

Full Length Research Paper

# Patterns of short- and long-term responses of honey bee (*Apis mellifera* L.) colony to changes in its internal environment

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Management of the honey bee (*Apis mellifera* L.) colony for honey production and pollination of crops includes the manipulation of its internal environment. It is not yet understood how the colony might respond to such manipulations in the short- (within four or five days post-treatment) and long-term (after 21 days post-treatment) and whether the once generated short-term response persists into the long-term. Five internal parameters (unsealed and sealed brood, pollen and honey area and colony strength) of the honey bee colony were manipulated and the patterns of resulting short- and long-term colony responses were studied. In the short-term, the honey bee colony showed a significant increase in pollen foraging and a decrease in nectar foraging following an increase in unsealed brood and honey stores; a significant decrease in pollen foraging and an increase in nectar foraging following an increase in pollen stores; and a significant increase in nectar foraging and no change in pollen foraging following an increase in colony strength. However, an increase in sealed brood did not cause any change in the colony foraging patterns. Majority of the short-term responses did not persist for long and wore off with the passage of time. Therefore, the patterns of the long-term responses were different from the short-term responses. In the long-term, only some responses were 'expected and similar to the short-term responses'; some were 'unexpected and different from short-term responses'; many were 'new (previously not reported) and expected'; and some others were 'new and unexpected'. The study reveals that knowledge of short-term responses would be helpful in devising management strategies to urgently stimulate a colony for nectar or pollen foraging. However, the results show that the short-term responses may or may not persist for long and the colony may need a fresh stimulus to sustain the desired response into long-term.

**Key words:** *Apis mellifera*, brood, colony, foraging, honey, honey bee, nectar, pollen, pollination.

## INTRODUCTION

Foraging in the honey bee colony is a social activity; the foraging bees work in response to a stimulus generated

by the specific need of the colony. The honey bees primarily forage for collecting nectar and pollen; the latter



are two unique and essential food types needed to meet the nutritional demand of the adult bees and the young brood (Seeley, 1989; Abou-Shaara, 2014). Five major parameters influence the foraging and other activities of the colony; these include colony brood (uncapped/unsealed and capped/sealed), colony reserves (pollen and honey) and the number of adult bees (colony strength). Colony management for honey production and pollination of crops requires frequent manipulation of these parameters. This study was conducted to investigate the short- and long-term effects of such manipulations on the foraging and some other activities of the honey bee (*Apis mellifera* L.) colony.

Earlier studies show that changes in the internal environment of the honey bee colony influence its foraging response. With an increase in the unsealed brood in the colonies, pollen foraging increases (Free, 1967; Cale, 1968; Al-Tikrity et al., 1972; Calderone, 1993; Dreller et al., 1999). Likewise, with an increase in the pollen in the colony, the latter shifts to nectar foraging; or the pollen foragers stop to forage (Camazine, 1993); or the pollen foraging activity decreases until it depletes the excess pollen and the amount of stored pollen returns to the pre-addition level (Barker, 1971; Free and Williams, 1971; Moeller, 1972; Fewell and Winston, 1992; de Lima et al., 2016). When the pollen is removed from a colony there is a concomitant increase in the number of pollen foragers (Free and Williams, 1971; Fewell and Winston, 1992), and their pollen load size also increases until the colony restores the amount of stored pollen to the earlier original level (Fewell and Winston, 1992; Eckert et al., 1994). Earlier reports also show that pollen collection varies with the stage of the brood; its collection and usage is maximal at larval stage (Hellmich and Rothenbuhler, 1986). An increase in the uncapped (unsealed) brood in the colonies results in a decrease in the nectar foraging (Hoopinger and Taber, 1979). A feedback mechanism seems to regulate the overall foraging activity and a positive or a negative feedback governs the switch-on mechanism (Cale, 1968; Antonsenko and Ermoleava, 1979). These researchers argued that incoming of plenty of one type of food in the colony inhibits its own supply by a negative feedback and stimulates foraging for the other food type. Likewise, the scarcity of anyone food stimulates its own storage. Further, reports also suggest that the pollen stores in the colony act as the main stimulus for the nectar foraging (Free, 1967; Cale, 1968; Barker, 1971; Fewell and Winston, 1992; Hoopinger and Taber, 1979). But a shortage in the pollen reserves of the colony decreases the brood rearing activity and the strength of the colony (Barker and Jay, 1974; Antonsenko and Ermoleava, 1979). This happens due to the lesser availability of food for brood rearing. However, the converse is true for honey stores; an increase in the latter parameter stimulates pollen foraging in the colony (Free, 1967; Cale, 1968; Barker, 1971; Fewell and Winston, 1992;

Hoopinger and Taber, 1979). Likewise, the colony strength also influences the colony foraging (Barker and Jay, 1974). The stronger colonies (with 20 thousand bees) produce three times more honey, pollen, and brood than the weaker colonies (with 10 thousand bees) (Bhusal et al., 2011; EL-Kazafy and Al-Kahtani, 2013). Thus, with any shortage or excess of an internal parameter, the honey bee colony changes its foraging response according to its emergent need.

But, there can be two types of colony responses viz. the short-term (within a few days post-treatment) and the long-term (after completion of one developmental cycle of 21 days post-treatment). All the aforementioned reports depict the short-term responses occurring due to changes in the colony parameters. However, we do not know about the patterns of responses the colony would show in the long-term due to changes in its internal environment. Do the short-term responses sustain for an unlimited period or do they wear off after some time? Revelation to these questions was the aim of this study. Insights into these revelations would be helpful in devising the strategies for the management and use of honey bee (*A. mellifera*) colonies for honey production and pollination of crops.

## MATERIALS AND METHODS

### Honey bee forage and conditions of the colonies

This study was carried out in the apiary of European honey bee (*A. mellifera* L. *ligustica*) at the Department of Zoology, CCS Haryana Agricultural University, Hisar (India). Honey bee colonies in this part of India rear new brood with the onset of flowering on pearl millet (*Pennisetum typhoides* (Burm. f.) Stapf & C. E. Hubb.). The brood rearing activity continues in September when pigeon pea (*Cajanus cajan* (L.) Millsp.) is in blooms. However, the leafy edible mustard (*Brassica juncea* (L.) Czern & Coss.) is the major source of pollen and nectar from early December to mid-February (Sihag, 1990). The inclement weather resulting from torrential rains or fog in the winter (December/January) interrupts normal foraging for seven to ten days when day temperature drops below 15°C. On all other days, availability of plenty of bee forage and favorable day temperature (>20°C) ensures normal foraging flights in the colonies. During this period, the colonies show brisk foraging and reproduction activities and need none supplementary feed. The growing colonies have full frames of unsealed and sealed brood and sealed honey frames in early December.

### Layout plan of the experiments

To record the short- and the long-term colony responses, the colonies were manipulated in the first week of December to use them for different tests. Two series of experiments were performed.

### *Experiments on the short-term responses of the honey bee colony to changes in its internal environment*

Honey bees visit flowers for two important food types viz. pollen and nectar (Figures 1 to 3), bring these to the colony (Figure 4) and store in the wax combs (Figures 5 to 7). Five experiments (one for



**Figure 1.** A forager bee of *Apis mellifera* foraging only for pollen on the flower of *Althaea rosea*. (Photo: Maria Isabel).



**Figure 2.** A forager bee of *Apis mellifera* foraging for pollen as well as nectar from the flower of a cruciferous plant (Photo: Dorin Gheorghe).

each parameter) were performed to record the short-term response of the honey bee colony to changes in its five internal parameters (viz. unsealed brood, sealed brood, honey stores, pollen stores and colony strength; Figures 5 to 11). Table 1 shows the layout plan of

these experiments (showing colony parameters, their treatments, and fixed values).

