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Impact of Supplementary Diets on Resistance of *Apis Mellifera* Colonies to Diseases and Pests

Mahesh Kumar, D.P.Abrol

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ABSTRACT

The study was conduct to determine the effect of artificial diet on colony build and resistance to bee diseases and enemies when enough floral resources were not available. Six diets were prepared which included Black gram (20)+ Yeast (20g) + Pollen (20g) + Honey (20g) + Glucose (20g), Soybean flour (60 g) + Honey (35g) + Yeast (4g) + Vitamins (1 g/kg) HAU Diet, Defatted soya flour 20g + skimmed milk powder 25g + sugar 5g + pollen (5g) +glucose 10g + h, Soybean flour (25 g) + Yeast (10 g) + Pollen (15 g) + Skimmed milk powder (5 g) + Honey (22.5 g) + Sugar (22.5 g) GBPUA&T diet, Brewer's yeast (42 g) + Gram (4g) + Skimmed Milk Power (4g) + Sugar (50g) + Pollen (10 g) PAU Diet and Control (sugar feeding). The studies revealed that *Apis mellifera* bees exhibited differential consumption pattern during different weekly intervals throughout the season as per the needs of the colony. The preference of different diets was in the order 3 >4>2>5>1>6. Diet 3 was most preferred followed by 4, 2, 5, 1 and 6 respectively.

The studies revealed that *Apis mellifera* colonies were attacked by various brood diseases which included American foul brood (AFB) Europen Foulbrood (EFB) Sac brood disease (SBV), adult diseases such as Nosema disease, several species of ectoparasitic mites such as *Varroa destructor*, Tropilaelaps clareae, phoretic mite Neocypholaelaps indica, wax moths Galleria mellonella, wasps *Vespa orientalis*, *V. Cincta* and birds such as *Meropes orientalis* and *Lizards varanus* spp. It was interesting to find that colonies fed on different diets were able to maintain sufficient strength , were healthy and able to resist against disease/ pest attack. The intensity of attack was far less in colonies given supplemental feeding as compared to control.

Keywords *Apis mellifera,* Protein diet, Bee flora, Beekeeping, Diseases.

INTRODUCTION

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

Mahesh Kumar, D.P.Abrol*
Division of Entomology, Sher-e-Kashmir University of Agriculture Sciences and Technology of Jammu, Main Campus, Chatha, Jammu 180009
Email: dharam_abrol@rediffmail.com
*Corresponding author

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, 1978, Maurizio 1975) and the hypopharyngeal gland development (Standifer 1978) 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 honeybees 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).

MATERIALS AND METHODS

The study was conduct to determine the effect of artificial diet on colony build, effect of different artificial diets on the incidence of diseases and enemies and qualitative and quantitative analysis of pollen loads to determine the major floral sources of *A mellifera* in the university apiary located at experimental farm of Sher-e-Kashmir University of Agricultural Sciences and Technology Jammu at Chatha during the period 2017- 2018.

Experimental site

The experimental site is located in the sub- tropical zone at 32.73°N and longitude of 74.87°E. It has an average elevation of 327m from the mean sea level. Most of the rains are received during July to October (about 70%). The annual mean maximum and minimum temperature are 29.60 and 16.70°C, respectively. Summer months are hot with temperature and humidity ranging from 23.50 to 35.50°C and 53.0 to 73.50%, respectively. The winter months experience mild to severe cold conditions with average temperature ranging from 6.50 to 21.70°C. December is the coldest month, when minimum temperature touches 4°C.The highest temperature is recorded in the month of June (4°C).The daily maximum and minimum temperature and evaporation rate rise from March onwards. The detailed material sand methods employed for these studies are reported in this chapter objective wise.

Details of experiments

Effect of artificial diet on colony build up

The experiment shall be conducted in the Apiary, Division of Entomology. The following pollen substitute treatments on weight basis shall be administered to three colonies in each treatment, which served as replicates.

Bee strength of experimental colonies:

- 6 bee-frame
- 8 bee-frame
- 10 bee frame

No. of colonies to be examined =
24 (8 each in 6, 8 and 10 bee-frame bee strength)

Diet -I Defatted soya flour 20g + skimmed milk powder 25g + sugar 5g + pollen (5g)+glucose 10g + honey 35g

Diet –II Black gram (20)+ Yeast (20g) + Pollen (20g) + Honey (20g) + glucose (20g)

Diet –III Brewer's yeast (42g) + Gram (4g) + Skimmed Milk Power (4g) + Sugar (50g) +

