources, microbe-based nutrition and their combination and commercial diet formulations that are required during off-season through which over all sustenance of honey bees can be achieved.

Keywords: artificial diet; dearth period; disease resistance; honey bees; microbial sources

Introduction

Honey bees are one of the most important insects for the economy and ecology, that pollinate crop and produce nutritional honey, propolis, venom and wax (1). The reduction in the bee population could have an adverse effect on agricultural productivity, since honey bees are involved in pollinating 3/4th of plants (2). One of the major reasons for population decline of bees is the lack of continuous food supply. The primary source of food for honey bees are pollen and nectar that are required for their growth and development (3). Changes in climate and weather parameters impairs the quality of flowers, whereby floral nutrition becomes inaccessible and ultimately reducing viability of bees. Scarcity of pollen results in reduced lifespan of worker bees, decreased honey production and brood rearing (4). In the earlier days of beekeeping, prior to the existence of artificial diets, beekeepers augmented honey bee nutrition by employing several methods. They followed supplementation of nutrition without any scientific understandings (5). The period between 1920 and 1930 is considered as an initial supplementation period, where beekeepers relied primarily on natural feed. Supplementation of food was sparse as well as experimental and the thought of artificial diets had not yet been formed. They supplied sugar syrup alone, especially when natural food sources were inadequate and this was an ancient method of nutrition which was more intended on supplying energy rather than complete nourishment.

On realizing the need of protein, fewer attempts were made to include some protein sources like soy bean flour or cornmeal or chickpea flour to the sugar syrup (6) . Despite these, earlier formulations were not precisely balanced, they just started a more focused strategy for bee feeding. Food additives which were meant to boost bee production and health were first introduced in the late 19th century. Even though the formulations were remained in underdevelopment, these diets were aimed to supplement more nutrients. Commercial pollen substitutes were first introduced in the early 20th century (7). These were made with an objective of supplying the similar protein, fat, vitamins and mineral composition as of natural bee food. Those substitutes include brewer's yeast, pollen patties, agar solution and vitamins (8). In the middle of 20th century, formulations for diet were refined, highlighting the importance of all essential nutrients. The introduction of hydrolyzed proteins which bees can digest easily, was a result of advancements in food science. The nutritional balance of artificial diets has significantly improved

in the latter half of 20th century. These formulated diets were designed to maintain bee health when there was a shortage of food source (9). In recent years, microbial sources are also used in diets to feed bees. Algal biomass act as a natural potential feed source that improves the health of honey bees. Because of its nutritional value, sustainability, algae could potentially be developed into a novel supplement for helping bee colonies against existing biotic and abiotic stress. In recent years, there has been increased interest in the role of probiotics in the diet of honey bees. It helps to maintain a healthy gut microbiome of honey bees and also confers immunity and enhance the resistance against *Nosema* and *Varroa* (10).

Research and review analysis on the bee nutritional studies

Literature collection

The literatures were collected from databases like Scopus and Google Scholar. Keywords such as “honey bees”, “bee nutrition”, “artificial diet”, “microbe-based nutrition” were used for collecting review papers which produced nearly 550 papers. Screened papers were helpful in extracting the relevant data using different visual representations such as tree maps, graphs, collaboration maps etc., The Bibliometric analysis were carried out using R Studio Software version 4.4.1.

Overview of the analysis

Fig. 1 represents the abstract graphically. Fig. 2(a) depicts the major author contributions on artificial diets from 1987 to 2023. Fig. 2(b) illustrates a frequent work on all aspects of honey bees. Fig. 2(c) and (d) displays the overall country wise production and their network on scientific outcomes. Fig. 2(e) demonstrates the country’s production, among all; USA involves in more research findings. Fig. 2(f) portrays annual contribution over year, but on 20th century more empirical evidence was recorded.

Bee nutrition

Nutrition of honey bee is a broad system and is not parallel to that of other animals, because it is well formulated. Honey bee nutrition is complex at different levels such as colony nutrition and larval nutrition. Strong and healthy colonies are extensively dependent on proper nutrition. More precisely, honey bee requires several nutrients such as carbohydrates, proteins, vitamins, minerals, amino acids and lipids (3). While minerals, vitamins and water are necessary for higher survival rate, honey bee uses carbohydrates as an energy source, proteins for growth and development and lipids for energy reserves (11). Nutrition has a major effect on a number of aspects of bee’s development, including caste development, increase in lifespan, immunity against diseases, development of hypopharyngeal gland, brain development and behavioural development. Both quality and quantity of diets influence the honey bee health. Appropriate distribution of nutrients plays a crucial role in honey bee caste development (queen, drone and worker bees) and also in hypopharyngeal glands development (12). Honey is an essential compound for the development of bees since it is rich in carbohydrates. In contrast, it is not advisable to feed fermented honey, because hydroxymethylfurfural (HMF) content increases consistently during fermentation process which ultimately causes diarrhoea, ulcer in gastro-intestinal tract and ultimately leads to the death of bees (13). Likewise, deteriorative foods condense the stability and survivability of bees. Some researchers found that adding probiotics or prebiotics to artificial diet can help lessen negative effects on gut health. Augmentation of protein sources in bee’s nutrition is a major consideration to maintain soundness and solidness of bee hive. Hence, it is necessary to formulate a well-nourished diet to maintain gut microbes and health of bees (14).

Essential nutrients for optimal bee health

Macronutrients

i) Carbohydrates : Carbohydrates, primarily in the form of sucrose, glucose, fructose provide essential energy for honey bee activities. It greatly influences the flight activity as it is a fuel for their foraging activity (15). A single larva of worker bee requires 59.4mg of carbohydrate right through its growth for further development. In the same way, for survival, an adult worker bee stands in need of 4mg of sugars every day. Worker bees use carbohydrates to generate heat and to regulate hive temperature during winter periods. Bees convert carbohydrates (sugars) into wax, which is used to construct honeycomb structures inside the hive (16). Appropriate availability of carbohydrates promotes brood development and overall growth of the colony. It is demanded for various

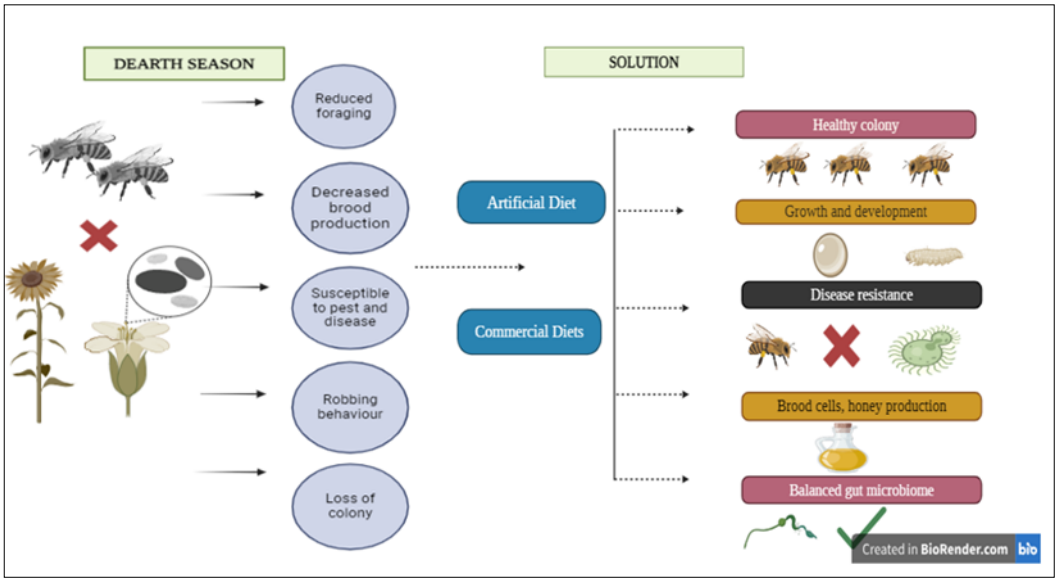

Fig.1. Graphical abstract showcasing the interplay between dearth period and role of artificial diet in enhancing colony resilience of honey bees.