The quantity of the colony parameters were selected according to the recommended management practices for beekeeping with



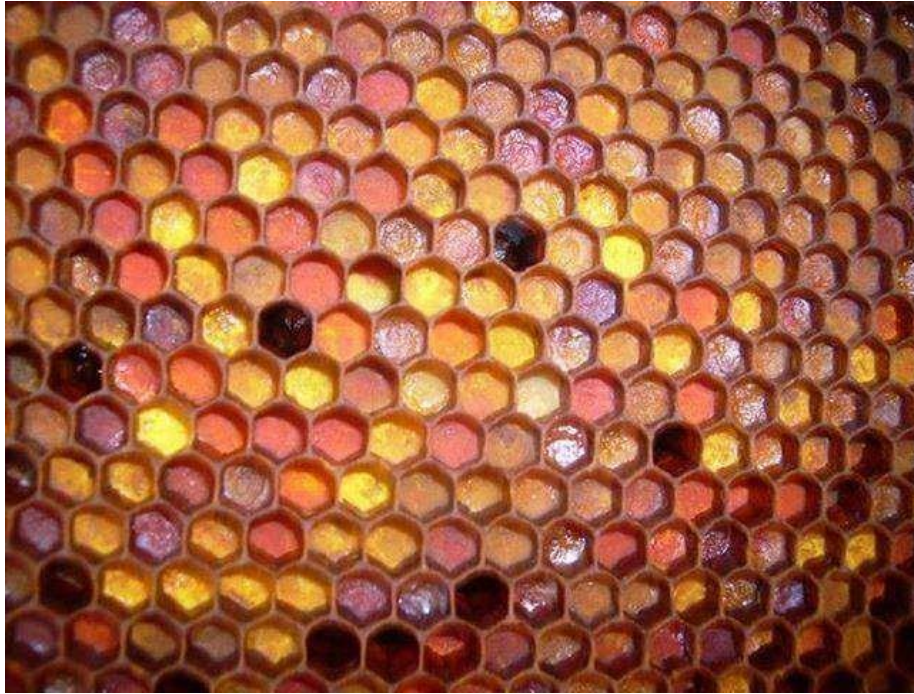
**Figure 3.** A forager bee of *Apis mellifera* foraging only for nectar from the flower of rose (*Rosa* sp.) (Photo: Dorin Gheorghe).



**Figure 4.** Forager bees of *Apis mellifera* at the hive entrance; here 6 bees are with pollen loads (pollen foragers) and 4 are without pollen loads (nectar foragers) (Photo: Apiculture Hamou).

European honey bee (*A. mellifera*) in this region (Sihag, 1990a, b). In each experiment, four parameters were kept at fixed levels and the remaining one parameter was changed to make three

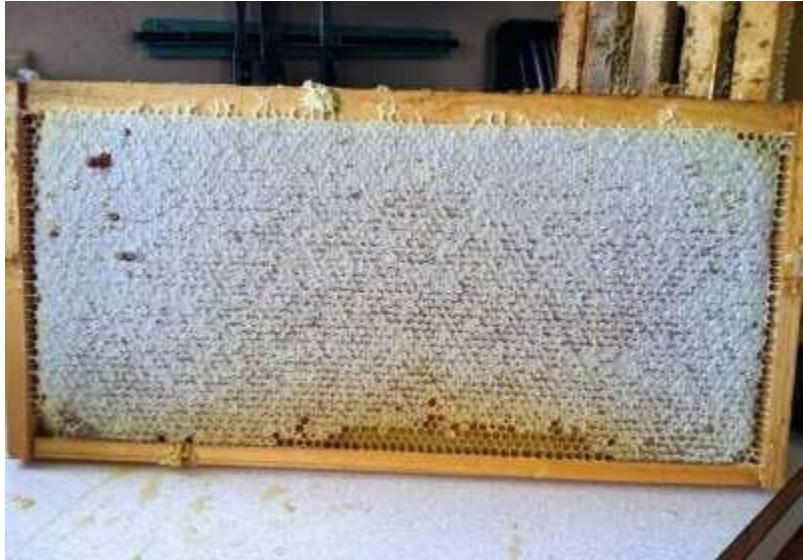
treatments. Each treatment had four replications (in four colonies), thus each experiment was performed on 12 colonies. The foraging response of the colonies was recorded by counting the number



**Figure 5.** A part of the comb frame of *Apis mellifera* showing stored pollen (Photo: Bees, Life and Harmony).



**Figure 6.** A part of the comb of the honey bee (*Apis mellifera*) showing the house bees storing nectar/honey in the comb (Photo: Apiculteur Miel).



**Figure 7.** A comb frame of the honey bee (*Apis mellifera*) showing the sealed honey (Photo: Oreiní Mélissa or Mountain Bee).



**Figure 8.** A part of the comb frame of the honey bee (*Apis mellifera*) showing the unsealed brood (Photo: Corona Apicultores).

of incoming bees at the hive entrance (Figure 4) on the post-treatment four consecutive days.

***Experiment on the long-term responses of the honey bee colony to changes in its internal parameters***

Table 2 shows the layout plan of the experiment on the long-term responses. This experiment was performed on the three types of colonies (viz. 5-frames, 7-frames and 9-frames). For each colony

strength (representing a treatment), the quantities of four internal parameters (viz. unsealed brood, sealed brood, honey stores and pollen stores) were selected as shown in Table 2, and data on the seven colony parameters (Table 2 and Figures 5 to 11) were recorded at 21 days interval on five observational days (that is, on 0, 21, 42, 63 and 84 days). Each treatment had four replication; thus, using four colonies for one treatment and twelve colonies for the experiment.

If a short-term response was sustained in the long-term too, it is designated as an 'expected response'; while a short-term response



**Figure 9.** A comb frame of the honey bee (*Apis mellifera*) showing the sealed brood (Photo: Oreini Mélissa or Mountain Bee).



**Figure 10.** A comb frames of the honey bee (*Apis mellifera*) fully covered with bees (Photo: Apicultor Adrian Stefanoli). This frame carries about 2800 bees on its two surfaces (Burgett et al. 1984).

turned different in the long-term was designated as an 'unexpected response'. Likewise, a response is designated as 'novel and expected' if it was new to science and seemed to be logical, and as 'novel and unexpected' if it was new to science and seemed to be illogical.

#### **Manipulations and measurement of the colony parameters**

Colonies were segregated in the apiary in two groups viz. the 'general pool colonies' and the 'experimental colonies'. This condition facilitated an easy manipulation of parameters in the





**Figure 11.** A 10-frames colony of the honey bee (*Apis mellifera*); frames are fully covered with bees (Photo: ashat oghaim). This colony has about 28000 bees (Burgett et al., 1984).

**Table 1.** Layout plan of experiments on the short-term responses of the honey bee (*Apis mellifera*) colony to changes in its internal parameters.

Experiment No.	Colony parameter	Treatments	Fixed values of other colony parameters
I	Unsealed brood	1. 775 cm <sup>2</sup> 2. 1250 cm <sup>2</sup> 3. 1725 cm <sup>2</sup> -	1. Sealed brood 775 cm <sup>2</sup> 2. Pollen area 150 cm <sup>2</sup> 3. Honey area 300 cm <sup>2</sup> 4. Colony strength 5 frames
II	Sealed brood	1. 775 cm <sup>2</sup> 2. 1250 cm <sup>2</sup> 3. 1725 cm <sup>2</sup> -	1. Unsealed brood 775 cm <sup>2</sup> 2. Pollen area 150 cm <sup>2</sup> 3. Honey area 300 cm <sup>2</sup> 4. Colony strength 5 frames
III	Pollen area	1. 150 cm <sup>2</sup> 2. 300 cm <sup>2</sup> 3. 450 cm <sup>2</sup> -	1. Unsealed brood 775 cm <sup>2</sup> 2. Sealed brood 775 cm <sup>2</sup> 3. Honey area 300 cm <sup>2</sup> 4. Colony strength 5 frames
IV	Honey area	1. 1200 cm <sup>2</sup> 2. 1800 cm <sup>2</sup> 3. 2400 cm <sup>2</sup> -	1. Unsealed brood 775 cm <sup>2</sup> 2. Sealed brood 775 cm <sup>2</sup> 3. Pollen area 150 cm <sup>2</sup> 4. Colony strength 5 frames
V	Colony strength	1. 5-frames 2. 7-frames 3. 9-frames -	1. Unsealed brood 775 cm <sup>2</sup> 2. Sealed brood 775 cm <sup>2</sup> 3. Pollen area 150 cm <sup>2</sup> 4. Honey area 300 cm <sup>2</sup>

Number of observations=48 (3 treatments × 4 replications × 4 observation days, each day having one mean observation of 4 daily observations).

