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Optimization of Artificial Diets for Management of *Apis mellifera* L. Colonies

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ABSTRACT

The necessity of artificial diets to honey bees has been long-standing interest to the beekeeping industry. The pollen substitute diets were even better than pollen/ pollen supplement with regards to acceptability and nutritional value for honey bees. The artificial feeding or supplementing pollen substitutes help to sustain colonies for honey production and pollination. Strong colonies produce more honey and serve as better pollinators. This is possible if sufficient brood stores, nectar pollen and bee population is available. Furthermore, provision of artificial diets is especially important in areas where stationery beekeeping is practiced.

Keywords Supplementary diets, Colony build up, Honey stores, Pollen stores, Brood development , Disease resistance, Palynology.

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INTRODUCTION

Honeybees besides producing a number of products (honey, beeswax, royal jelly, bee venom and propolis) are important pollinators of a wide variety of crops increasing productivity (quality and quantity) of agricultural crops through cross pollination (Free 1993). It has been recognized as one of the essential inputs in agriculture (Sihag 2001). However, the success of beekeeping depends upon the prevailing weather conditions, availability of bee flora and management practices. Proper manipulative and management practices are required for honey production and successful pollination in an area.

In northern part of our country, summer season is little longer and harsh as compared to other parts. The rainy period from June/July to August/September is the most difficult time for bees as floral dearth coupled with attack of diseases and enemies require extra management practices like providing pollen substitutes or artificial diets. The natural bee flora starts disappearing in the month of may causing dearth of food (pollen and nectar) for bees. The dearth periods result into low nutritional reserves which seriously affects the egg laying and brood rearing activity. Also due to poor strength, colonies may be attacked by various bee enemies and diseases. All these factors lead to quick dwindling and sometimes even perishing of bee colonies. To avoid this situation, colonies are either physically moved to a bee flora rich area or provision of feeding artificial diet in form of pollen substitutes and supplements for maintenance. Several investigators have attempted different types of pollen substitutes/ diets for honey bee colonies during floral dearth periods such as pollen substitutes with skimmed milk whey and wheat, soy products, brewer's yeast , fish meal and meat scraps (Haydak 1967, Standifer *et al.* 1978, Herbert 1979, Chalmers 1980, Kulincervic *et al.* 1982, Rana *et al.* 1996). The necessity of artificial diets to honey bees has been long-standing interest to the beekeeping industry. The pollen substitute diets were even better than pollen/pollen supplement with regards to acceptability and nutritional value for honey bees. The artificial feeding or supplementing pollen substitutes help to sustain colonies for honey production and pollination. Strong colonies produce more honey and serve as better pollinators. This is possible if sufficient brood stores, nectar pollen and bee population is available. Furthermore, provision of artificial diets is especially important in areas where stationery beekeeping is practiced (Nabors 2000, Van der Steen 2007, De Grandii-Hoffman *et al.* 2008, De Jong *et al.* 2009, Sihag and Gupta 2011). Some commercial pollen substitutes are available in international market, but they are formulated and tested in different eco-climatic conditions and their efficacy may not be satisfactory.

