

BROOD-REARING ENHANCING POTENTIAL OF MANUALLY PACKED POLLEN FEEDING IN COMPARISON WITH POLLEN AND POLLEN SUPPLEMENTS IN PATTY FORMS

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Abstract

Dearth-period pollen or pollen supplement feedings have become a common practice to maintain and stimulate the early buildup of honeybee colonies. Not only amount, type and consumption rate of feeds are important but the conversion and its nutrient availability are essential. In the current study, we investigated how honey bee colony's brood rearing enhanced by feeding natural pollen powder (packed on cells of drawn-out combs) in comparison with patty forms natural-pollen, Mega-bee and Ultra-bee pollen substitutes during dearth period. The study showed that colonies given the pollen powder (packed on cells of drawn out combs) immediately converted it into beebread similar to naturally collected pollen. The colonies in the packed pollen-powder group showed consistent increment and on average reared 86.3% more brood than the patty-form natural pollen, Mega-bee and Ultra-bee pollen substitutes, without having significant differences in their consumption rate. The colonies in the packed-pollen group performed better might be because the conversion of the packed-pollen powder into beebread allows better nutrient availability due to its fermentation by nest microbes and further biosynthesis process. Moreover, the presence of beebread from packed-pollen adjacent to the brood combs represents the natural way of feed reserve storage which might also contribute to the stimulation of brood rearing in colonies better than feeding in patty-forms of natural pollen, Mega-bee and Ultra-bee. Packed-pollen feeding as an alternative to patty forms could improve dearth period maintenance and early population building of colonies.

Keywords: bee bread, brood rearing, honeybee feed, packed pollen, pollen supplement, population buildup

INTRODUCTION

Seasonal unavailability and limited diversity of pollen source bee forage plants become very critical in brood rearing, timely population buildup and ultimate productivity of honey bee colonies all over the world. As modern land-use practices reduce dependable nectar and pollen supplies and their diversity (Standifer, 1980; Kremen et al., 2007), the need for supplemental feeding of bee colonies is becoming indispensable. In arid

regions where rainfall is very short and dearth periods are commonly very long; shortage of pollen sources is very critical, which directly affects brood rearing and timely population buildup of honey bee colonies. Generally, supplemental feeds are important in the development and maintenance of honey bee colonies with optimum populations for peak nectar flow periods, crop pollination, spring divisions of colonies and queen and package-bee production (Standifer, 1980).

Pollen provides honey bee colonies with lipids, proteins, vitamins and mineral nutrients (Seeley, 1982; Huang & Robinson, 1996). However, only colonies with access to optimum amounts of pollen are able to perform successful brood rearing (Dietz & Stephenson, 1980). Moreover, Kunert & Crailsheim (1988); Crailsheim & Stolberg (1989) reported that insufficient pollen supply during larval periods leads to decreased lifespan and smaller hypopharyngeal glands of worker honey bees. Schmickl & Crailsheim (2001) demonstrated that the larval survival rate declined from 83% to 53% as result of a shortage of protein source feeds or restriction of honey bee colonies to the incoming and stored pollen.

The scarcity of stored pollen in hives leads to a gradual shifting of nursing efforts from young larvae to focus more on the very old larvae, which are close to the final capping (Schmickl & Crailsheim, 2002; Schmickl et al., 2003). Fewell & Winston (1992), Blaschon et al. (1999) indicated the presence of direct relationship between stored pollen and brood production efficiency of colonies. Moreover, Schmickl, & Crailsheim (2002), Schmickl et al. (2003) indicated the strong influence of the ratio of the amount of stored pollen to the number of larvae in a colony. This has been interpreted as an adaptive response by workers to reduce the brood population to a size that would likely be successfully reared when food is limited (Schmickl & Crailsheim, 2001; Brodschneider & Crailsheim, 2010). However, these strategies influence the spatial organization, the age demography and polyethism of a honey bee colony (Schmickl & Crailsheim, 2004).