**Table 2.** Layout plan of the experiment on the long-term responses of the honey bee (*Apis mellifera*) colony to changes in its internal parameters.

Experiment No.	Colony strength (Number of frames fully covered with bees)	Initial fixed values of other colony parameters
I	5-frames (Approximately 14,000 bees)	1. Unsealed brood 775 cm <sup>2</sup> 2. Sealed brood 775 cm <sup>2</sup> 3. Pollen area 150 cm <sup>2</sup> 4. Honey area 1200 cm <sup>2</sup>
II	7-frames (Approximately 20,000 bees)	1. Unsealed brood 1250 cm <sup>2</sup> 2. Sealed brood 1250 cm <sup>2</sup> 3. Pollen area 300 cm <sup>2</sup> 4. Honey area 1800 cm <sup>2</sup>
III	9-frames (Approximately 25,000 bees)	1. Unsealed brood 1725 cm <sup>2</sup> 2. Sealed brood 1725 cm <sup>2</sup> 3. Pollen area 450 cm <sup>2</sup> 4. Honey area 2400 cm <sup>2</sup>

experimental colonies.

#### **Manipulations of the colony parameters**

The initial levels of the parameters in the experimental colonies of two series of experiments (as given in Tables 1 and 2) were completed by manually adding or removing the comb frames. The colonies of the general pool acted as the reservoirs to accept or donate the required parameter. For example, to overcome any deficiency in the unsealed brood of the experimental colonies, the frames with the desired level of unsealed brood (without adult bees) were brought from the general pool colonies and added to the experimental colonies. However, to manipulate the colony strength, the frames covered with bees were added to or taken out from the experimental colonies as the need was. General management practices recommended for the maintenance of the honey bee (*A. mellifera*) colonies in this region were followed (Sihag, 1990a, b).

#### **Measurement of the colony parameters**

In the two series of experiments (short and long-term), observations on the numbers of incoming nectar and pollen carrying bees at the hive entrance for 5 min at 2-h intervals with the help of a tally counter and a stopwatch were recorded (thus six observations per day). The bees carrying pollen were considered as pollen foragers and those without pollen as nectar foragers (Figure 4), and the mean of six observations of a parameter on a single day was derived.

The unsealed brood, sealed brood, pollen and honey stores were measured in terms of area (cm<sup>2</sup>) following Sihag and Gupta (2011, 2013) (Figures 6 to 9). Total honey stores in the test colonies were measured at the end of the experiment. The bee strength was measured in terms of the number of frames fully covered by bees following Burgett et al. (1984); one fully covered frame carried about 2800 bees (Figures 9 and 10).

#### **Randomization, statistical design, and analysis**

In the short-term experiments, three treatments were selected for

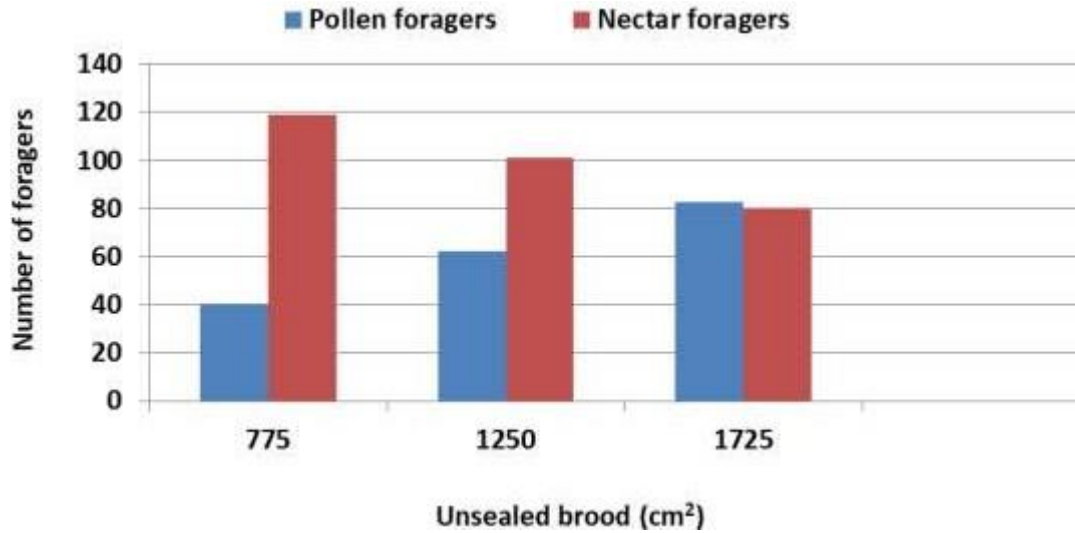
each parameter (Table 1) and four replications for each treatment (thus 12 colonies for one experiment and 60 colonies for the five short-term experiments). In the long-term experiment, the values of parameters of three treatments on five observational days were compared; here too there were four replications of each treatment (thus 12 colonies for an experiment). The randomization process of treatments was completed by the draw of lots. The experiments were laid down in a 'Completely Randomized design' for one factor 'Analysis of Variance' for the short-term experiments and two-factor 'Analysis of Variance' for the long-term experiment. The treatments were compared at 5 and 1% levels of significance with the help of derived 'least significant difference' (LS) values (Snedecor and Cochran, 1967). The degree of relatedness between any two parameters was also determined by deriving correlation coefficient 'r' and testing at 5 and 1% levels of significance with n-2 degrees of freedom (n = number of observations) using 'Independent Sample t-Test' (Snedecor and Cochran, 1967; SPSS Tutorials, 2014).

## **RESULTS**

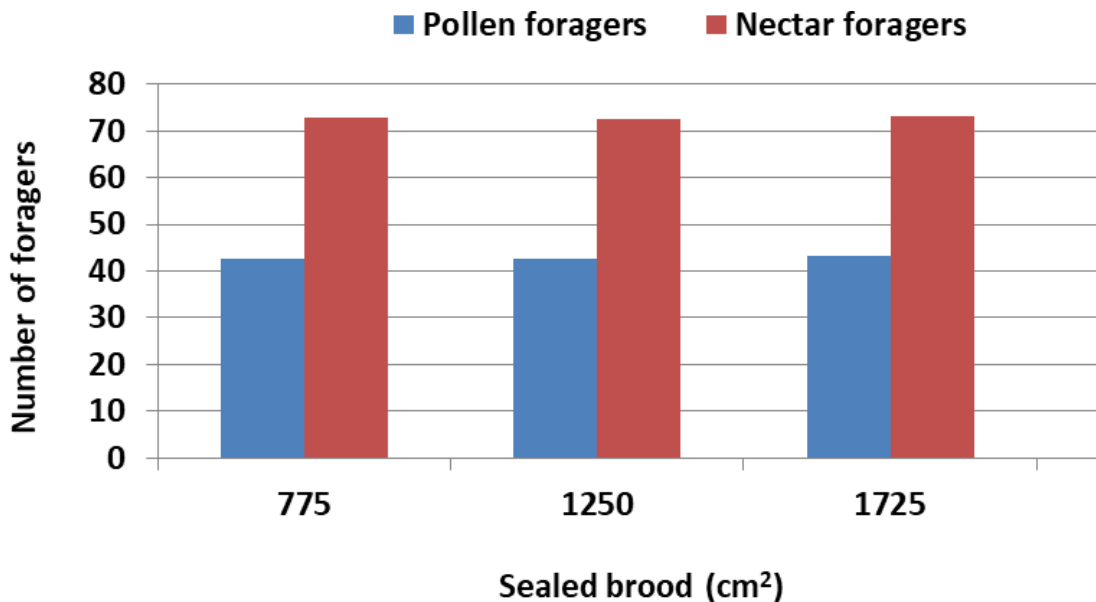
### **Short-term responses of the honey bee colony to changes in its internal environment**

The patterns of short-term responses of the honey bee colony to changes in its internal environment are as shown in Figures 12 to 16 and Tables 3 and 4.