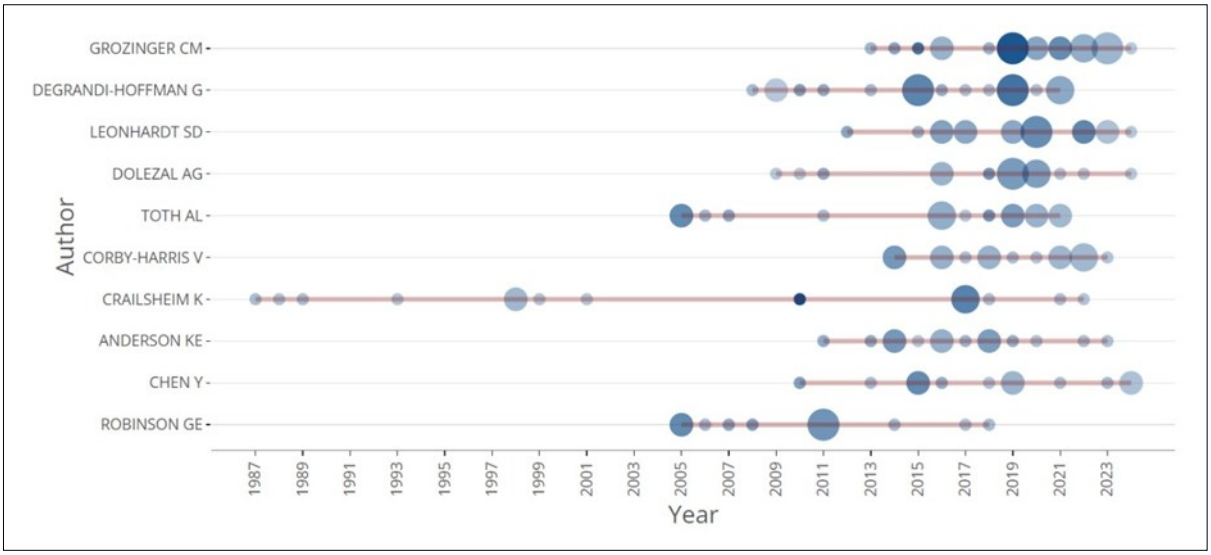

Fig. 2. Bibliometric analysis (a) Longitudinal bibliometric profile of honey bee research, showing yearly author involvement over a 37-year period.

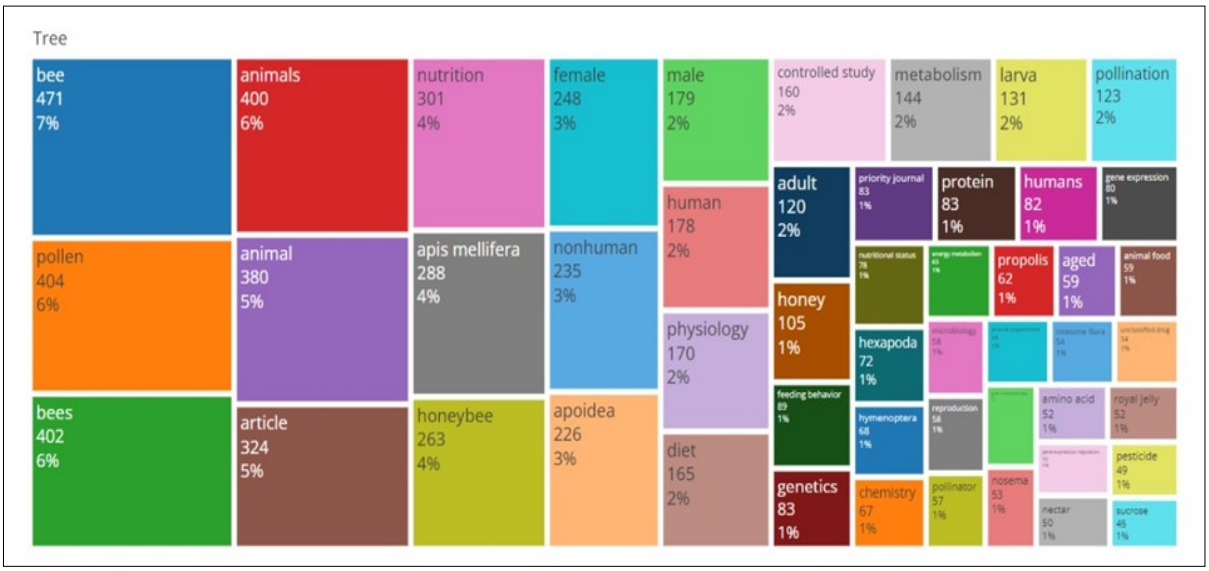

Fig. 2(b). Comprehensive world frequency tree map of honey bee related publications.

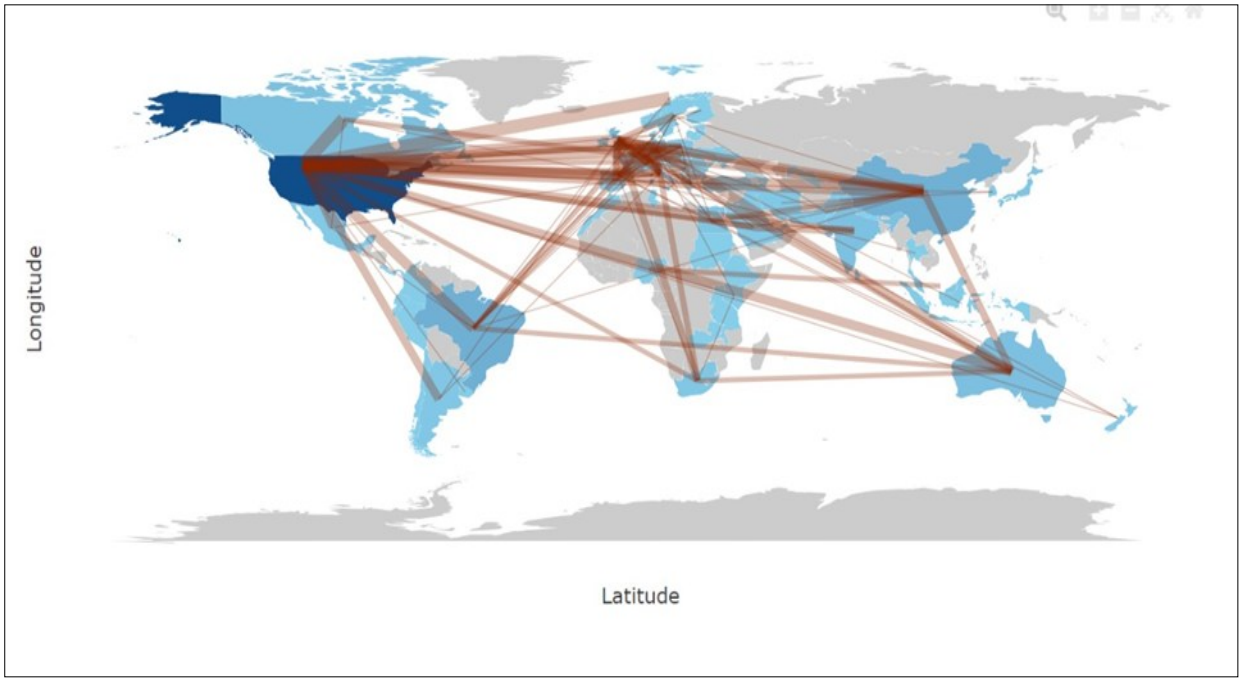

Fig. 2(c). Country collaboration network map illustrating international research partnerships in honey bee research.

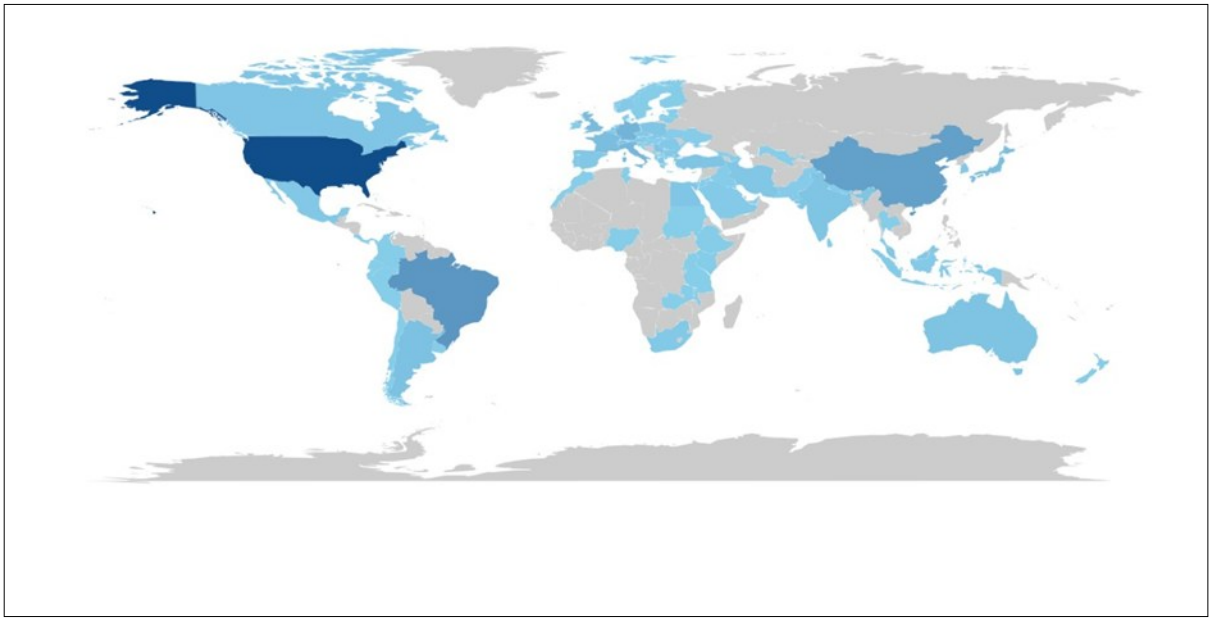

Fig. 2(d). Geographical visualization of honey bee studies, showcasing publication frequency.