Not only the availability, but also the location of storing of the incoming pollen in the hive is very important. In this regard, Taber (1973), Doull (1974), Camazine (1991) empirically demonstrated the presence of preferences by nurse bees for better utilization of pollen stored adjacent to open brood areas. Moreover, Dreller, & Tarpy (2000), demonstrated that returning pollen foragers preferentially deposit their pollen loads in those frames containing open brood, thus again minimizing transport distances. In relation to this, the brood nest of a honey bee

colony is spatially organized in such a way that broods are kept at the centre area surrounded by cells containing pollen insuring that pollen is easily transported to the brood and has been considered as a logistic optimization (Schmickl & Crailsheim, 2004). Deposition of pollen near to open brood areas, is representing a short-term reserve of nutrients as close as possible to areas of high consumption (Schmickl & Crailsheim, 2004).

Many commercial pollen substitutes such as: Feed Bee®, Bee-Pro®, Mega-bee and Ultra-bee have been developed and widely used as pollen substitutes to maintain colonies in dearth period. The exact chemical compositions of the pollen substitutes remain trade secret, but the claimed crude protein percentage is equal and sometimes more than the amount of crude protein reported in the natural pollen. As labeled on the package, the crude protein contents of Mega-bee and Ultra-bee are not less than 38.3% and 60% respectively. Many studies reported for significant contribution of pollen substitutes towards better over-wintering of colonies, early population build up and better honey yield of colonies (Matilla & Otis, 2006; Oliver, 2014). However, natural pollen with even the lowest crude protein percentage reported to perform better than pollen substitutes with a higher crude protein percentage (Oliver, 2014).

During a dearth period when there is a shortage of incoming pollen, it is a common management practices to provide colonies with pollen or pollen substitute in the form of a patty. However, the patties are usually kept on top of frames which do not represent the bees' natural way of storing pollen and also do not undergo fermentation as that of pollen stored in combs (Oliver, 2014). The conversion of stored pollen into beebread and its fermentation process are reported to render its nutrients more available to bees (Mattila et al., 2012). When pollen is converted into beebread some of its proteins are broken down into amino acids, starches are metabolized into simple sugars and vitamins become more bio-available (Degrandi-Hoffman et al., 2013). In general, the amino acid concentration in beebread is observed to be higher than in pollen

(Degrandi-Hoffman et al., 2015).

According to the beebread-maturation hypothesis, stored pollen is fermented by colony microbes into a more palatable, nutritionally-superior form either through biosynthesis of new nutrients (i.e. vitamins) or greater bioavailability of refractory nutrients (i.e. amino acids) (Brodschneider & Crailsheim, 2010; Vasquez & Olofsson, 2009). This indicates the importance of the way of feeding and further bioavailability of feeds' refractory nutrients.

With this background information, we hypothesized that in dearth periods feeding of colonies with pollen (packed manually in cells of full drawn out combs) and keeping adjacent to their brood area facilitates the conversion of the pollen into beebread and this in turn enhances brood rearing better than colonies given pollen and pollen substitutes in the form of patty on top of frames. To our knowledge, no study has been conducted on the stimulating potential of manually packed pollen on dearth- period brood-rearing performances of colonies in comparison with that of pollen and pollen substitute patties. The main objectives of this study were to assess the possibility of bees' converting manually packed pollen into beebread and to investigate its brood rearing enhancing potential in comparison with patty-form pollen and pollen substitutes.

MATERIAL AND METHODS

The study was carried out in the hot climatic region (Saudi Arabia, Al-Baha), where major dearth periods occur from September to January and the flowering of honey bee plants and incoming pollen were absent or very insignificant. Twenty-five *Apis mellifera jemenetica* colonies lead by queens of similar ages of relatively medium and similar strength (occupying six combs) were used. Prior to the commencement of the experiment, the colonies were equalized according to the standard methods of Delaplane et al. (2013). The average brood and adult bee population occupied areas of the experimental colonies were 11.8 dm² and 37.6 dm², respectively. In one dm² an average of

450 broods and 225 adult bees were recorded. During data recording, the same unit area was used to estimate the brood and adult bee population of the experimental colonies.