When the unsealed brood in the colony was increased, there was a significant increase in the numbers of pollen foragers and a decrease in the nectar foragers ( $P < 0.05$ ;  $F_{2, 45} = 5.301$ ; ANOVA; Figure 12, Table 3). Presence of a positive and highly significant correlation of unsealed brood with the pollen foragers and a negative with the nectar foragers ( $P < 0.01$ ,  $df = 46$ , t-test, Table 4) supported these results. This shows that if the unsealed brood is added in the colony, the latter would need more pollen for the rearing of developing larvae. To fulfill this need, more



**Figure 12.** Short-term effect of variation in the unsealed brood on the foraging activity of honey bee (*Apis mellifera*) colony.



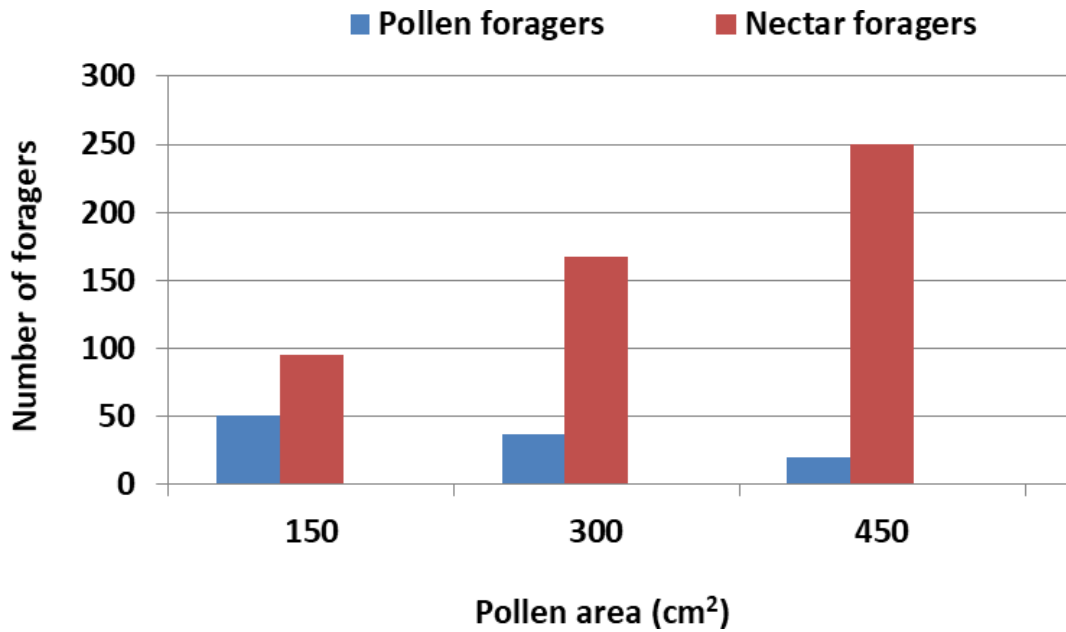
**Figure 13.** Short-term effect of variation in the sealed brood on the foraging activity of honey bee (*Apis mellifera*) colony.

and more foragers would shift to the pollen foraging; thus the proportion of pollen foragers increases and that of nectar foragers decreases.

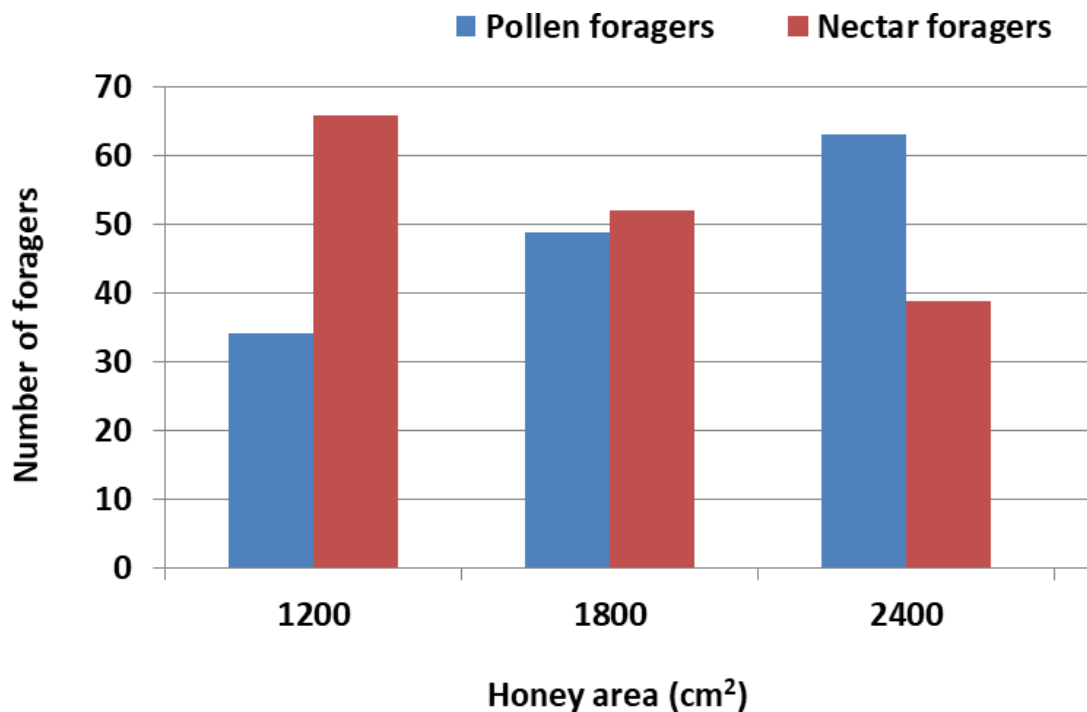
However, the addition of sealed brood in the colony did not influence the pollen or nectar foraging ( $P > 0.05$ ,  $F_{2,45} = 2.326$ ; ANOVA; Figure 13 and Table 3), as both categories of foragers had non-significant correlations with the sealed brood ( $p > 0.05$ ,  $df = 46$ , t-test, Table 4). This indicates that keeping all other colony parameters at a constant level when the sealed brood in the colony is

increased, the pollen and nectar foraging remains unaffected. This is because the addition of sealed brood in the colony does not need more pollen or nectar as food types; hence, the colony is not stimulated for more pollen or nectar foraging.

On the other hand, with an increase in the pollen stores in the colony, the number of pollen foragers decreased and the nectar foragers increased significantly ( $P < 0.05$ ,  $F_{2,45} = 5.402$ ; ANOVA; Figure 14 and Table 3); presence of a negative and highly significant correlation of pollen