The presence of ample food in the colonies keeps them healthy and more honey can be harvested from such colonies during next blooming season. The performance of honey bee colonies gets affected due to non-availability of flowers during summer and winter season (Avni *et al.* 2009, Brodschneider and Crailsheim 2010). The performance of honey bee colonies depends on a number of factors, including amount of food stores to be used as food source during dearth periods (Kumar and Agrawal 2014). The conditions become more severe when beekeepers tend to remove complete honey stores before a dearth period (Mishra 1995). During this season, all hive activities like egg-laying by the queen, rearing of unsealed/ sealed and drone brood almost stops due to paucity of food inside the hive (Abdul Rouf *et al.* 2016). If dearth periods prolongs for an extended duration, worker bees may even stop to attend un-sealed brood and / or they may even throw the immature stages out of the hives (Kumar 2013). The colony strength has also bearing on the incidence of various disease and natural enemies. Honey bees try to sustain the adversity with the help of supplementary food reserves in the hive. In short, honey bees struggle for existence during dearth periods; however, they try to maintain a large enough population of workers of all age groups so that they can again built up quickly and take full advantage of a blooming season. In India, migratory beekeeping is the only solution available to the beekeepers to save their colonies during dearth periods. But long migration has its own challenges/ stressor (Mishra 1995, Gemeda 2014). An alternative strategy that can be adopted to overcome nutritional stress during shortage of pollen and pesticide damage is artificial feeding to honeybee colonies (Sihag and Gupta 2013). It is often emphasized by bee scientists that provision of pollen supplements or substitutes will be helpful in strengthening weaker colonies and maintaining enough bee population to derive benefits of future non-dearth period (Skubida *et al*. 2008). The development of a nutritive artificial diet for honey bees has long been an area of interest to researchers and apiculturists (Mishra 1998, Saffari *et al.* 2004, Kumar *et al.* 2013). In India, most of the beekeepers either do not provide any external food to bee colonies or just supply sugar syrup. Attempts have been made by scientists to formulate food to be fed to bee colonies during dearth period so as to solve the problems of food shortage and to get better output (Chhuneja *et al.* 1992, Sihag and Gupta 2011, Kumar *et al.* 2013) but a standard and well accepted formulation is still not available in our country.

Honey bees depend on pollen and nectar for their food but these provisions are not available to the bees right through the year, which causes the depletion of the on hand honey stores in the colony. There may also be a shortage of pollen, bees then reduce the amount of brood that they rear resulting in quick dwindling and perishing of honey bee colonies. The major problem in beekeeping is to maintain good strength of honeybee colonies during dearth periods (summer and winter) as enough bee flora is not available during this period. The condition is more severe in hilly areas where dearth periods are little longer as compared to other parts sometimes resulting in the death of bee colonies. Therefore, during these periods, special care should be taken in management of bees in terms of feeding artificial diets (Haydak 1936).

The studies are therefore needed to compare the effect of various diet formulations fed to bee colonies during dearth periods on colony parameters and diseases and enemies incidence so that suitable pollen substitute can be developed to improve beekeeping practices besides conducting palynological studies for exploitation and conservation for the development of apiculture in an area. In view of the above the information on supplementary diets and their impact on colony build up is reviewed as under.

Artificial pollen substitutes and supplemental diets for honey bee colonies

The need for artificial diets has been must for sustainable beekeeping (Haydak 1935, 1936). In India, work on the artificial diet formulation has been carried and various substitutes have been suggested with different compositions has been suggested (Chhuneja *et al.* 1992, Srivastava 1996, Sihag *et al.* 2011). Kumar *et al.* (2013) studied the preference of six protein rich artificial to *Apis mellifera* colonies in the form of patties and found that containing defatted soy four, brewer's yeast and soy protein hydrolysate powder proved to be most effective to produce higher sealed brood area, total bee strength in terms of frames. covered frames and 9138.6 bee population. Tomar and Singh (2014) fed artificial diet (Sugar solution and pollen substances) to honeybee colonies and found 7.8, 6.2, 4.4 and 3.3 frame bee strength after passing dearth period. The colonies whom artificial diet was not provided timely, could not survive till last week of June. Kumar and Agrawal (2014) developed highly palatable, nutritionally balanced and economically viable pollen supplement or substitute for *Apis mellifera*. Diet composed of soy flour-1part, brewer's yeast-1part, soy protein hydrolysate-1part, sugar-1part, glucose-1part proved to be best for honeybees in terms of biochemical composition, net consumption, positive influence on colony parameters and input cost involved. Abd El-Wahab (2016) evaluated pollen supplement diet, the main components of orange juice, mint oil, Turmeric and Fenugreek powders and vitamins (diet E) traditional pollen supplement diets, A (powdered sugar + sugar syrup), B (diet A+ date pollen grains), C (diet A+ bee honey) and D (date pollen grains + powdered sugar + sugar syrup). The diet E was found to be most preferred one resulting in higher brood production and population of bees.