The colonies were randomly assigned in five treatment groups with five replications each and kept in the same apiary. Colonies in the first (packed pollen) group were given ground bee-collected pollen powder that was packed manually on cells of drawn out combs (Fig. 1). The pollen was imported but fresh. The pollen pellet was first homogenized and ground in to a fine powder, which was then carefully packed manually in cells of fully drawn out and clean combs. For this purpose, relatively strong combs which had been previously used for brood rearing were chosen. The compacting of the pollen powder was done easily by repeated filling of the powder on the cells of a comb and gently pressing it on a back of spoon until the cells become completely compacted and full (Fig. 1).

The surface of the packed pollen was then brushed off to the periphery cells using soft drawing brush to remove the excess pollen from the surface of the cells and to keep the filling of the cells about 1 mm below the rim of the cells. The pollen powder was packed only on one side of the combs in about 13 x 25 cm area avoiding the peripheral parts. The combs with packed pollen powder were kept in a deep freezer to kill any wax moth larvae and eggs before given to the experimental colonies. Then the combs with packed pollen were placed next to the brood combs in the respective treatment colonies. Each colony in this treatment group was given one comb packed with 185 g pollen powder at a time. When the packed pollen was consumed, other combs with packed pollen were inserted immediately replacing the previous one.

In the second treatment group, colonies were fed the same bee collected pollen but in patty form. The homogenized pollen pellet was taken and formed into soft patty with pure water and placed on the top of frames to be fed to the experimental group.

Colonies in the 3rd and 4th treatment groups were given two commercially available and