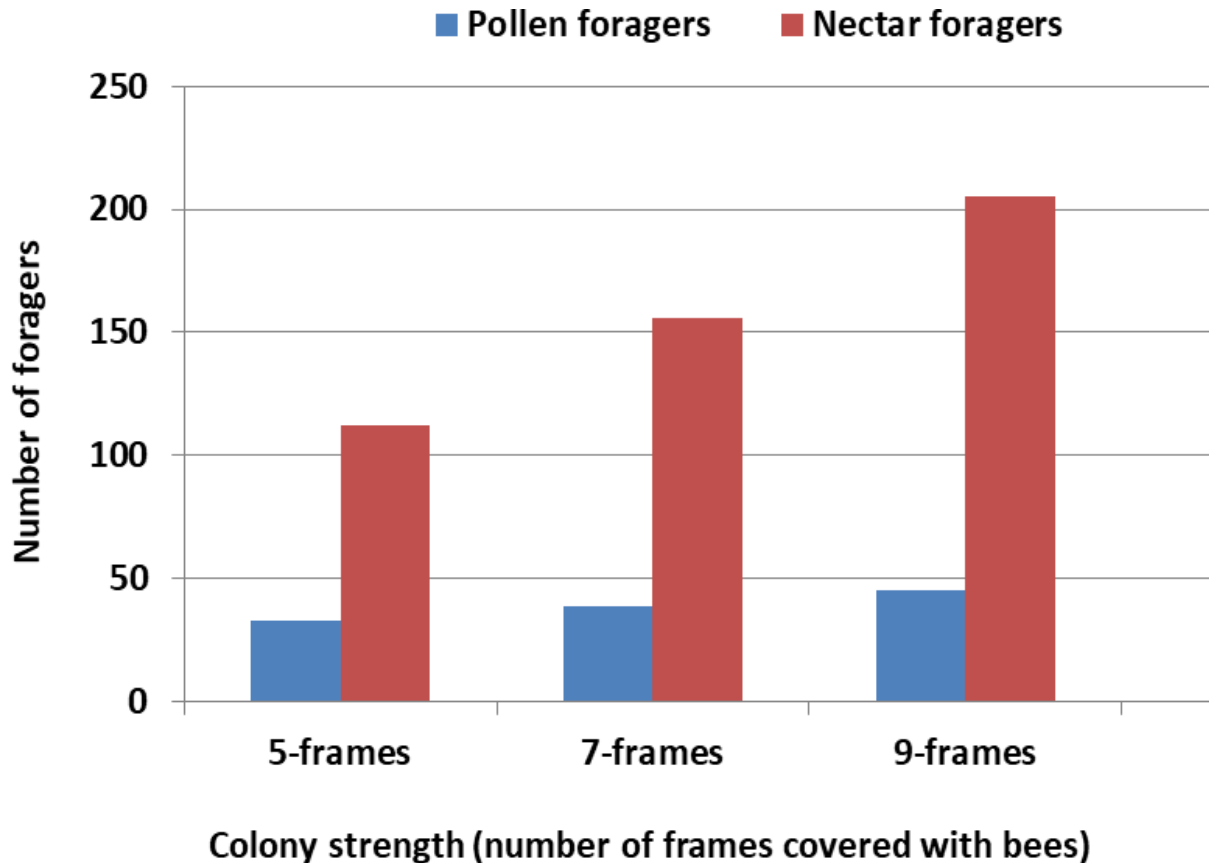
**Figure 14.** Short-term effect of variation in the pollen store on the foraging activity of honey bee (*Apis mellifera*) colony.



**Figure 15.** Short-term effect of variation in the honey store on the foraging activity of honeybee (*Apis mellifera*) colony.

reserves with the number of pollen foragers, and a positive and significant correlation with the nectar foragers strongly supported these finding ( $P < 0.01$ ,  $df = 46$ ,

t-test, Table 4). Likewise, the addition of more honey to the colony significantly increased the pollen foraging and decreased the nectar foraging ( $P < 0.05$ ,  $F_{2, 45} = 5.314$ ,



**Figure 16.** Short-term effect of variation in the colony strength on the foraging activity of honey bee (*Apis mellifera*) colony.

ANOVA; Figure 15 and Table 3). These results were supported by the presence of a positive and highly significant correlation of honey reserves with the number of pollen foragers and a negative and significant correlation with the nectar foragers ( $P < 0.01$ ,  $df = 46$ , t-test, Table 4). This indicates that the addition of pollen in the honey bee colony decreases the pollen foraging and increases the nectar foraging (Figure 14 and Table 3). However, the addition of honey in the colony increases the pollen foraging and decreases the nectar foraging (Figure 15 and Table 3).

With an increase in the colony strength, the nectar foraging increased significantly ( $P < 0.05$ ,  $F_{2,45} = 5.413$ ; ANOVA; Figure 16 and Table 3), but the pollen foraging remained unaffected as this activity did not increase significantly ( $P > 0.05$ ,  $F_{2,45} = 2.286$ ; ANOVA; Figures 16 and Table 3); presence of a positive and significant correlation of the colony strength with the number of nectar foragers ( $P < 0.01$ ,  $df = 46$ , t-test, Table 4) and a positive and non-significant correlation with the pollen foragers ( $P > 0.05$ ,  $df = 46$ , t-test, Table 4) strongly endorsed these results. This indicates that keeping all other parameters at fixed levels, when the colony strength is increased, honey bee colony shifts to more

and more nectar foraging, while the pollen foraging activity is not affected and remains unchanged (Figure 16 and Table 3).

#### **Long-term responses of the honey bee colony to changes in its internal environment**

The patterns of long-term responses of the honey bee colony to changes in its internal environment are as shown in Figures 17 to 19 and Tables 5 to 10. The following four types of long-term responses were observed.

##### **Expected responses**

There were only two such short-term responses of the honey bee colony that sustained in the long-term too. For example, with an increase in the unsealed brood and the honey area, the number of pollen foragers increased significantly ( $p < 0.05$  and  $p < 0.01$ , respectively, ANOVA, Figures 17 to 19 and Tables 5 to 7); and with an increase in the colony strength, the nectar foragers increased

**Table 3.** Short-term effects of variations in the internal parameters of honey bee (*Apis mellifera*) colony on its foraging activity.

Colony parameter	Value of colony parameter <sup>a</sup>	Kind of foragers			
		Pollen foragers <sup>b</sup>		Nectar foragers <sup>b</sup>	
		Mean ± s.d.	LSD value <sup>c</sup>	Mean ± s.d.	LSD value <sup>c</sup>
Unsealed brood (cm <sup>2</sup> )	775	40.2±2.8		118.8±5.3	
	1250	62.5±3.9	1.8**	101.2±4.7	4.5**
	1725	82.8±4.7		80.1±3.7	
Sealed brood (cm <sup>2</sup> )	775	42.5±2.6		72.7±3.1	
	1250	42.6±2.8	NS	72.5±2.8	NS
	1725	43.1±2.6		73.1±2.7	
Pollen area (cm <sup>2</sup> )	150	50.4±2.7		95.8±4.1	
	300	36.7±2.3	2.5**	167.2±7.3	2.7**
	450	20.2±1.7		250.1±12.4	
Honey area (cm <sup>2</sup> )	1200	34.2±2.5		65.9±2.4	
	1800	48.8±3.7	1.5**	52.1±2.6	4.9**
	2400	63.1±3.7		38.7±1.3	
Colony strength <sup>d</sup>	5-frames	32.8±2.4		112.5±8.4	
	7-frames	33.5±2.6	NS	155.8±5.8	3.9**
	9-frames	34.0±2.9		205.6±6.3	

<sup>a</sup>Other colony parameters were changed as shown in Table 1. <sup>b</sup>Each value represents mean ± s.d. of 16 observations (4 colonies × 4 days). <sup>c</sup>(\*\*p (<0.01, 45 d.f., Completely Randomized Design, ANOVA)=significant. <sup>d</sup>Number of frames fully covered with bees.

**Table 4.** Correlations between internal parameters of the honeybee (*Apis mellifera*) colony and its foragers (short-term response).