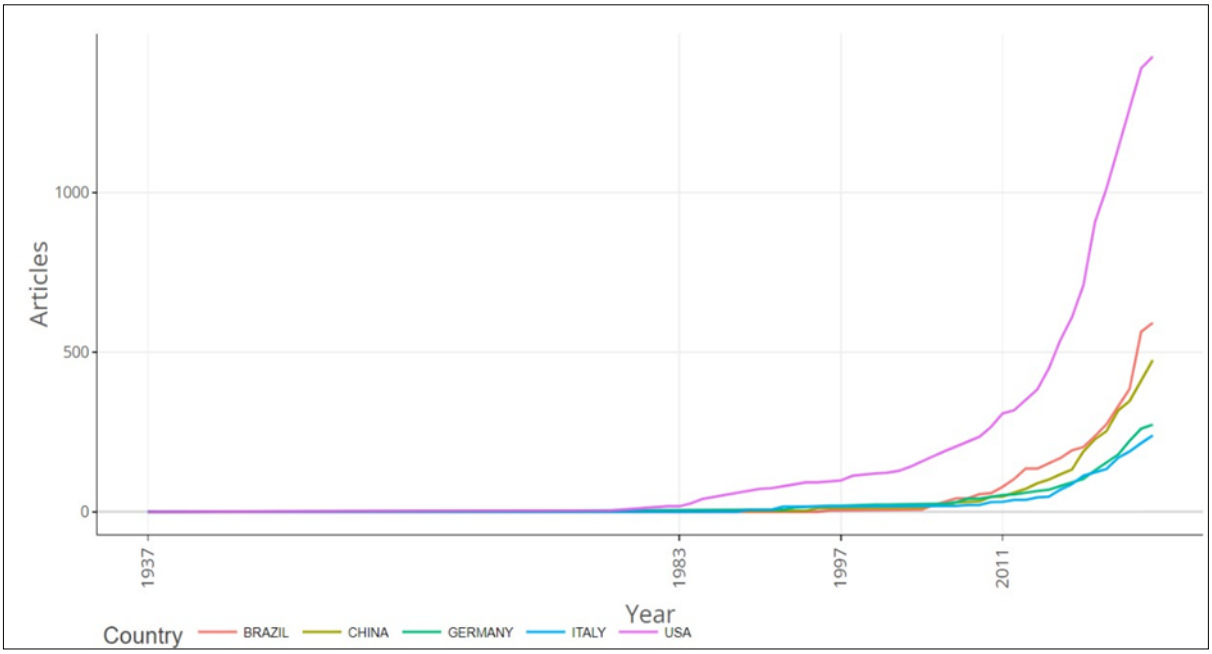

Fig. 2(e). Assessing a global contribution to honey bee science: A bibliometric perspective.

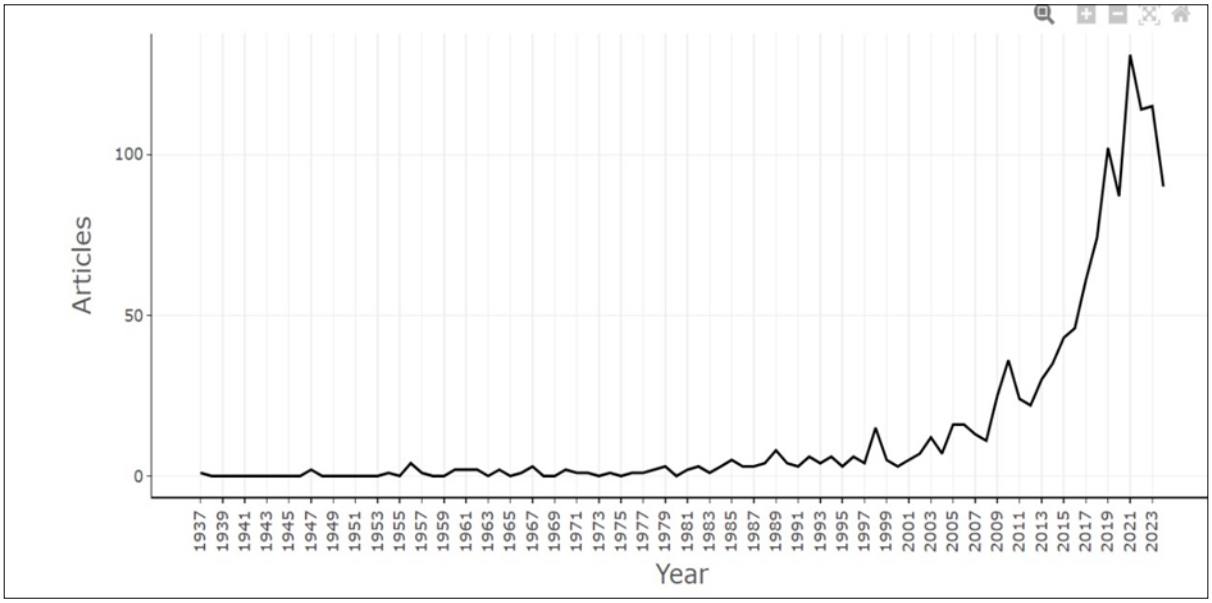

Fig. 2(f). Visualization of yearly scientific contributions to honey bee research, indicating emerging and sustained interest.

physiological process in particular with immune reactions, physiological metabolism and microbial resistance (17). Generally, carbohydrates are fed to colonies after harvesting of honey or during dearth period. This can be done by feeding sugar supplements (sucrose solution) and other fruit syrup like banana syrup, pumpkin syrup etc.

Over 14 types of sugar forms as a carbohydrate source have been listed by many researchers and they are: sugar in the form of solution (18), syrup (19), honey (20), sugarcane juice (21), glucose (20), fructose, mixtures of sucrose and dextrose, starch syrups (22), inverted saccharose syrups (22), high fructose corn syrup (23) and royal jelly (with honey)

ii) Proteins: The primary source of protein for honey bee is pollen. Pollen comprises about 10-36 % of protein but its content may vary in different regions under varied climatic conditions based on its origin (24). 22.2 % of proteins of pollen are essential for progressive development of mandibular gland and hypopharyngeal gland that is accountable for royal jelly secretion (25). Essential amino acids required for growth and development of bees are: threonine 3g, valine 4g, methionine1.5g, leucine 4.5g, isoleucine 4g, phenylalanine 2.5g, lysine 3g, histidine 1.5g, arginine 3g and tryptophan 1g (26). If any one of the amino acids is deficit in diet, it results in hindrance of growth and thereby affects the whole life process (27). Royal jelly is secreted by nurse bees when the pharyngeal glands are fully active and it becomes milky or light pale in colour. It is fed to young worker larva, queen larva and adult since it is considered as brood food. It contains 15-18 % of proteins, 6-8 % of lipids and distinct minerals like zinc (Zn), copper (Cu), sodium (Na), potassium (K) and magnesium (Mg).