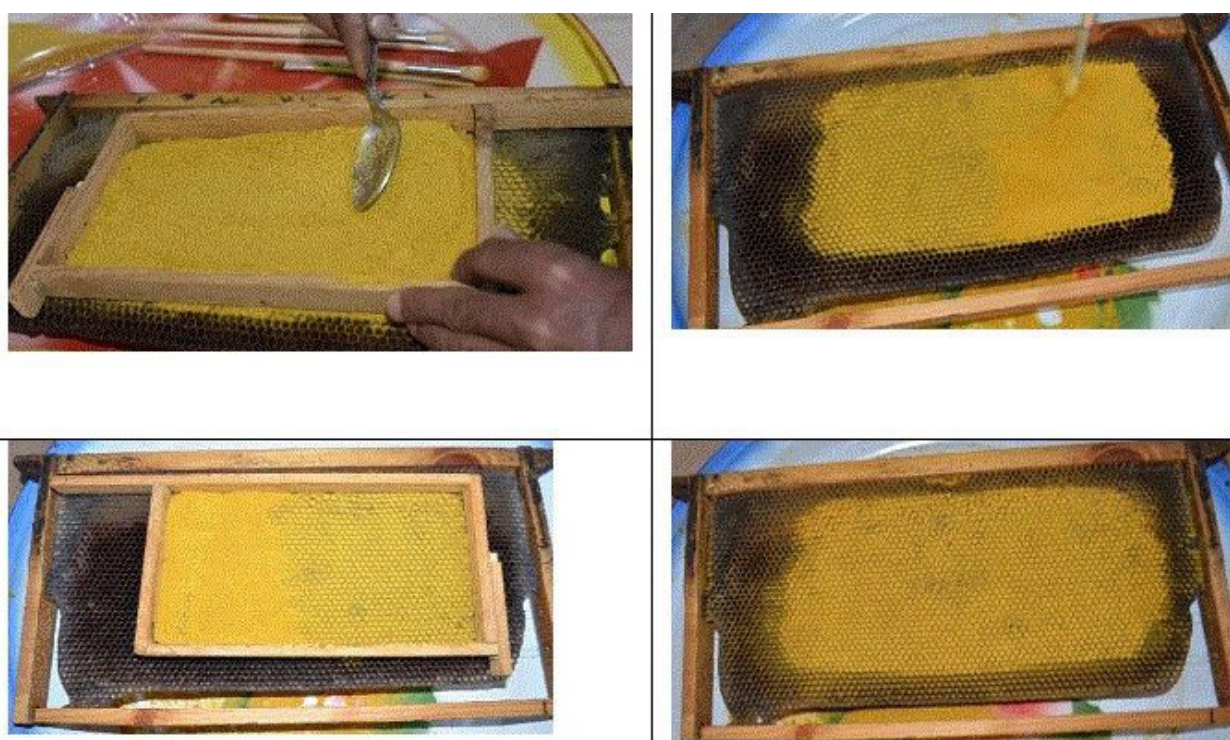


Fig. 1. Process of pollen powder packing on cells of drawn out beeswax combs.

widely used pollen substitutes, Mega-bee and Ultra bee, respectively. As labeled on the package, the crude protein content of Mega-bee and Ultra bee were $>38.3\%$ and $>60\%$ respectively. Ultra bee is supplied in a patty form and was given directly. Mega-bee is supplied in a powder form and was prepared in to patty form according to the procedures described in the packaging. Each colony under the patty form treatment groups was given 185 g patty at a time. However, since the consumption rates of colonies varied within and among the treatment groups, the feeds were given upon their consumption without interruption in ad-libitum condition. Colonies in the 5th treatment group were left as control without any pollen supplements. However, a sugar syrup 1:1 sugar water ratio solution was given to all colonies including the control group as source of energy. After the colonies were assigned to the different treatment groups, they were acclimatized by being fed their respective feeds for one month in September before the recording of the actual data was started. The colonies' feed consumption status was monitored every after five days and given for those have finished the feeds. When new feed was given, any leftover

feed was collected, weighed and recorded. Total consumption rate was calculated as total dry matter of the feeds. The total dry matter was determined through drying the feeds in an oven at 65°C for 24 hr. Data on total brood and adult bee population, and pollen stored were estimated every twenty-one days using grid frame wire (5x4 cm unit area) following Jeffree's (1958) procedures with some modifications. The number of adult bees per unit area was determined by taking pictures of adult bees on the surface of combs gently superimposing the grid wire. Then the number of bees per unit area was counted from the images with the use of a computer screen and marked for each bee with overlapping transparent paper.

Statistical analysis

ANOVA procedures were used to analyze for the presence of significant variations in the performances of colonies among the different treatment groups. Means were compared with the Tukey-Kramer HSD test. Moreover, pair-wise correlation and descriptive analysis were also conducted. Computations were made with the use of JMP-5 statistical software (SAS, 2002) at 95% level of significance.

RESULTS

Conversion of packed pollen into beebread

Colonies provided with manually packed pollen observed to properly repack it into beebread as rapidly as for naturally collected pollen. Fig. 2A shows how the nurse bees were busy repacking the packed pollen powder after 24 hours it was given to the colony. Depending on the strength of a colony, the packed pollen was observed to be fully converted into beebread by adding the necessary secretions from nurse bees within three to five days (Fig. 2B). However, the speed of conversion of the packed pollen into beebread varied from colony to colony.

Consumption rate of feeds

The experimental colonies consumed a significant amount of feeds which on average varied from 252 ± 110.10 - 373 ± 69.50 g/colony/month as dry matter intake (Tab. 1). The average highest dry matter intake of 373 ± 69.50 g/colony/month was recorded for colonies in the packed pollen treatment group. The lowest dry matter intake of 252 ± 110.10 g/colony/month was recorded for colonies under Mega-bee patty treatment group (Tab. 1). The daily average dry matter consumption rate of 12.4 g, 12.1 g, 9.1 g and 8.4 g was recorded for packed pollen, pollen patty, Ultra bee and Mega-bee patties, respectively. Generally, the consump-