Colony parameter	Value of correlation coefficient (r)	
	Pollen foragers	Nectar foragers
Unsealed brood	0.89**	-0.88**
Sealed brood	0.05	0.13
Pollen area	-0.95**	0.99**
Honey area	0.79**	-0.92**
Colony strength	0.35	0.90**

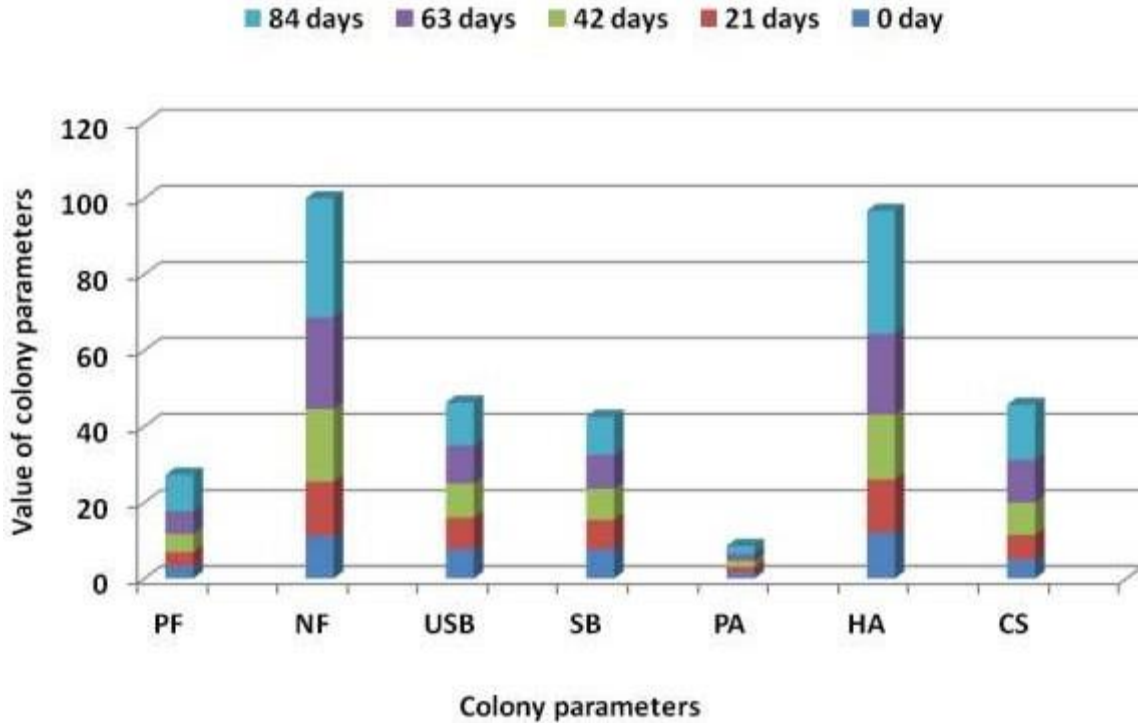
\*\*p<0.01 = significant, \*p<0.05 = significant (n=48, d.f.=46, Independent Sample t-test).

significantly (p<0.01, ANOVA; Figures 17 to 19 and Tables 5 to 7). Presence of a positive and significant correlation between each stimulus and its response confirmed these results (P<0.05 and p< 0.01, respectively, df=18, t-test; Tables 8 to 10, digits in green color). These responses existed in the short-term and sustained in the long-term too; hence, these were the expected responses. All other colony responses did not sustain in the long-term.

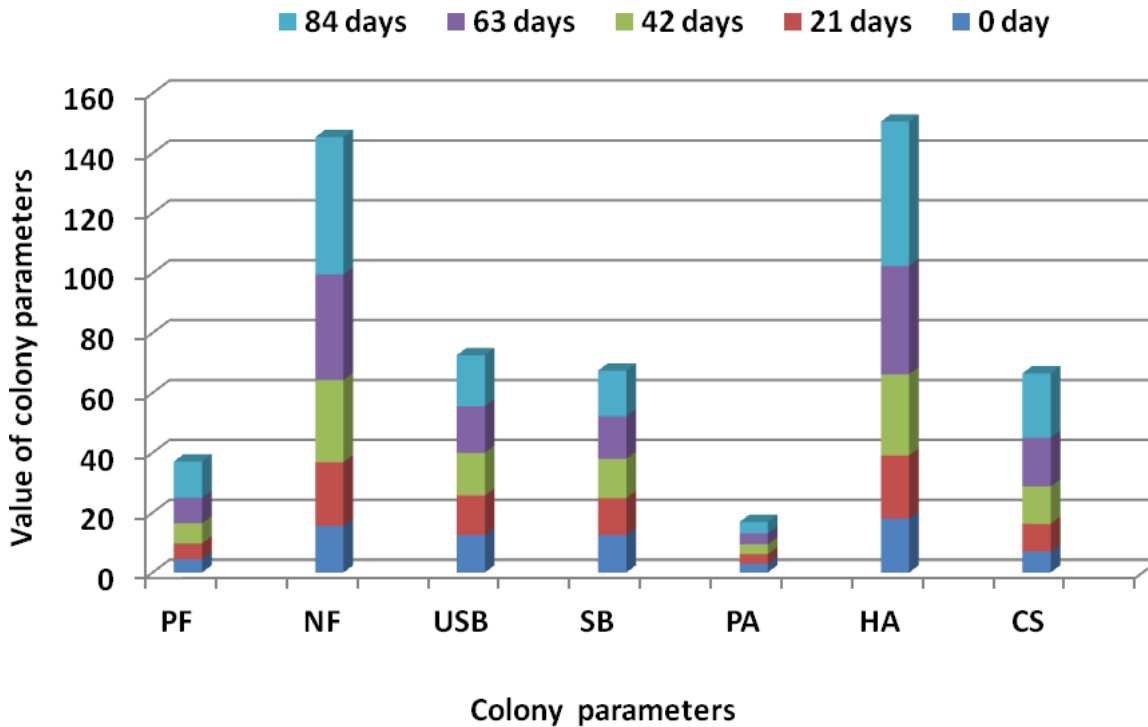
### Unexpected responses

In the long-term, the number of pollen foragers increased

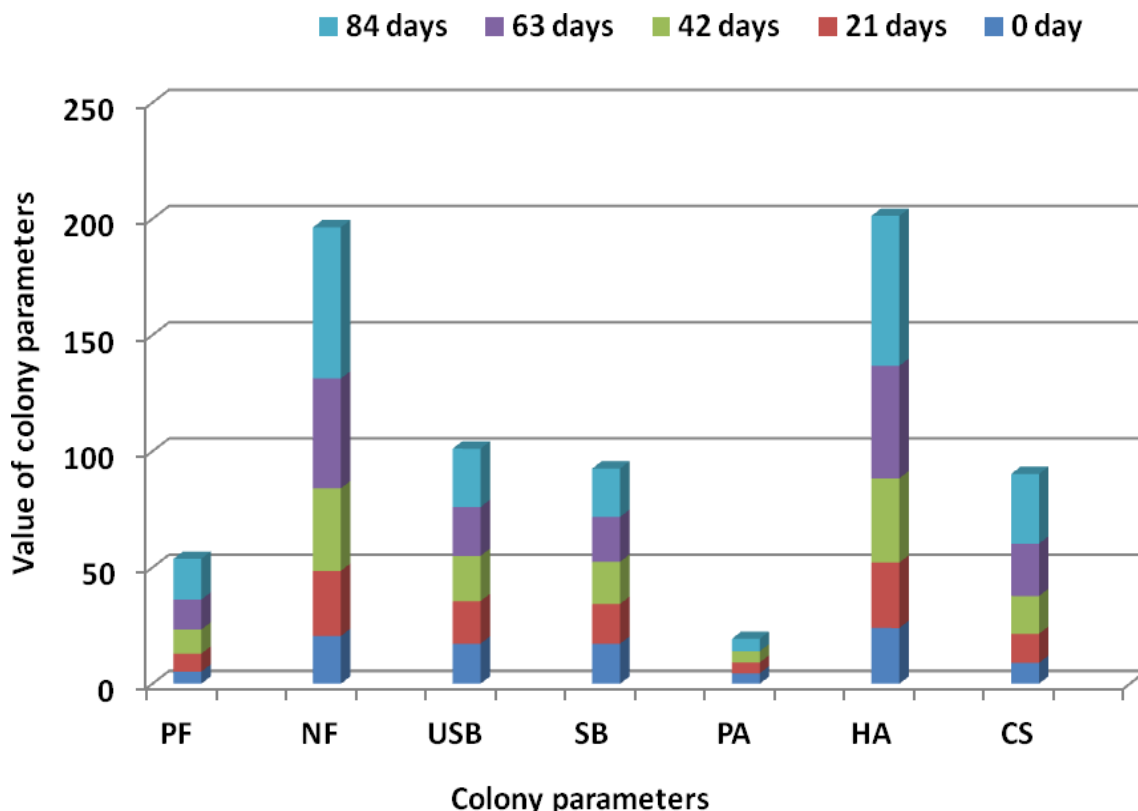
significantly with an increase in the number of nectar foragers, the colony strength (p<0.01, ANOVA; Figures 17 to 19 and Tables 5 to 10), and the sealed brood (p<0.05, ANOVA; Figures 17 to 19 and Tables 5 to 7). In the short-term, the number of pollen foragers has decreased with an increase in the number of nectar foragers and vice versa (Figures 12, 14 and 15), remained unaffected with an increase in the colony strength (P>0.05, F<sub>2,45</sub>=2.286, ANOVA; Figure 16 and Table 3) and the sealed brood (P>0.05, F<sub>2, 45</sub>=2.326, ANOVA; Figure 13 and Table 3). These responses in the long-term appeared contrary to the expectations; hence, were designated as unexpected responses. Some other such responses included: a significant increase in the