Abdulraoufmro *et al*. (2015) tested the efficacy of 5 proteinaceous diets soybean meal, mesquite pod powder, date paste, Feed bee and corn gluten, on honey bees along with control (bees fed naturally on pollens). They found that date paste was most preferred followed by Feed bee, mesquite and corn gluten, respectively. Brodschneider and Crailsheim (2010) emphasized on nutritional demands of honey bee workers that strongly affected by shortages of this nutrient. Muhammad *et al.* (2016) reared artificially *Apis mellifera* L. (Hymenoptera: Apidae) at varying diets including royal jelly and reported that survival rates of the larvae and queen bee were higher in 8-g food as compared to 4 and 6 g.

Sihag and Gupta (2011) used four pulses viz, soybean (*Glycine max* L. Merr.), mungbean (*Vigna radiata* L. Wilczek), chick pea (*Cicer arietinum* L.) and pigeon pea (*Cajanus cajan* L. Mill SP) for the preparation of artificial diets to feed colonies of honeybee (*Apis mellifera* L.) during the dearth period. Of all these pulses, soyabean was the most preferred with higher brood area and population of bees.

Soybean have been recommended as highly palatable proteinaceous diets (Sihag and Gupta 2011, Mahfouz 2016). They have been shown to play a substantial role in enhancing activities of colonies (De Groot 1953, De Grandi Hoffman *et al.* 2008).

Artificial diets played a predominant role in enhanced brood development (Chhuneja *et al.* 1993, Abd El-Wahab *et al.* 2016). Shoreit and Hussein (1993) also reported higher honey production rates in colonies fed with proteinaceous diets as compared to those fed on sugar alone. Sometimes pollen depression in colonies may be responsible for reduced rates of honey collection (Peixin and BaoHua 2010). Kumar and Agrawal (2013) found a direct correlation between developments of colonies with artificial diets. Mahfouz (2016) reported that colonies fed with artificial diets showed improved rates of honey and pollen collection.

Pollen analysis

Marcos da Costa Dórea *et al.* (2010) identified the botanical origin of pollen loads collected by *Apis mellifera* L. in Canavieiras municipality, Bahia state. They found that pollen types Elaeis (23.99%), *Mimosa pudica* (22.78%) and *Cecropia* (13.68%) were the most abundant among the samples. Diver (2002) suggested that calendar for beekeeping is a time-table that indicates the approximate date and duration of the blossoming periods of the important honey and pollen plants. The distribution and type of honeybee plants as well as their flowering duration vary from one place to another place due to variation in topography, climate and farming practices (Liseki and Boniphace 2008).

In Bure District also the potential bee floras are studied with their flowering calendar by Tessega (2009) and the flowering time of *Biden* spp. *Clematis hirusta, Pisumsativum, Zea Mays* was found to be from September to October, and that of *Carissa edulisans Eucalptus* spp was from March to May whereas for *Crotonmacrostachy* it was from March to April. From the analysis of the flowering periods of the bee plants and field interviews, it was possible to identify honey flow seasons (Admasu and Debissa 1996). Generally, flowering calendars can make easier to plan various beekeeping management operations such as the sitting of hives near to particular crops and deciding the best time for honey harvest and/ or colony swarming (Bista and Shivakoti 2001)