Fig. 2A

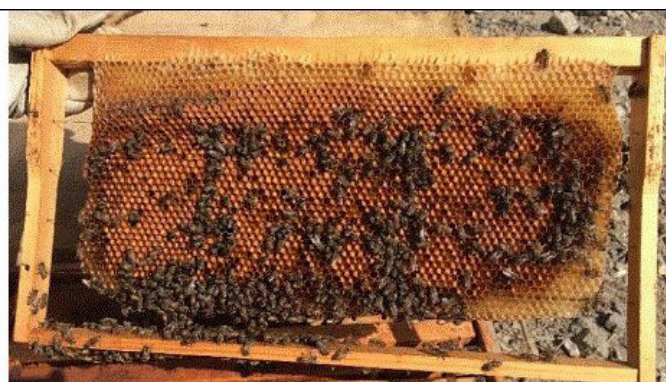


Fig. 2B

Fig. 2. (A) Process of repacking packed pollen by the bees after 24h, (B) the conversion of the packed pollen into bee bread after three days being given to the colony

Table 1.
Monthly mean and standard deviation ($\pm$) of dry matter intake (g/colony)
of different supplement groups

Treatments	Oct	Nov	Dec	Jan	Monthly Average
Packed pollen	510	204	408	372	373.5 ± 127.2^a
Pollen patty	288	366	471	330	363.8 ± 78.3^a
Ultrabee patty	231	270	372	219	273.0 ± 69.5^a
Megabee patty	228	147	408	231	253.5 ± 110.1^a
P-value					= 0.255

Average values within the column that denoted with similar superscript letter (a) are not significantly different

tion amount of natural pollen was higher than that of commercial pollen substitutes (Tab. 1). However, there was no statistically significant difference in the amount of daily or monthly dry matter intake of colonies among the different treatment groups, $P = 0.255$. Moreover, it was noted that bees do not store any patty form of feeds in their comb as they do with nectar and pollen collected from the field or as sugar syrup.

Brood population

The lowest average brood population of $12.3\pm5.77\text{ dm}^2$ area was recorded for colonies in the control group while the maximum average brood population of $24.6\pm13.29\text{ dm}^2$ area was recorded for colonies in packed pollen treatment groups (Tab. 2); (1 dm^2 area consists, an average of 450 broods). The brood population of the packed pollen treatment group was significantly ($P < 0.0001$) higher than that of all other treatment groups. However, the variations in average brood population of other treatment groups were not significant (Tab. 2). The average brood population growth trends were generally increased for colonies in packed-pollen and pollen-patty feed groups (Tab. 3) which indicates

the positive effect of natural pollen on brood rearing and maintaining colony strength during dearth periods. However, the rate of increment in the packed- pollen treatment group was significantly ($P < 0.0001$) higher than that of other treatment groups (Tab. 3) and the group on average reared 73.2% more brood than the average brood population of other groups. Even if the average brood population of colonies under Mega-bee patty was higher than that of the pollen patty group (Tab. 2), the colonies in the natural-pollen patty group showed consistent increment in their average brood population growth and at the end of the experiment surpassed all other groups except for the packed-pollen group (Tab. 3). At the end of the experiment, (end of January) the colonies in the packed pollen treatment group reared 86.3% more brood than the average brood population of other groups.

Adult bee population

The highest average adult bee population of $51.3\pm18.17\text{ dm}^2$ area was recorded for colonies in packed pollen treatment group. The lowest average adult bee population of $33.5\pm12.27\text{ dm}^2$ areas was recorded for colonies in control group.

Table 2.

Effects of different feeds on the performance parameters of honey bee colonies during dearth period (Oct. – Jan.)