**Figure 17.** Long-term variations in the internal parameters of honey bee (*Apis mellifera*) colony with initial 5- frames strength; PF= Number of pollen foragers  $\times 10$ ; NF= Number of nectar foragers  $\times 10$ ; USB= Unsealed brood ( $\text{cm}^2 \times 10^2$ ); SB= Sealed brood ( $\text{cm}^2 \times 10^2$ ); PA= Pollen area ( $\text{cm}^2 \times 10^2$ ); HA= Honey area ( $\text{cm}^2 \times 10^2$ ); CS= Colony strength (number of frames fully covered with bees).



**Figure 18.** Long-term variations in the internal parameters of honey bee (*Apis mellifera*) colony with initial 7- frames strength (Abbreviations are same as in Figure 17).



**Figure 19.** Long-term variations in the internal parameters of honey bee (*Apis mellifera*) colony with initial 9-frames strength (Abbreviations are same as in Figure 17).

**Table 5.** Long-term responses of the honey bee (*Apis mellifera*) colony to changes in its internal parameters (with an initial 5-frames strength).

Colony parameter	Values of various colony parameters after fixed interval of days					*LSD Values
	0 day	21 days	42 days	63 days	84 days	
Pollen foragers <sup>a</sup>	32.2±2.3	38.1±3.6	47.1±4.6	60.3±5.8	95.4±6.4	2.13
Nectar foragers <sup>b</sup>	112.2±4.4	142.4±8.2	191.3±10.7	241.2±13.4	313.2±14.6	2.16
Unsealed brood (cm <sup>2</sup> ) <sup>c</sup>	775.0±0.0	823.25±18.6	901.5±21.4	1003.5±25.8	1126.25±31.4	26.3
Sealed brood (cm <sup>2</sup> ) <sup>d</sup>	775.0±0.0	771.25±17.2	815.5±20.5	892.25±23.8	1001.25±26.1	22.7
Pollen area (cm <sup>2</sup> ) <sup>e</sup>	150.0±0.0	156.5±5.3	165.25±6.8	181.25±8.7	203.25±9.2	4.03
Honey area (cm <sup>2</sup> ) <sup>f</sup>	1200.0±0.0	1407.5±32.7	1713.5±43.3	2122.25±58.6	3234.5±62.2	54.2
Colony strength <sup>g</sup>	5.0±0.0	6.5±0.2	8.5±0.4	11.25±0.5	14.5±0.8	0.37

a-g: Mean± s.d. of 4 observations; g: Number of frames fully covered with bees; \*p<0.05 (15 degrees of freedom, Completely Randomized Design, ANOVA).

nectar foragers with an increase in the honey stores ( $p<0.01$ , ANOVA; Figures 17 to 19 and Tables 5 to 7); and the unsealed brood and the sealed brood ( $p<0.05$ , ANOVA; Figures 17 to 19 and Tables 5 to 7). Likewise, with an increase in the pollen area, the nectar foraging did not increase significantly ( $p>0.05$ , ANOVA; Figures 17 to 19 and Tables 5 to 7). These results were further supported by the respective correlations (Tables 8 to 10).

### Novel and expected responses

In the long-term, with an increase in the colony strength, the unsealed brood, the sealed brood, and the honey area increased significantly ( $p<0.01$ , ANOVA; Figures 17 to 19 and Tables 5 to 7). Likewise, with an increase in the pollen area, the unsealed brood increased significantly ( $p<0.01$ , ANOVA; Figures 17 to 19 and Tables 5 to 7).



**Table 6.** Long-term responses of the honey bee (*Apis mellifera*) colony to changes in its internal parameters (with an initial 7-frames strength).

Colony parameter	Values of various colony parameters after fixed interval of days					*LSD
	0 day	21 days	42 days	63 days	84 days	Values
Pollen foragers (cm <sup>2</sup> ) <sup>a</sup>	44.7±2.6	52.2±3.4	67.6±4.3	84.8±5.9	121.5±6.2	2.3
Nectar foragers (cm <sup>2</sup> ) <sup>b</sup>	155.3±6.8	212.9±8.6	274.7±13.2	352.2±18.3	458.4±26.4	9.2
Unsealed brood (cm <sup>2</sup> ) <sup>c</sup>	1250.0±0.0	1324.25±37.4	1426.5±42.7	1551.5±52.6	1703.25±63.9	34.4
Sealed brood (cm <sup>2</sup> ) <sup>d</sup>	1250.0±0.0	1236.5±33.3	1311.5±37.7	1415.25±43.2	1532.5±48.5	30.3
Pollen area (cm <sup>2</sup> ) <sup>e</sup>	300.0±0.0	312.25±12.8	334.25±14.6	356.5±15.4	383.5±18.9	7.6
Honey area (cm <sup>2</sup> ) <sup>f</sup>	1800.0±0.0	2107.25±61.7	2712.5±74.3	3618.25±95.6	4823.25±118.2	96.6
Colony strength <sup>g</sup>	7.0±0.0	9.25±0.2	12.5±0.4	16.25±0.7	21.5±1.2	0.8

a-g: Mean± s.d. of 4 observations; g: Number of frames fully covered with bees; \*p<0.05 (15 degrees of freedom, Completely Randomized Design, ANOVA).

**Table 7.** Long-term responses of the honey bee (*Apis mellifera*) colony to changes in its internal parameters (with an initial 9-frames strength).

Colony parameter	Values of various colony parameters after fixed interval of days					*LSD
	0 day	21 days	42 days	63 days	84 days	values
Pollen foragers <sup>a</sup>	52.1±2.5	77.3±3.6	103.6±4.8	128.8±5.7	175.7±8.3	7.4
Nectar foragers <sup>b</sup>	205.4±8.6	280.4±11.3	354.7±13.8	472.9±18.7	650.3±24.6	13.6
Unsealed brood (cm <sup>2</sup> ) <sup>c</sup>	1725.0±0.0	1827.25±42.6	1948.25±44.8	2103.5±48.9	2511.5±52.7	50.3
Sealed brood (cm <sup>2</sup> ) <sup>d</sup>	1725.0±0.0	1710.25±36.8	1812.25±42.5	1931.25±45.7	2083.5±47.9	42.43
Pollen area (cm <sup>2</sup> ) <sup>e</sup>	450.0±0.0	464.5±13.6	486.5±15.1	507.25±16.8	533.5±18.9	11.7
Honey area (cm <sup>2</sup> ) <sup>f</sup>	2400.0±0.0	2812.5±68.8	3627.5±82.4	4839.25±93.8	6451.25±126.7	131.6
Colony strength <sup>g</sup>	9.0±0.0	12.5±0.8	16.25±1.2	22.5±1.6	30.0±1.8	0.7

a-g: Mean± s.d. of 4 observations; g: Number of frames fully covered with bees; \*p<0.05 (15 degrees of freedom, Completely Randomized Design, ANOVA).

**Table 8.** Correlations between different parameters of the honeybee (*Apis mellifera*) colony with an initial 5-frames strength (long-term response).