Impact of nutrition on health of honey bees

By ensuring reproduction of many plants, pollinators, like honey bees, are essential to the functioning of natural and agricultural ecosystems (Klein *et al.* 2006, Gallai *et al.* 2009, Morse 1991). Pollinators benefit by collecting nectar and pollen needed for their growth and health (Brodschneider and Crailsheim 2010). The development and the survival of honey bee colonies are therefore intimately associated with the availability of those environmental nutrients (Brodschneider and Crailsheim 2010, Keller *et al.* 2005, Haydak 1970) which suggests that the alteration of bee foraging areas due to the current intensification of agriculture and landscape changes might provide a deficient nutrition and therefore affect honey bee populations (Decourtye *et al.* 2010, Naug 2009). Therefore, studying the link between nutrient availability and bee health might help to better understand the current bee losses observed throughout the world (Neumann and Carreck 2010, Van Engelsdorp and Meixner 2010). Among different flower rewards and nutrients pollen is the main source of proteins, amino acids and minerals (Roulston and Buchmann 2000, Stanley and Linskens 1974) is a major factor influencing the longevity of individuals (Haydak 1970, Crailsheim *et al.* 1992, Crailsheim 1992). Therefore, a direct impact of nutritional deficiency is a decrease in the colony population (Keller *et al.* 2005) and likely a deficient health of individuals, which could also affect the resistance threshold of bees to other stress (pathogens or pesticides) (Naug 2009, Le Conte *et al.* 2011). Despite some studies showed that pollen quality can affect the longevity of bees (Schmidt *et al.* 1987, Schmidt *et al.* 1995, Standifer 1967, Maurizio 1950) and the hypopharyngeal gland development (Standifer 1967) and, more recently, that pollen diversity might improve some immune functions (Alaux *et al.* 2010), our knowledge of the influence of quality and diversity of pollen diets on bee health is rather limited. Branchiccela *et al.* (2019) reported that nutritional stress due to habitat depletion, infection by different pests and pathogens and pesticide exposure has been proposed as the major causes of decline of honey bees worldwide. Nutritional stress affects colony strength and health. Colony losses are likely the result of the effect of multiple stressors (Steinhauer *et al*. 2018, Carreck and Neumann 2010). Pollen nutrition affects bee lifespan, their immunecompetence, their resistance to pathogen infection (De Grandi-Hoffman *et al.* 2010, Basualdo *et al.* 2014, Alaux *et al.* 2011) and behavioral transition (Toth and Robinson 2005 Ament *et al. 2010).* Among the pathogens affecting honey bee health, *Varroa destructor*, RNA viruses (Dainat *et al*. 2012, Cox-Foster *et al.* 2007) and the microsporidia *Nosemaceranae* (Higes *et al.* 2013, Higes *et al*. 2009) have the most important impact on colony losses (Higes *et al.* (2009, Higes *et al.* 2008). It has been reported that colonies having poor nutrition are more susceptible to nosema infections.

Honey bee Nutrition and diet

The honey bee diet requires proteins, carbohydrates, lipids, vitamins, sterols and minerals. All nutrients are derived from flowering plants. However, very few flowering plants meet the nutritional requirements completely. Deficient nutrition can impair immune function and increase the susceptibility of individuals to disease (Aluax *et al*. 2010).

The nutritive capability of the colony as a whole all falls on the ability of the nurse bees to process the pollen gathered into royal jelly. The honey bee forager gathers two components, pollen and nectar, from varieties flowering plants to provide the colony with these nutrients. As a polyletic forager, she will collect pollen and nectar from different species of flowering plants ensuring immunity of the hive.

In a study of diet related immunocompetence, Aluax *et al.* (2010) determined that the polyfloral diets enhanced immune functions, glucose oxidase activity, when compared with monofloral diets. Mixed pollen given to caged bees has been shown to have greater longevity than those on a single species of pollen. The nurse bees eat the pollen in the form of beebread that is stored in the cells. It is converted into royal jelly using the vitogellin it does not use in the development of eggs. The hypopharyngeal gland and mandibular glands are essential in the production of royal jelly. The jelly or bee milk is composed of proteins, lipids and vitamins. The queen and drones do not possess a hypopharyngeal gland. The worker loses her ability to produce royal jelly around 16 days post emergence due to the shrinking of the hypopharyngeal glands.

However, crowded conditions, warm temperatures, high concentrations of resources and periods of confinement in the nest are ideal for pathogen invasion and transmission that can lead to epidemics (Caron and Sagili 2011, Calderone 2012). Honey bees are important pollinators in undisturbed ecosystems, but are essential for the production of numerous high-value crops (Sumner and Boriss 2006). Viruses have received much attention due to the significant loss of colonies especially over winter from *Varroa* mite and virus associations (Naug 2009, van Engelsdorp and Meixner 2010).

The role of nutrition in immune response to viral pathogens is of utmost importance. There is need to understand the connections between nutrition and individual immunity and speculate on the possible changing nutritional requirements of colonies throughout the year. It has been found that the effects of parasitism by Varroa when the mite is present, optimal nutrition alone might not be sufficient to keep virus levels low (Spivak *et al.* 2011, Claassen *et al.* 2011).