Protein directly influences the royal jelly production, which is crucial for feeding the larva, as it activates growth-promoting pathways and also influence the hormonal balance in bee larvae. It regulates bee's immune system and capacity to evade diseases and parasites (28). Besides, it becomes essential for defensive mechanisms like encapsulation, phenyl oxidase and lysozyme activities and it directly helps in egg production which greatly supports the population of the colony. Leucine regulates protein turnover via gene expression and cellular mTOR signalling, whereas lysine is directly implicated in the creation of nitric oxide, a neurotransmitter that has been shown to strengthen memory in honey bees (29). When deprived pollen quality is available, protein-rich diet can be fed to colonies during the dearth period for worker bee longevity, fat body maintenance and to enhance growth in the spring. The amino acid levels of haemolymph in captive bees are boosted by the diets supplemented with enriched proteins

Many researchers have identified some of the commercial and isolated proteins which can be added to artificial diet of honey bees and they are: albumin (30), casein (30), crude protein (31), isolated soy protein (32), protein hydrolysate (33), sodium caseinate, tankage protein and whey protein (34). Out of all the proteins that have been documented, albumin and casein have been the most commonly utilized in bee artificial feeding.

iii) Lipids: Lipids and fats function as structural elements in cell membranes, provide energy and involved in various physiological process which includes development, reproduction, fecundity and biosynthesis of hormones (35). Linoleic acid (LA) and alpha linoleic acid (ALA) are the two most important essential fatty acids crucial for queen bee's reproductive health and energy metabolism. Dietary lipids alter the fatty acid composition of body and lipid peroxidation. During brood stage, lipids are considered as a salient factor for synthesis of bio-molecules (36). Sterol, a form of lipid act as a precursor molecule for ecdysteroid synthesis which help in moulting process. Scantiness of linoleic and linoleic acids content leads to slow down of ecdysis process in pupal and adult stages (37). In addition to energy reservoir, they exert an influence in behavioural maturation of bees, while they change from nursing to forager bees is accompanied by a reduction in the amount of fat stored in the body. There may be an early transition of nurse bees to foragers at a younger age that results due to lipid depletion in diet. The ability of bees to identify diseased brood is further impaired by a varying lipid content in diet which may have a disruptive effect on colony health and hygiene (38). Furthermore, age and caste determination of bees is correlated with lipid unsaturation of membrane phospholipids. Diminished amount of lipid content directly affects the social behaviour, health, productivity and colony duties. For instance, adding 0.5 % to 1 % of coconut oil to diet for newly emerged and forager bees, enhances the lipid content and survival capacity of honey bees (39).

Micronutrients

i) Vitamins: Vitamins are vital micronutrients that are crucial for healthy growth and act as an enzyme precursor which helps in maintaining internal metabolism. They are prerequisites for growth and development of healthy body of bees. Table 1 represents the essential vitamins and their role in honey bees. Plenty of vitamins, in particular with vitamin C had a major effect on hypopharyngeal gland (40). Pollens are rich in B-complex vitamins, carotenoids with all other vitamins. For the production of royal jelly, bees need a vitamin rich diet and also the vitamin-B complex for brood rearing. Vitamin-B complex, so-called methyl donors and methionine, an essential amino

Table 1. Essential vitamins and their role in honey bees

S.No	Vitamins	Role/Function	Reference
1.	Vitamin A(Retinol)	Body structure development Cellular differentiation	(82)
2.	Vitamin B2 (Riboflavin)	Energy production Cellular respiration Reproductive function in queen bees	(23)
3.	Vitamin B1 (Thiamine)	Carbohydrate metabolism Foraging activities	(23)
4.	Vitamin B6 (Pyridoxine)	Amino acid metabolism Production of antibodies against diseases	(23)
5.	Vitamin B12	Energy metabolism for foraging activity	(23)
6.	Vitamin C (Ascorbic)	Act as Anti-oxidant	(19)
7.	Vitamin E (Tocopherol)	Egg production of queen bees Larval development Cellular health	(82)
8.	Vitamin K3 (Menadione)	Proper coagulation of haemolymph Synthesis of protein which helps in physiological process	(23)
9.	Ca-d-Pantothenate	Synthesis and degradation of fatty acids	(23)
10.	Multivitamin mixtures	Supports metabolic process Maintain immune function	(23)

acid cum antioxidants, essential for gene expression, caste determination and also, they ward off bees from hypomethylation, a process where methylation of cytosine and adenosine residues in DNA are lessened (41). Vitamin B_5 also called pantothenic acid, is a precursor of coenzyme (Co-A) biosynthesis that becomes an essential co-factor for metabolic reactions. In earlier studies it was established that, pantothenic acid, thiamine and riboflavin act as prime component for hypopharyngeal gland development. Conversely, increased concentration of pantothenic acid also decreases the survival rate of honey bees. Vitamin C fortified diet helps in increasing anti-oxidative enzyme activity such as peroxidase, catalase, glutathione transferase activity, protein content, TAS content with mortality rate during winter season. Previous studies stated that vitamin B and protein sources are the key factors for maturation of newly emerged bees (42).

ii) Phytochemicals: They are chiefly regarded as beneficial nutrient to honey bees; even they are micronutrients and are derived from plant products. Many phytochemicals have antioxidant properties that help to overcome radical causing oxidative stress. Previous studies stated, phytochemicals foster immunity and microbial stability of bees (43). They are usually found in floral nectar and pollen. Nectar is a good source of phytochemicals which has phenolic acid, terpenoids and flavonoids which act as primary phytochemicals (44). While some phytochemicals involving thymol and eugenol have positive effects on bee health. At the same time, some phenolic acids, flavanones and flavones have unclear effects. Adverse effects to phytochemicals, like thymol, might involve loss of phototaxis in adult bees and the expression of vitellogenin in larvae(1). Earlier studies stated that feeding bees with phytochemicals, develops immunity against protozoan parasite, *Nosema ceranae* which causes symptoms like reduced strength, loss of hive productivity, increased death rate of adult bees and absence of crawling behaviour of bees. Increased longevity of worker bee can be achieved by infusing some of the phytochemicals such as p-coumaric acid, gallic acid, kaempferol and caffeine into the diet (45). Among all compounds, p-coumaric acid helps in regulation of microbial activity in gut by elimination of toxic substance and boosting immunity and also reducing pesticide stress of honey bees (46).

iii) Phytosterols: Honey bees are sterol dependent but they are unable to bio synthesise sterols, so called auxotrophs (47). Sterols act as signalling molecules and also support for biological changes. In addition to cellular membrane development, it serves as a precursor for moulting hormone, an ecdysone (48). The chief component of phytosterol for honey bee is 24, methylene-cholesterol (24MC). It increases survival rate and brood production when they are fed artificially under caged conditions. Some studies have recorded the accumulation of large amount of 24MC in honeybee pupae and also proven that increased survival rate and increased head protein content can be accomplished when there is an increased uptake of sterols. The sterol level can be maintained by transferring from sterol pools of worker bees to young larva by brood food (49).

Navigating the dearth period - to fill nutritional gaps

Dearth is a shortage of food i.e. reduced nectar and pollen availability. Usually, summer and rainy season are considered as dearth period. For instance, the Africanized bees replaced European bee (*Apis mellifera*) with hybrid bees and population of these bees become weakened during dearth period in the Central and South America, thereby population size became diminished and mortality rate increased (50). Changes in availability of food alter the foraging pattern in that way all the colonies started to abandon. (51) highlighted that winter months and occasionally during the summer months is considered as scarcity period in Northern part of India. (52) stated that Western part of India faces dearth period during hot summer and winter due to reduced flowering. (53) noted that dry season (Mar-May) and the winter period (Dec-Feb) can lead to decreased nectar availability in Southern India. indicated that nectar and pollen scarcity often occur during November to March and occasionally during early summer in Eastern India. Commonly during dearth time, colonies become weak and pollination activity also get reduced which leads to loss of colony population. During lean period, many of the pests such as greater wax moth, lesser wax moth, ants, yellow banded hornet and wax beetles cause drastic attack to honey bees. Among all pests, the Oriental wasp, *Vespa orientalis* causes greater damage and becomes more sustainable during August-September. Greater wax moth becomes more active during summer season and this season assists their development and reproduction. During these period, tropical bee colonies such as *Apis cerana* took to abscond in search of food resources (54).

Dearth period management- use of artificial diet supplements

Nectar and pollen from natural resources serves as a nutritious and appropriate food for honey bees. However, uninterrupted supply of food sources is unfeasible at all times (19). Pollen and nectar scarcity periods of honey bees vary across diverse regions of India due to varied climatic conditions and floral availability. Contrarily, in wasteland, farming fields and desolate land, a large number of weeds and herbs emerge throughout the summer and monsoon seasons and two such weeds are *Parthenium hysterophorus* and *Cyperus rotundus* which are abundant in North India and bees gather pollen from such weeds when there is insufficient bee flora during periods of famine (55). Egg laying and brood rearing activities are negatively impacted by poor nutritional reserves. Bee enemies such as black ants, bee-eating birds and varroa mites may target colonies due to their weaker strength. As a result, bee colonies quickly diminish and occasionally even dies (56). To overcome this adverse situation, colonies are either physically transported to abundant bee floral regions or supplementing pollen and nectar artificially. Artificial diet is a man-made supplement or equivalent that is designed to meet all essential nutrients and energy required by honey bees. Thus, adequate artificial feeding with enriched nutrients is the sole component to overcome off-season period.

Importance of artificial diet to bees

Feeding honey bees with artificial diet supplements not only stimulates colony and brood performance but also increases reproductive rates and improves immunity (57). In the course of scarcity period of natural resources (both pollen and nectar), feeding bees with artificial pollen or nectar substitute improves colony strength for production of honey and other by-products. As a result, their physiological reciprocation gets increased and its result is determined in terms of disease resistance, natality,

mortality, number of individuals in hive etc (58). Moreover, addition of antibiotics to diet, significantly reduced survivability that may be neglected by adding some pollen which could enlarge head mass and requite the loss of vitellogenin (vg) and five major royal jelly protein genes (mrjp 1-5) to a certain extent. Fig. 3 picturizes the uses of artificial diet in honey bees (59).