Treatments	Variables adult bee and brood population area in dm^2 (Mean $\pm$ SD)				
	N	Utilized combs	Adult bees	Brood area	Stored pollen
Packed-pollen	30	110.4 $\pm$ 16.99 ^a	51.3 $\pm$ 18.17 ^a	24.6 $\pm$ 13.29 ^a	1.2 $\pm$ 1.85 ^b
Pollen-patty	30	93.9 $\pm$ 10.06 ^b	38.8 $\pm$ 10.56 ^b	14.8 $\pm$ 7.50 ^b	2.8 $\pm$ 3.25 ^a
Ultra Bee-patty	30	86.4 $\pm$ 24.72 ^b	36.9 $\pm$ 16.43 ^b	13.0 $\pm$ 9.12 ^b	2.3 $\pm$ 1.91 ^{ab}
Megabee patty	30	94.4 $\pm$ 16.99 ^b	42.6 $\pm$ 18.92 ^{ab}	16.8 $\pm$ 8.72 ^b	2.0 $\pm$ 1.25 ^{ab}
Control	30	84.3 $\pm$ 8.33 ^b	33.5 $\pm$ 12.27 ^b	12.3 $\pm$ 5.77 ^b	1.5 $\pm$ 1.36 ^{ab}
P-value		0.000	0.000	0.000	0.032

* Values within the column that denoted with similar superscript letters ("a" or "b") are not statistically significantly different,
1 dm^2 area consists an average of 450 broods and 225 adult bees

Table 3.

Broods and adult bee population performances of colonies of different treatment groups over the experimental periods

Broods reared in dm ² area (Mean $\pm$ SD); one dm ² area consists 450 broods						
Months	Packed pollen	Pollen patty	Ultra Bee patty	Mega-bee patty	Control	P-Value
October	14.8 $\pm$ 14.8 ^a	11.8 $\pm$ 6.9 ^a	9.6 $\pm$ 12.4 ^a	14.5 $\pm$ 13.7 ^a	8.7 $\pm$ 1.7 ^a	0.206
November	22.3 $\pm$ 15.1 ^a	13.4 $\pm$ 6.6 ^a	11.8 $\pm$ 9.8 ^a	19.2 $\pm$ 1.6 ^a	12.3 $\pm$ 4.9 ^a	0.200
December	27.7 $\pm$ 9.8 ^a	17.3 $\pm$ 4.5 ^a	16.0 $\pm$ 5.8 ^a	16.8 $\pm$ 5.9 ^a	13.2 $\pm$ 3.9 ^a	0.081
January	31.2 $\pm$ 11.1 ^b	19.6 $\pm$ 7.3 ^b	16.1 $\pm$ 7.6 ^b	16.1 $\pm$ 5.7 ^b	15.3 $\pm$ 6.6 ^b	0.000
Adult bee population in dm ² area (Mean $\pm$ SD), one dm ² area consists 225 adult bees						
Months	Packed pollen	Pollen patty	Ultra Bee patty	Mega-bee patty	Control	P-Value
October	44.8 $\pm$ 9.1 ^a	41.6 $\pm$ 8.8 ^a	36.8 $\pm$ 12.1 ^a	40.2 $\pm$ 20.9 ^a	36.8 $\pm$ 4.4 ^a	0.280
November	50.4 $\pm$ 21.7 ^a	40.0 $\pm$ 9.2 ^a	36.0 $\pm$ 20.7 ^a	44.6 $\pm$ 20.0 ^a	36.4 $\pm$ 11.2 ^a	0.355
December	54.0 $\pm$ 19.6 ^a	34.4 $\pm$ 11.5 ^a	37.6 $\pm$ 18.7 ^a	45.3 $\pm$ 23.3 ^a	32.8 $\pm$ 15.1 ^a	0.434
January	54.5 $\pm$ 18.6 ^a	38.4 $\pm$ 12.8 ^{ab}	37.6 $\pm$ 14.8 ^{ab}	38.8 $\pm$ 16.8 ^{ab}	30.2 $\pm$ 14.3 ^b	0.019

Values within the column connected with similar upper case letters ("a" or "b" are not significantly different)

The average adult bee population of colonies significantly varied among the different treatment groups ($P < 0.0001$) (Tab. 2). Generally, in all the treatment groups there were strong positive correlations ($r = 0.37 - 0.76$), $P < 0.05$ between the brood and adult bee population. However, both the brood and adult bee populations in the packed pollen treatment group, besides the positive correlation, it showed consistent and steady growth throughout the experimental period (Tab. 3). In other treatment groups there was no significant increment in their adult bee population and remained more or less the same throughout the study periods (Tab. 3).