Pollen (10 g) PAU Diet
Diet –IV Soybean flour (60 g) + Honey (35 g) + Yeast (5g) + Vitamins (1 g/kg) HAU Diet
Diet-V Soybean flour (25 g) + Yeast (10 g) + Pollen (15 g) + Skimmed milk powder (5 g)
+ Honey (22.5 g) + Sugar (22.5 g) GBPUA&T diet
Diet -VI Control (sugar feeding)

Days to consume the diet

Provision of feeding the diet formulations to honeybees shall be made in the form of patties for a feeding period of 14 days. Weighed amount of patties shall be fed to *Apis mellifera* colonies by top bar method of feeding. The number of days taken by bees to consume the diet fully shall be recorded. Observations shall be recorded on daily basis to find out which diet is consumed fully. The least number of days taken to complete the diet can be an indication of its palatability and feeding preference to bees. In order to estimate the effect of diet formulations, the overall performance of colonies shall be judged by observing and comparing all the parameters viz, sealed and unsealed brood, egg laying, honey stores, and bee activity with control colonies. For studying the economics of feeding the pollen substitutes and supplements, the prices of the feedstuffs used in the various formulations shall be collected from the market and cost per kg of each of the pollen substitute and supplement shall be calculated.

Effect of artificial diet on seasonal incidence of diseases and enemies

The seasonal incidence of natural enemies shall be observed weekly throughout the study period. The observations on the incidence of enemies and diseases shall be recorded at weekly interval. Each sample shall consist of minimum of 20 larvae, pupae and adults from each colony and 20g debris from each colony. The collected samples shall be analyzed for mites and associated pathogens if any. The samples of the honeybee, *A. mellifera* shall be examined for the presence of mites. Each sample consisted of 100 suspect bees along with a part of the comb containing immature stages of bees and stored food materials. Samples shall be collected randomly from each hive at fort nightly intervals and examined directly under a stereo binocular for the presence of mites. Besides, hive debris shall also be collected and examined for the presence of mites. The collected mites shall be preserved in 70% alcohol and permanent slides prepared using Hoyer's medium. The mites shall be identified with the help of available literature and the identifications later got confirmed from the mite taxonomists.

The combs of *A. mellifera* shall be examined for the presence of eggs, larvae, pupae and adults of wax moths. If any of the stage shall be found in debris of combs and /or on combs, the hive shall be considered to be infested. Based on this the seasonal incidence pattern shall be worked out. The debris combs containing eggs/larvae shall be maintained in glass jars covered with muslin cloth and supplemented with the wax from old combs as food for developing larvae. The bee's population in the apiary shall be recorded by visual counts while walking from one corner of the apiary to the other. Weekly observations recorded thrice a day (morning, noon and evening) and diurnal fluctuation shall also be recorded.

RESULTS AND DISCUSSION

Impact of artificial diets on colony diseases and enemies of honeybees

The data presented in Table 1 reveals that *Apis mellifera* colonies were attacked by various brood diseases which included American foul brood (AFB) Europen Foulbrood (EFB)Sac brood disease (SBV), adult diseases such as Nosema disease, several species of ectoparasitic mites such as Varroa destructor, Tropilaelaps clareae, phoretic mite Neocypholaelaps indica, wax moths Galleria mellonella, wasps *Vespa orientalis, V. cincta* and birds such as *Meropes orientalis* and *Lizards varanus* spp. It was interesting to find that colonies fed on different diets were able to maintain sufficient strength , were healthy and able to resist against disease/ pest attack. The intensity of attack was far less in colonies given supplemental feeding as compared to control.

The role of nutrition in immune response to viral pathogens is of utmost importance. There is need to

Table 1. Impact of artificial diets on the attack of various brood, adult diseases, mites and various pests and predators of honeybee *Apis mellifera*

Disease	Causative agent	Intensity of attack	
		Fed colonies	Control
Brood diseases			
American foul brood (AFB)	Bacteria, Bacillus larvae	+	+++
Europen Foulbrood (EFB)	Bacteria, *Melissococcus plutonius*	+	+++
Sac brood disease (SBV)	Virus, *Morator aetatulus*	+	+++
Adult diseases			
Nosema disease	*Nosema ceranae*	+	+++
Mite species			
Ectoparasitic mite	Varroa destructor	+	+++
Ectoparasitic mite	Tropilaelaps clareae	+	+++
Phoretic mite	Neocypholaelaps indica	+	+++
Moths			
	Galleria mellonella		
Wasps			
	Vespa orientalis	+	+++
	V. cincta		
Birds			
	Meropes orientals	+	+++

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 honeybee nutritional needs. This includes the pollen of numerous crops that depend heavily on honeybees 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 honeybee colonies.

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