Vital elements for bees

i) Pollen: Several variable weather parameters like light, wind, temperature along with other elements like foraging activity, incidence of pests and disease and reproduction capacity of queen controls the number of bees in a colony (60). flower-bloom season, the population of worker bee is normally large enough to supply food under suitable temperature. While on the other extreme, the population of worker bee and egg production level become truncated during lessened pollen season (61).

Pollen is the dominant source of protein, essential for the structural development of muscles, glands and other tissues. When offsprings are fed with pollen free diet, growth size and development of hypopharyngeal glands got arrested. Enriched pollen diets, imparted more nitrogen content to bees. Pollen supplies required nutrients for the secretion of royal jelly by worker bees. 8-40 % of protein, lecithin and cholesterol are present in pollen (27). When lecithin and brewer's yeast are mixed and provided as feed, it nurtures colony during spring season and also helps in pollen shortage problem. When bees are fed with pollen at first five days of their life, directly influence the elevation of nitrogen level in their three segments of body such as head, thorax and abdomen by 92.6 %, 76 % and 37.5 % respectively (62).

Some micronutrients are also present in considerable amount in pollen. Among them, Zn constitutes 5.1 to 340mg/kg. To make sure healthy and superior brood and to bring out royal jelly with high Zn content, colonies stand in need of 60mg Zn/kg pollen (31). 100g of dried pollen has more B-complex vitamins such as thiamine (Vit-B1) 6-13g, riboflavin (Vit-B2) 6-20mg, niacin (Vit-B3) 40-110mg, pantothenic acid (Vit-B5) 5-20mg, pyridoxin (Vit-B6) 2-7mg, ascorbic acid (Vit-C) 70-560mg, biotin (Vit-H) 0.5-0.7mg and folic acid 3-10mg. Pollen quality also determines the physiology and parasitic tolerance of nurse bees (24). Daily consumption of pollen for about 3.4-4.3mg is required for worker bees due to the unavailability of marked protein reservoirs. Depending on the age of bees, pollen requirement also varies. So, it is necessary to supply enough pollen artificially for ensuring colony health (63). Table 2 depicts the different pollen substitutes and their role in honey bees.

ii)Nectar: Nectar is the richest source of carbohydrates and comprises of water and simple sugars such as sucrose, fructose, glucose differing from 7-70 % w/w (64). Pure sucrose is preferred by honey bees over pure glucose or fructose solution, that are not common in flower nectars but their combinations are better suitable for preference assessments. Nectar is considered as notorious plastic because its character is being modified by interactivity of bees in flowers and thus change the quality and property of nectar (65). Floral nectar is a sugary saturated solution that conciliate reciprocation between flora and mutualists i.e. pollinators especially honey bees. Sugar syrup is the one which is predominantly used as nectar substitute in supplementary diet of bees. Some researchers suggested the alternative nectar substitutes and they are sugar powder and honey, royal jelly and fructose, royal jelly and honey, royal jelly and sugar, powdered sugar and sugar syrup, sucrose and magnetized water, honey, sugar powder and sugar syrup, worker jelly, glucose and fructose, linseed oil (*L. usitatissimum*) and palm oil (66). Nectar substitute can be mixed with vitamins such as A, E, C and B-complex and yeast such as brewer's yeast, torula yeast, commercialized yeast, difco yeast and dry yeast. Preparation of inverted sugar syrup by adding water and sugar in the ration of 2:1 together with acidifying chemical can be provide as additional feeding of bees (67). When sugar syrup excluded with acidified solution i.e., lemon content and HCl are fed to bees, it resulted in increasing survivorship of bees. Without

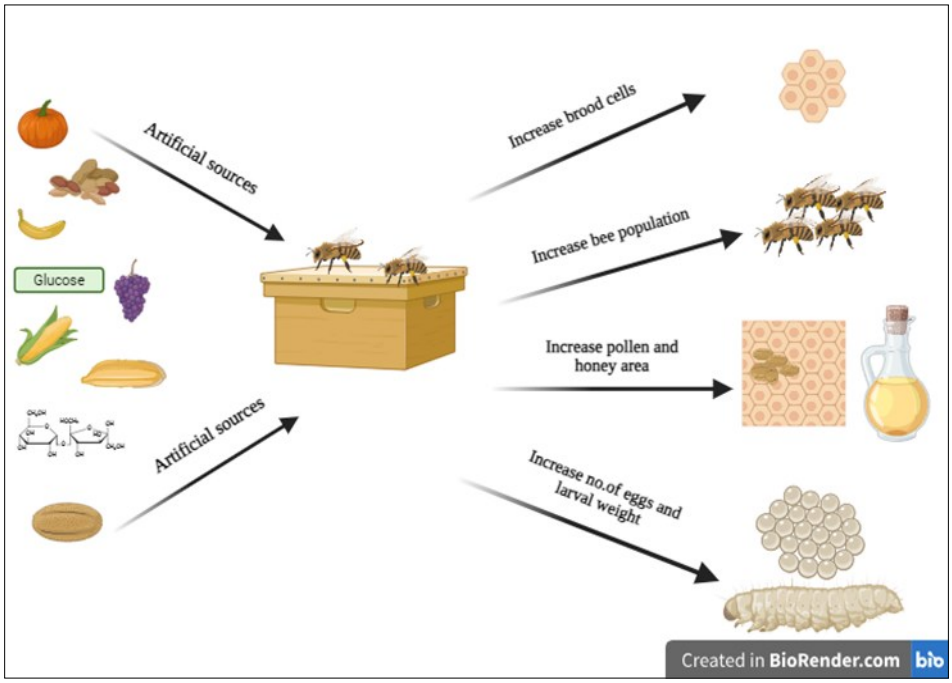

Fig. 3. Uses of artificial diet.

Table 2. Pollen substitutes for honey bees

S.No	Pollen Substitute	Nutritional value of	Uses/Role	Reference
1.	20 g soybean flour + 5 g brewer's yeast + 7 g honey + 8 g turmeric and fenugreek powders + 0.5g A, D and E vitamins + 9.5 ml orange juice + 150 ml sugar syrup	**Soybean** Protein-36g/100g Carbohydrates-30g/100g **Fenugreek** Minerals-28 % Proteins-22 % **Turmeric** Crude protein-9.40g Carbohydrate-67.3g	Increase brood area (1567.3 cm^2) Increase bee population (8)	(83)
2.	20 g maize flour + 5 g brewer's yeast + 7 g honey + 8 g turmeric and fenugreek powders + 0.5 g vitamins A, D and E + 9.5 ml orange juice + 150 ml sugar syrup	**Maize** Carbohydrates-71.88g Protein-8.84g	Increases brood area (1456.3 cm^2) Increase bee population (6)	(83)
3.	Redgum (*Corymbia calophylla*) + Soyflour protein	Total sugars-6g	Increases head weight of bees Enhances the development of Hypopharyngeal gland	(84)
4.	Artificial pollen+ Zn level of 60-70mg/kg	Protein-22.7 % EAA-10.4 %	Increases royal jelly production Improves health of bee larvae	(31)
5.	Opium poppy or bread seed poppy	Fructose-19.4 % Glucose-14 % Protein-21.5 % DPPH-88 %	Increases brood area-1184.14 cm^2 Increases wintering ability- 92.19%	(4)
6.	Soybean 30 % + Brewer's yeast 10 %+ Fenugreek 5 %+ Pollen 5 %+ Sugar 50 %	Protein-30-40g Vitamin C-10 %	Increases honey storage area- 637.29 $inch^2$ Increases colony population strength (no.of.covered combs-5.9)	(85)
7.	Crushed *Panicum* 46 % +Soybean 23 % + Mixed from melon & orange shell juice 23 %+ Agwa 8 %	***Panicum*** Protein- 12.5 % Carbohydrates- 70.4 %	Increases sealed brood area with 196.67 $inch^2$ in Carniolan hybrid bees	(86)
8.	Crushed *Phalaris* 46 % + Soybean 23 % + Mixture of melon and orange shell juice 23 % + Agwa 8 %	***Phalaris*** Protein- 92.5 % Carbohydrates- 70.4 %	Increases honey production in Carniolan hybrid and Italian hybrid with 6.33kg and 7.33kg respectively	(86)
9.	Chickpea flour	Protein-22.7 %	Increases brood area, honey yield and pollen area by 266.93±12.63 cm^2/colony, 8.4k/colony and 202.63±10.66 cm^2/colony respectively	(87)
10.	10g bee honey + 8g Turmeric and Fenugreek powders + 0.5g A, D and E vitamins + 45g powdered sugar + 20ml orange juice + 10ml mint oil + 30ml sugar syrup	**Orange juice** Vitamin C-80 % **Mint** Protein-0.12 % Carbohydrates- 60g Fiber- 7g	Increases biological activity, consumption rate and honey yield. Increases worker brood area with value 61.66 $inch^2$	(8)
11.	Date paste + 5 % *Spirulina* diet		Lowers mortality rate- 26.57 %	(88)
12.	Grinded groundnut enriched diet	Carbohydrates- 16.13g Protein- 25.80g	Increases area of worker bee 790.31cm^2/ colony and high proportion of foragers-0.17 %	(88)
13.	Pollen grain with yeast	Carbohydrates-5g Protein-8g	Increases brood area by 157.4cm^2 with high percentage of honey production	(19)
14.	Dried brewers' yeast (*Sacharomyces* sp.) at 25 % concentration + 400 g soya bean (lipid free) + 400 g barley (apical + roots) +1000 g sugar.	**Brewers' yeast** Protein- 46.67g Carbohydrates-43.33g	Increases pollen storage area	(19)
15.	Horse gram 10 %+ skimmed milk powder 1 % + glucose 1 % + honey 6 %	**Horse gram** Protein- 22.5g Carbohydrates- 66.6g	Increases sealed brood area, bee population, no.of.eggs and honey storage area in respect of 378cm^2, 133.1, 405.46, 210cm^2	(89)
16.	Chickpeas+ corn+ sugar+ powdered milk+ brewers' yeast fortified with chamomile extract	**Powdered milk** Carbohydrates-12 % Protein-52 %	Increases survival rate, thoracic weight, mandibular gland and hypopharyngeal acini diameter	(90)
17.	50g Brewers' yeast+ chickpea cake fortified with 4.2 % pollen+ 250ml sugar syrup	**Brewers' yeast** Protein- 46.67g Carbohydrates-43.33g	Increases ATPase activity (51.73mmol/mg tissue)	(19)