Amount of stored pollen

Since the experiment was conducted in the dearth period, the amount of incoming and stored pollen was generally low in all treatment groups which varied from 1.2 $\pm$ 1.85 dm² area in the packed pollen group to 2.8 $\pm$ 3.25 dm² in the pollen patty treatment group. Except for a slight increment in the stored natural pollen amount in November for pollen patty, Ultra bee patty and Mega-bee patty groups, the general incoming stored pollen amount was declined for all groups.

DISCUSSION

The high brood population of colonies in the packed-pollen treatment group could be due to the conversion of the packed pollen into beebread which allows for better palatability and nutrient availability than patty-form feeds. This agrees with the findings of Vasquez & Olofsson (2009); Brodschneider & Crailsheim (2010); Vasquez & Olofsson (2011) & Mattila et al. (2012), who reported the fermentation of stored pollen by colony microbes and its conversion into microbial-driven nutrients which are more palatable. Since the pollen and other pollen substitute patty-form feeds are consumed directly without the fermentation process (Oliver, 2014) it may not have an equally positive effect as that of beebread.

In another way, the high performances of colonies in the packed-pollen treatment group could be due to the possible brood rearing stimulating potential of the beebread of manually packed pollen which is similar to their natural way of storing collected pollen adjacent to the brood. In relation to this, Fewell & Winston (1992), Blaschon et al. (1999) and Schmickl et al. (2003) have reported the presence of direct relationship between the stored-pollen

and brood production of colonies and its strong influence and determining role in increasing the brood rearing efficiency of a colony. The patty-form feeds kept on the top of frames cannot be converted into beebread and restored on combs. So it may not have the same brood rearing stimulating effect on honey bee colonies which are evolutionarily adapted to store their reserves on the comb which may guide them to strategize their brood rearing conditions. The absence of fermentation and nutrient release, lack of its storing in a comb as beebread and its direct consumption as raw were indicated as some of the limitation of patties (Oliver, 2014). Even if the amount of monthly dry-matter intakes by colonies in different treatment groups did not significantly vary, colonies in the packed-pollen treatment group attained significantly higher brood and adult populations. This clearly indicates not only the amount of feed intake is important, but the availability and utilization of essential nutrients are more important.

Though there were no statistically significant differences in the general performances of colonies among the different patty-form feeding groups, the pollen-patty group showed consistent and steady increment in its brood population and lastly surpassed the other patty forms both in its brood and adult population, which agrees with Standifer (1980) and Oliver (2014) who reported the absence of pollen substitutes better than the natural pollen. Though the commercial pollen substitutes patties did not contribute to the significant growth of the adult bee population, but it assisted to the maintenance of colonies in a dearth period.

Packed pollen feeding can be more important in hot and dry climatic regions where the dearth period is very long and the flowering period is very short to stimulate a colony for early population build-up to match their strength with the flowering period of specific honey source plants in the region. In such areas, before the colonies manage to attain their optimum strength, the honey flow period may be over. Packed-pollen feeding can also be very useful to prepare a strong colony early for queen and drone rearing and pollination services. Mattila

& Otis (2006) have reported the role of dearth period pollen supplement for faster colony spring build-up and higher honey yield of honey bee colonies.

Although the conversion and utilization of packed pollen varied among colonies within the same treatment group, from the current study it was concluded that when there is no incoming pollen or forage, manually packed pollen feeding would be better than the patty form of feeding for both dearth period maintenance and stimulate early population buildup of colonies for a specific purpose.

In the case of feeding very weak colonies, packed pollen should be given in small amounts and gradual. Packing pollen might be made more efficient and faster on drawn out plastic combs than beeswax combs and also some mechanical means can be used to pack a large number of combs faster and easier for its large scale utilization. However, this might need further testing and evaluation for its acceptance by the bees in comparison with beeswax combs.

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