Pollen nutritional content is highly variable. The pollen of some species of flowers lacks key nutrients necessary for honey bee nutritional needs. This includes the pollen of numerous crops that depend heavily on honey bees for their pollination. Areas with more developed land were associated with greater colony loss compared to areas with more open, undeveloped land; similarly, uncultivated land has been positively associated with honey production and survival and physiological health.

Some previous studies have identified their importance, particularly in brood rearing, but there has not been exploration of how these nutritive components could contribute to pathogen resistance or susceptibility in bees. Bee nutrition is only one of the many environmental stressors that impact honey bee colonies. Honey bee viruses, of which more than 20 are known, are widespread and often persist as asymptomatic infections, even in otherwise healthy colonies. Bee viruses were a relatively minor problem until the spread of the parasitic mite, *Varroa destructor* which supports replication of some viruses and serves as a virus vector, delivering high viral titres to mite-infested bees with severe pathological consequences.

Palynological analysis of pollen loads of *A. mellifera*

Melissopalynology helps (Nair 1964, Maurizio 1975, Moar 1985, Jones and Bryant 1992, Ramanujam *et al.* 1992 Sajwani *et al.* 2007 Song *et al.* 2012) to determine origin of floral based on samples from honey. It also helps to assess the weather conditions of the area (Jato *et al.* 1994, Bilisik *et al.* 2008) and factors influencing pollinators and pollination (Herrera 1995, Jens *et al.* 2008, Rands and Whitney 2008, Thomas *et al.* 2009, Baldock *et al.* 2011, Selva *et al.* 2011, Nascimento and Nascimento 2012).

Melissopalynology is one of the best option method to identify lant resources. However, the rapid advances in gene technology method are likely to make this method less reliant. The presence of pollens in honey plants is the index of bee foraging plants. Bees have been reported to forage on specific plants (Dimou 2007). The pollen grains are likely to vary from one location to another and floral resources (Song *et al.* 2014).

In Uttar Pradesh, India pollen from *Antegonon* and *Moringawere* found to be more predominant (Nair and Singh 1974). Other plant species with abundant pollen included *Rumex* sp., *Nephelium* sp., and members of Myrtaceae, Liliaceae, Rosaceae and Euphorbiaceae (Sharma and Nair 1965, Gaur and Nanwani 1989). Singh *et al.* (1994) reported that honey from Himachal Pradesh had dominance of *Brassica, Adathoda, Clematis, Mussenda* and *Helianthus* sp. Kalpana and Ramanujan (1997) from the honey samples *Sapindus, Eucalyptus, Anacardium* and *Cleome* as the major pollen types. In Karnataka, honey samples were having pollens from *Cocos, Eucalyptus, Schefflera* and *Mimosa* (Suryanarayana *et al.* 1997). House (1997) reported that the samples collected in October, November, December and January were rich in pollens of *Eucalyptus*. *Eucalyptus* species have become a an important source of nectar in tropical and subtropical areas (Chauhan *et al.* 2017, Daners 1998, Bonilla *et al.* 2016, Carroll 2006, Rasoloarijao 2014, Seijo *et al.* 2003, Terrab *et al.* 2003, Feas *et al*. 2010). The presence of pollen in the honey of particular plant species during different months is related to the blooming of that particular plant species from which the bees have collected pollen during foraging activity (Joshi *et al*. 1998). The major honey sources included *Bombax, Lannea, Limonia, Moringa, Peltoforum, Pongamia, Syzygium,* and *Tamarindus* during February- July and the pollens of *Eucalyptus* and *Alternanthera* most abundant during September-December.

CONCLUSION

The dearth periods result into low nutritional reserves which seriously affects the egg laying and brood rearing activity. Also due to poor strength, colonies may be attacked by various bee enemies and diseases. All these factors lead to quick dwindling and sometimes even perishing of bee colonies. To avoid this situation, colonies are either physically moved to a bee flora rich area or provision of feeding artificial diet in form of pollen substitutes and supplements for maintenance. Supplementary diets help the bees to overcome dearth periods, buildup colony strength , produce more honey, help better pollination and to resist diseazses and enemies. Hence optimization of diets is essential foe best beekeeping practices.

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