doing pollination, nectar robbers such as wasps, ants, humming birds and some passerine birds steal away nectar by making floral punctures which leads to decrease in nectar quality and also alters sugar concentration which is main component required by bees for growth. This may also be an indirect issue for nectar scarcity. So, supplementation of nectar is essential during mortality period. Table 3. represents the alternative nectar substitutes with their nutritional content and its role in honey bees.

Cutting-edge natural boosters for bee diet

i) Onion waste: The price of sugar increasing every day, to compensate price unfairness it is necessary to find a better alternative diet sources for honey bees. It was found that onion waste was one such best source. So, onion wastes act as a novel diet supplement for honey bees. It has sucrose 9.4±0.31 %, glucose 29.3±0.19 %, fructose 27.2±0.14 % and fructose and glucose ratio with 0.9 and these nutrients are on par with honey. Onion was subjected to popping pre-treatment and enzymatic hydrolysis to convert into bio sugar which comprises

Table 3. Nectar substitutes for honey bees

S.No	Nectar substitute	Family	Nutritional value of Nectar substitute	Uses	Reference
1.	Neem fruit pulp (*Azadirachta indica*)	Meliaceae	Carbohydrates-6.5 % Protein-6.5 % Ascorbic acid-0.0024 %	Increase brood area June-21.87 % July-50.55 % Increase honey area June-8.56 % July-47.40 % Increase pollen area June-195.10% July-341.66%	(91)
2.	Mahua flower syrup (*Madhuca longifolia*)	Sapotaceae	TSS-19 %	Increases larval weight, honey storage area (41.432 cm^2) and brood area (152.90 cm^2)	(92)
3.	Barhal fruit juice syrup (*Artocarpus lacucha*)	Moraceae	TSS- 16.24 % Fructose, Sucrose	Increase propolis production Increase brood area (93.01 cm^2)	(92)
4.	PAU Bee feed	-	Supply proteins	Increases brood area and honey storage area	(92)
5.	Rice bran syrup (*Oryza sativa*)	Poaceae	Supply vitamins, minerals, lipids, phytochemicals-α-oryzanol and tocopherols	Increases honey content in brood cells Increases no.of.brood frames with a mean of 3.26	(93)
6.	Pumpkin syrup (*Curcubita pepo*)	Cucurbitaceae	TSS-3.42±0.19 Reducing sugars- 2.15±0.03 Total carbohydrates- 8.06	Increases no. of. cells with honey (824.10±557.8) Increase no.of.brood frames covered by bees	(93)
7.	Banana syrup (*Musa sp*)	Musaceae	TSS-23.2 (^{0} Brix) RSS-10.02 (^{0} Brix)	Increase no. of. brood per colony (6420.80±736.8) Increase no.of.covering frame of bees and brood frames by 5.2 and 3.8 respectively	(93)
8.	Plum syrup (*Prunus domestica*)	Rosaceae	Total sugars-13.79g Protein-0.37g Carbohydrates-14.3g	Increase brood area (770 cm^2) Increase honey store area (814cm^2) Increase pollen store area (311.667 cm^2)	(94)
9.	Apricot syrup (*Prunus armeniaca)*	Rosaceae	Total sugar-13.79g Protein-0.37g Carbohydrates-14.3g	Increase brood area (743 cm^2) Increase honey store area (802 cm^2) Increase pollen store area (305 cm^2)	(94)
10.	Grape syrup (*Vitis vinifera)*	Vitaceae	Total Brix-17.5g/100g Total phenols-36.2g/100g Antioxidants-20mg Tartaric acid-339mg/100g	Increase no.of.capped cells of honey Increase wax production	(95)
11.	High fructose corn syrup (*Zea mays)*	Poaceae	Total carbohydrtaes-26 % Potassium-1 % Sodium-6 %	Increase honey comb area-4571.63 cm^2	(96)
12.	Sugarcane syrup (*Saccharum officinarum*)	Poaceae	Total carbohydrates-24 % Potassium-1 % Magnesium-2 %	Increase bee strength and proportion of foragers in respect of 7 bee frames/colony and 0.20 %	(20)

mainly glucose, fructose, galactose and mannose. It was recorded that the mortality rate was decreased by 5.4 times when bees were fed with pollen and alternative bee feed (after removal of sulphuryl compounds from pre-treated popped onion sugar) (68)

ii) Melon extract: Agwa increased brood in autumn and also supplied amino acids. It can be supplemented with yeast, sugar, glucose and cane syrup, through which higher honey production can be achieved (69).

iii) *Castanea* and *Asparagus* sp: Chestnut helps in the development of hypopharyngeal gland in caged bees at an age of 5. Pollen of *Asparagus* promotes the growth of acid gland sac than the *Castanea* pollen. But feeding preference was more in *Castanea* i.e. 4.83g/day due to the presence of amino acid such as leucine, phenylalanine, histidine, lysine, arginine, threonine, methionine, tryptophan and isoleucine at the rate of 1.15 %, 0.69 %, 0.50 %, 1.11 %, 0.76 %, 0.60 %, 0.43 %, 0.24 % and 0.67 % respectively (70)

iv) Sweet Potato dough: Sweet potato dough offers vital nutrients like vitamins and carbohydrates, thus serving as a potential supplement for bees. When nectar is scarce, the natural sugars and carbohydrates in sweet potatoes may provide bees with an energy boost. Sweet potato dough with wheat starch and potato starch influences the average population of colony, honey production and average of brood which results in 7120per/cm^2, 2750g, 4176g/cm^2respectively (15).

v) *Agaricus brasiliensis* extract: One of the most notable components in *Agaricus* extract is beta-glucans that have anti-oxidant and immune supporting qualities. The extract is a good source of Vitamins-B complex essential for cellular functions. Combined feed of *Agaricus* with honey, sugar powder and water enhance bee colony strength, prevent mortality and its applicable under both lab and field trials (41).

vi) Chamomile, Caraway and Sesamum: Antioxidant found in chamomile, like flavonoids, can protect bees from oxidative stress. Reducing oxidative stress can lengthen life span of bees. *Caraway* helps in digestion and has anti-parasitic effect on *Varroa* (71).

vii) Fishmeal diet: Fishmeal increases longevity of bees due to its lack of poisonous substance, but when it is combined with raw soybean, decreases the longevity of honey bees from 112 to 65 days (72).

viii) Meat scrap: When 3 part of soybean flour, 1 part of brewers' yeast, 1 part of meat scrap, 1 part of dried skimmed milk and 1part of pollen are incorporated to honey bees, it increases brood cells (5)

ix) Liquorice extract: When the combined diet of 30 % liquorice extract, sugar, honey, soybean, vitamin-B complex, tetracycline and water was fed to *Apis mellifera*, it leads to increasing the area of honey produced, area of brood produced and area of pollen in respect of 7800 inch^2, 10780 inch^2 and 7730 inch^2 (73).

Microbes: a potential food source for honey bees

Microbes are also a distinguished and great source of B-complex vitamins. In addition to vitamins, minerals such as chromium (Cr), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), selenium (Se) and zinc (Zn) helps in increasing foraging activity and vigourness of bees (74). In microbes-based nutrition, yeast, bacteria and cyanobacteria play a superior role. The widely used yeast is commercial yeast, brewer's yeast, pie mixed yeast, yeast extract, dry yeast, bacto-yeast, difco yeast, torula yeast, bread yeast, beerswort and inactive yeast. Among all, Brewer's yeast and commercially available yeast are the most commonly used (19). When fortifying bee feed with Torula yeast, pollen and sucrose syrup, it enhanced adult bee population and it was preferable for autumn or fall season. On the contrary supplementing bee with royal jelly, water, D-glucose, D-fructose, Difco bacto-yeast extract and charcoal-treated yeast extract brings about increasing larval growth with decreasing adult yield due to disproportionate supply of amino acid.

Bacteria (EM® Probiotic's live micro-organisms) act as popular microbe-based nutrition contributes in maintaining well-balanced gut microbiome which assists digestion, nutrition absorption and overall health (75).

Microalgae are used as productive and functional feed source (pollen substitute) for honey bees under caged condition. Algae has abundant source of carbohydrates, protein, lipids, vitamins, minerals and amino acids (76) in addition to carotenoids such as astaxanthin and poly unsaturated fatty acids which help to overcome stress reciprocation. Proteins of algae have more essential amino acids (EAA) besides act as bioactive potentials of lectin, enzymes and peptides. Fig. 4 illustrates a diverse algal source employed in artificial diets. Spirulina, *Arthrospira platensis* can be used as protein source for diet. When queens were fed with freshwater green algae *Oocystis borgei*, it increased fecundity by 8.3% than control sugar fed syrup (77). Similarly, *Spirulina* also increased egg-laying rate by 44.7 %. *Chlorella vulgaris* and *Spirulina* stimulate brood production. When *Spirulina* fed with sugar syrup it increases brood area by 54.2 %. When bees were fed with *Scenedesmus quadricauda,* it increased honey production level to 27.6 %. When *Porphyridium marinum* and *P. purpureum* extracts were added to diet and fed to *Nosema ceranae* infected colonies, it resulted in reduced parasitic loads due to its tolerance capacity and they also ameliorate the physiology of honey bee. Increased mRNA level of vitellogenin and transcript levels of shock protein 90 was activity exhibited when honey bees were fed with *Chlorella vulgaris* and *Spirulina vulgaris* respectively. In contrast, the direct feeding of *Chlorella* caused increased mortality due to the failure of advanced or preceding digestion by nurse bees (78).

Commercial diet formulations

In recent years, pollen and nectar nutrition is an important concern in the beekeeping industry. Pollen is the only supply of protein, lipids, vitamins and minerals; nectar mostly provides carbohydrates. During unfavourable foraging season, honey bees failed to prepare bee bread due to the limited pollen supply. Hence, enough pollen is required during the brood-producing season (64). Therefore, floral diversity is crucial to provide an ideal and varied diet for honeybees, since various floral source yield pollen and nectar with unique nutritional

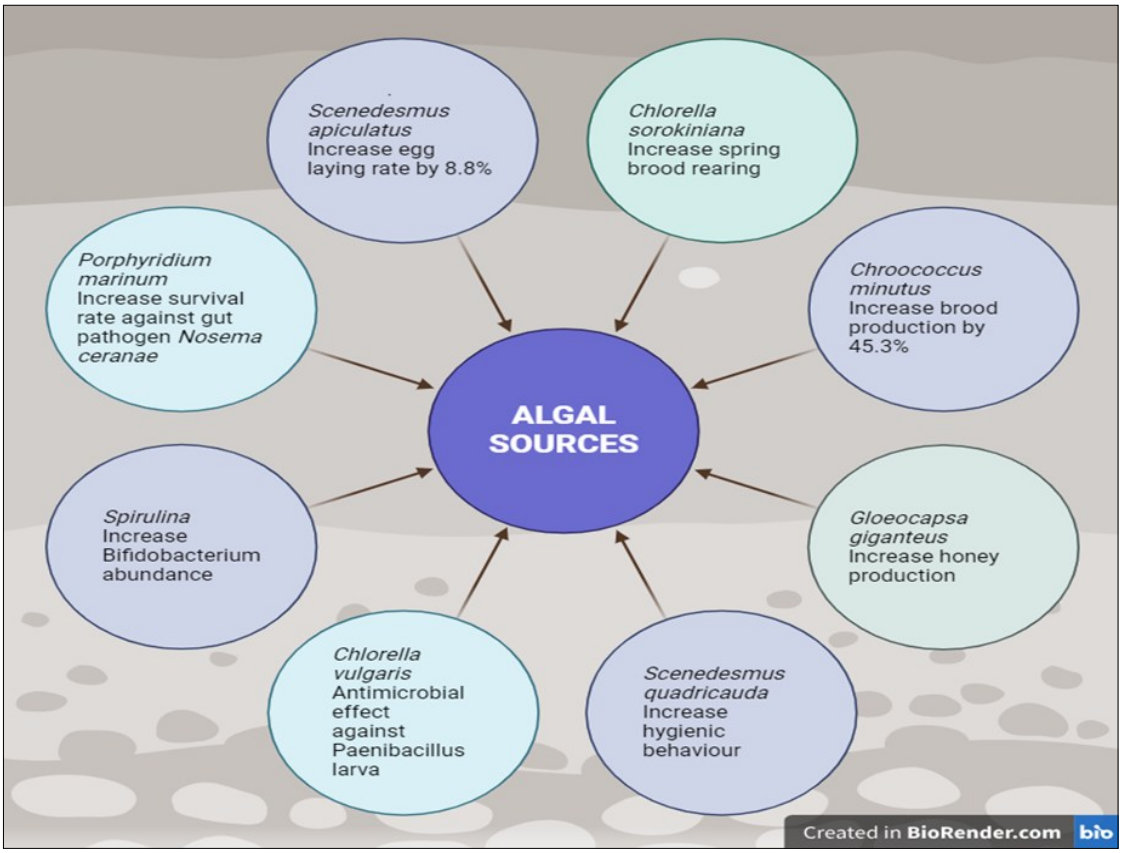

Fig. 4. Algal sources employed in artificial diet.

value. Individual honey bee health and colony performance are adversely affected by inadequate pollen nutrition, which also makes bees less resilient to other pesticide stress (79). In order to combat these issues, many beekeepers and beekeeping industries involved in formulating commercial diets enriched with all essential nutrients. Many companies came forward to produce an effective artificial diet. Fig. 5 portrays the available commercial diets with their respective available nutrients. Here, some of the commercial diets are listed, they are: Homebrew, Ultra bee, Bee-Pro, Feed-Bee, Mega bee, Hearty Bee, Star (Egyptian product), Royal King 4H (German product) and Royal Star (German Product). Diet with enriched pollen are Global patties and Homebrew with 15 % and 20 % pollen respectively; Diet without pollen constituents are Ultra bee, Bee-Pro, Feed-Bee, Mega Bee and Hearty Bee. Pollen less diet has 18 %, 29.9 %, 36.4 %, 40 % and 56 % of crude protein respectively (80). Fig. 6 shows total amino acids content in commercial diets.

Increased vitality, increased productivity and longer acini in the lobules of hypopharyngeal gland can be achieved by introducing diets such as Bee Tonic; Beeodine, Immunebee solution and Nektapol (73). The most important product Beltsville bee comprises lactalbumin and yeast. When it is incorporated to honey bees with sucrose syrup and water, results in increased trypsin activity which helps in the digestion of protein in their midgut.

Bio-active compounds in artificial diets - tools for bee disease management

Many bioactive compounds alleviate bee diseases when used as feed supplement. Lack of nutrition in honey bees can cause a number of illness and health problems. Feeding bees with higher pollen and fat enriched diet increases the synthesis of hypopharyngeal gland and simultaneously decreasing deformed wing virus (DWV) suspension under caged conditions (81) and those viral loads can also be reduced with supplementing the combination diet which comprises Soytide

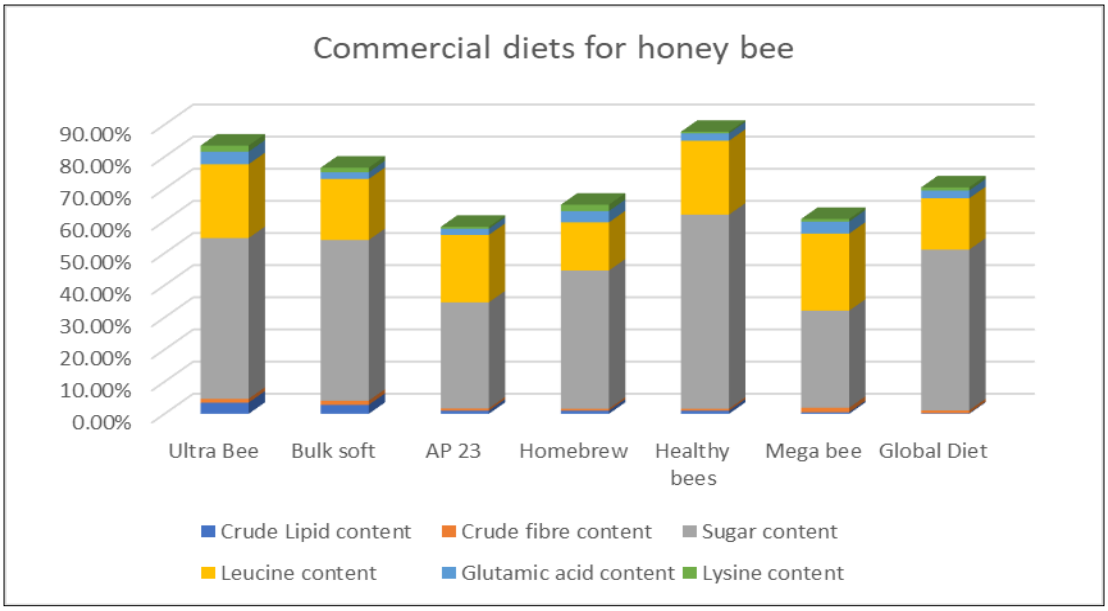

Fig. 5. Commercial diets for honey bee with their nutrient profile.

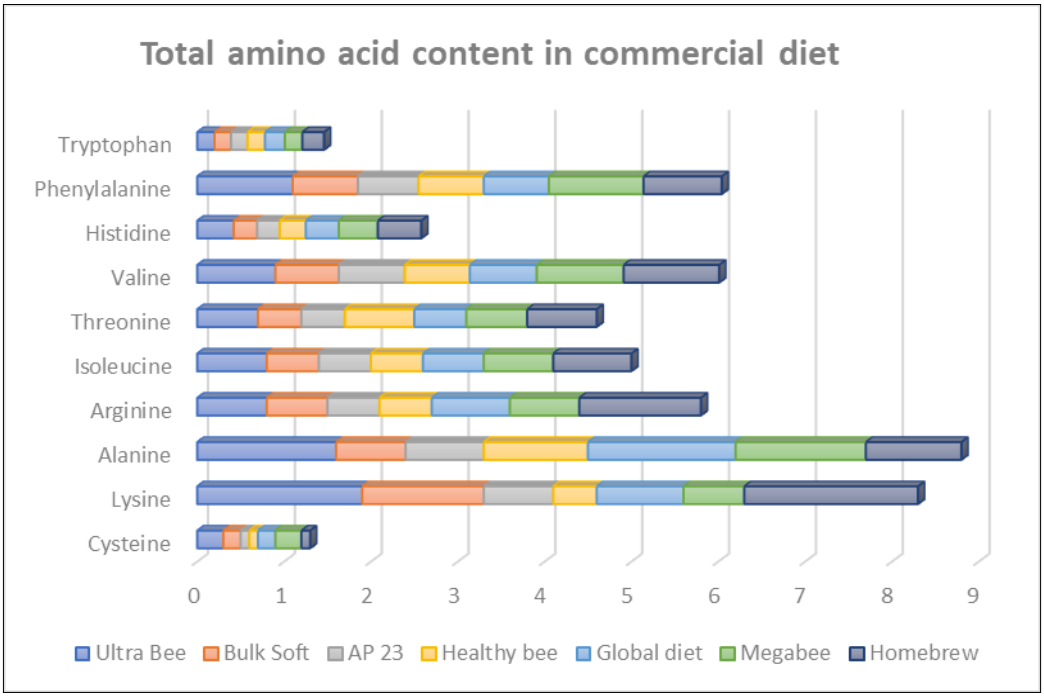

Fig. 6. Total amino acid content in commercial diets.

powder 2.21 %, apple juice 4 %, chlorella powder 0.08 %, tangerine juice 4 %, citric acid 1.85 %, sugar 35.36 %, canola oil 1.01 %, wheat bran powder 0.88 % and multiple vitamins 0.44 % . Augmentation of arachidonic acid, a poly-unsaturated omega fatty acids in diet enhances enzyme activity such as phenol oxidase, antitrypsin, lysozyme in immune system.

It is advisable to add coconut oil to artificial diet since it has positive correlation on bee health but has negative correlation on glucose oxidase activity since increased glucose oxidase activity has decreased survival rate (39).

Extracts from Anise hyssop (*Agastache foeniculum*), common worm wood (*Artemisia absinthium*), oakmoss (*Evernia prunastri*), hops (*Humulus lupulus*), bay laurel (*Laurus nobilis*), oregano (*Origanum vulgare*) and blue berry (*Vaccinium myrtillus*) have antibacterial and antifungal properties (82). Those extracts can be mixed with diet and feed as supplements to enhance resistance. The following combinations shows resistance against pathogens: 40 % *Artemisia* extract in diet show resistance to *Paenibacillus alvei*(82); 40 % *Artemisia* and 5 % *Laurus nobilis* extract show resistance against *Ascosphaera apis*, *Paenibacillus larvae* and bee virus Black queen cell virus; inoculation of *Oregano vulgaris* resisted *Brevibacillus laterosporus* and essential oil resisted against gram positive bacteria *Staphylococcus saprophyticus; Cryptocarya alba* exhibited maximum resistance activity against *Nosema ceranae*

Melissococcus plutonius, a gram-positive bacterium causes European foul brood disease in honey bee larva and kills them at coiled stage and creates foul smell. This disease can be overcome by using hydroalcoholic laurel extract of *Syzygium aromaticum*, *Cinnamomum zeylanicum*, *Thymus vulgaris* and *Cinnamomum zeylanicum* in the feeds of honey bee. Feeding the isolate of *Calothirx parietina* reduces varroa mite infestation by 2.7 %. Sugar syrup and Fumagilin were used as feed for *Apis mellifera ligustica*, with positive effects on wintering ability, survival rate and brood area. When administering sucrose and chlortetracycline, that drastically reduce the larval and post defecation mortalities caused by American Foulbrood disease (23). Hence, it is proven that many of the diseases of honey bees can be reduced through artificial diet supplementation

Future outlook

i) Gut health microbiomes : Microbes in gut are crucial for bees' health. More research ought to be done to explore impacts of artificial diet supplements and algal products on gut health (microbiomes) of honey bee

ii) Disease management: Many of the disease cannot be controlled, but can be managed through artificial diet. For instance, some people suggested that artificially banana fed bees can overcome chalkbrood disease. But it is plausible. So, researchers should take this fluctuation into consideration and provide an apt solution to it.

iii) Sustainability considerations: Artificial diet of honey bees should be commercialized cost effectively and economically feasible for all beekeepers.

iv) Longevity: Shelf life and longevity of the diet should be focused to improvise the product for utilization during scarce period.

Conclusion

One of the reasons for decreasing bee colonies is dietary imbalance. Nectar and pollen are natural diet resources of honey bees which could not be obtained year around. By incorporating of supplements into the bee's diet, we can overcome the problem occurring during resource scarcity period to sustain colonies and productivity. They supply diversified nutrients like essential proteins, carbohydrates vitamins and minerals required for betterment of colony performance such as increasing brood area, honey production, wax production, hive vigour and reducing mortality rate. In artificial feeding, diet combination can be prepared by using natural food components to provide a balanced and enriched source of nutriments. Thereby, it imparts wholesomeness of nutrients to bees. It is the seasonal supporter especially during summer and monsoon. By providing well-rounded diet with target supplements and immune boosting components, beekeepers can lessen the impact of disease and ensure healthy and well-being life of bees. Overwintering mortality can be reduced through diet supplements. In addition to natural components, microbes such as algae and yeast helps in improving colony parameters. Some algae contain antioxidants that may boost immune system to resist pest (varroa mite) and disease. Therefore, artificial diet is a better way to furnish all nutrients and enhancing strength of colony through controlled feeding (both in quantity and quality) and get better off from food shortage season. At the same time, all the supplemented diets might not be readily accepted by bees. So, beekeepers should concentrate on palatability of diet to ensure their consumability.

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Authors' contributions

SP participated in collection of reference articles and drafting of original manuscript. KA and SVR conceptualized the experiment. RV and GI edited and refined the manuscript. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

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