Colony parameter	Value of correlation coefficient (r)						
	Pollen foragers	Nectar foragers	Unsealed brood	Sealed brood	Pollen area	Honey area	Colony strength
Pollen foragers	1.00						
Nectar foragers	<b><u>0.98**</u></b>	1.00					
Unsealed brood	<b><u>0.55*</u></b>	<b><u>0.54*</u></b>	1.00				
Sealed brood	<b><u>0.63*</u></b>	<b><u>0.65*</u></b>	<b><u>0.73*</u></b>	1.00			
Pollen area	<b><u>0.41</u></b>	<b><u>0.42</u></b>	<b><u>0.81**</u></b>	<b><u>0.92**</u></b>	1.00		
Honey area	<b><u>0.88**</u></b>	<b><u>0.84**</u></b>	<b><u>0.37</u></b>	<b><u>0.43</u></b>	<b><u>0.35</u></b>	1.00	
Colony strength	<b><u>0.98**</u></b>	<b><u>0.98**</u></b>	<b><u>0.64*</u></b>	<b><u>0.56*</u></b>	<b><u>0.44</u></b>	<b><u>0.83**</u></b>	1.00

N=20 (4 colonies × 5 days); \*p<0.05, \*\*p<0.01 (18 degrees of freedom, Independent Sample t-test). Numbers in simple and green letters represent 'expected' responses (already reported by the earlier researchers); bold, italic, underlined and red represent 'unexpected' responses (contrary to the earlier reports); bold and blue as 'novel and expected' responses (new to Science) and bold, italic and pink as 'novel and unexpected' responses.

Presence of a positive and significant correlation between each stimulus and the respective response further supported these results (Tables 8 to 10). Also, an increase in the honey area did not increase the unsealed and the sealed broods, as the correlations between

respective parameters were non-significant (Tables 8 to 10). All these responses are novel (new to science) and seemed to be behaviorally logical. For example, under the persistent pollen and nectar supply, with an increase in the colony strength, there is an increase in the pollen

**Table 9.** Correlations between different parameters of honeybee (*Apis mellifera*) colony with an initial 7-frames strength (long-term response).

Colony parameter	Value of correlation coefficient (r)						
	Pollen foragers	Nectar foragers	Unsealed brood	Sealed brood	Pollen area	Honey area	Colony strength
Pollen foragers	1.00						
Nectar foragers	<b><u>0.99**</u></b>	1.00					
Unsealed brood	<b>0.56*</b>	<b><u>0.57*</u></b>	1.00				
Sealed brood	<b><u>0.67*</u></b>	<b><u>0.65*</u></b>	<b>0.74*</b>	1.00			
Pollen area	<b><u>0.41</u></b>	<b><u>0.44</u></b>	<b>0.80**</b>	<b>0.93**</b>	1.00		
Honey area	<b>0.87**</b>	<b><u>0.85**</u></b>	<b>0.49</b>	<b>0.44</b>	<b>0.33</b>	1.00	
Colony strength	<b><u>0.99**</u></b>	<b>0.97**</b>	<b>0.61*</b>	<b>0.55*</b>	<b>0.42</b>	<b>0.85*</b>	1.00

N=20 (4 colonies × 5 days); \* p<0.05, \*\* p<0.01 (18 degrees of freedom, Independent Sample t-test). Numbers in simple and green letters represent 'expected' responses (already reported by the earlier researchers); bold, italic, underlined and red represent 'unexpected' responses (contrary to the earlier reports); bold and blue as 'novel and expected' responses (new to Science) and bold, italic and pink as 'novel and unexpected' responses.

**Table 10.** Correlations between different parameters of honeybee (*Apis mellifera*) colony with an initial 9-frames strength (long-term response).

Colony parameter	Value of correlation coefficient (r)						
	Pollen foragers	Nectar foragers	Unsealed brood	Sealed brood	Pollen area	Honey area	Colony strength
Pollen foragers	1.00						
Nectar foragers	<b><u>0.98**</u></b>	1.00					
Unsealed brood	<b>0.54*</b>	<b><u>0.57*</u></b>	1.00				
Sealed brood	<b><u>0.65*</u></b>	<b><u>0.66*</u></b>	<b>0.73*</b>	1.00			
Pollen area	<b><u>0.43</u></b>	<b><u>0.46</u></b>	<b>0.92**</b>	<b>0.82**</b>	1.00		
Honey area	<b>0.87**</b>	<b><u>0.84**</u></b>	<b>0.48</b>	<b>0.44</b>	<b>0.35</b>	1.00	
Colony strength	<b><u>0.99**</u></b>	<b>0.99**</b>	<b>0.63*</b>	<b>0.58*</b>	<b>0.43</b>	<b>0.84*</b>	1.00

N=20 (4 colonies × 5 days); \*p<0.05, \*\*p<0.01 (18 degrees of freedom, Independent Sample t-test). Numbers in simple and green letters represent 'expected' responses (already reported by the earlier researchers); bold, italic, underlined and red represent 'unexpected' responses (contrary to the earlier reports); bold and blue as 'novel and expected' responses (new to Science) and bold, italic and pink as 'novel and unexpected' responses.

and nectar foraging activity. The latter situation causes an increase in the pollen as well as nectar storage in the colony, and consequently an increase in the brood rearing activity too (Figures 17 to 19 and Tables 5 to 7). Hence, these responses are new and logical, and were designated as the 'novel and expected responses'.

### ***Novel and unexpected responses***

In the long-term, still some other responses were novel but these were contrary to our expectations. For example, with an increase in the sealed brood, the unsealed brood increased significantly. Similarly, with an increase in the honey area and the colony strength, pollen area did not increase significantly (Figures 17 to 19 and Tables 5 to 7). These responses are new to science but the relatedness of the stimulus and the respective response is illogical; hence, these are novel

and unexpected responses.

The colony parameters in the long-term grow and interact with each other in a complex way to generate some new and unexpected responses (Figures 17 to 19 and Tables 5 to 10). However, due to the persistent brood rearing activity, the colony simultaneously keep on utilizing the stored pollen. Thus pollen area does not increase much but nectar storage area increases significantly.

### **Effect of initial colony strength on the colony buildup**

Irrespective of the initial colony strength, general patterns of the long-term responses in the honey bee colony were same. There was about 1.5 times increase in the unsealed brood, 1.25 times in the sealed brood, 1.33 times in the pollen stores, 2.66 times in the honey stores, 3 times in the colony strength and about 3 times in the

nectar and pollen foragers in the three types of colonies; there was significant increase in each parameter ( $p < 0.05$ ,  $F_{4, 15} = 5.375$  for pollen foragers, 5.387 for nectar foragers, 5.462 for unsealed brood, 5.384 for sealed brood, 5.384 for pollen store and 6.124 for honey stores, ANOVA; Figures 15 to 18 and Tables 5 to 7).

However, the initial colony strength and colony stores had direct effects on the colony buildup and resources collection. A five frame colony (with the initial resources presented in Table 2) could collect  $203.25 \pm 9.2$  cm<sup>2</sup> of pollen,  $3234.5 \pm 62.2$  cm<sup>2</sup> of honey, and the final colony strength reached  $14.5 \pm 0.8$  frames. The corresponding figures for a 7-frames and a 9-frames colony were  $383.5 \pm 18.9$  and  $533.5 \pm 18.9$  cm<sup>2</sup> pollen,  $4823.25 \pm 118.2$  and  $6451.25 \pm 126.7$  cm<sup>2</sup> honey and  $21.5 \pm 1.2$  and  $30.0 \pm 1.8$  frame strength, respectively; the differences in the respective parameters were significant ( $p < 0.05$ ;  $F_{2, 12} = 6.235$  for pollen stores, 5.821 for honey stores and 5.917 for colony strength, respectively) (Figures 17 to 19 and Tables 5 to 7). In terms of honey weight, a 9-frames colony stored maximal honey (8.06 kg) followed by a 7-frames (6.03 kg) and a 5-frames colony (4.04 kg). Therefore, a strong colony is expected to be the better pollinator and honey gatherer than a weak colony.

## DISCUSSION

A honey bee colony is a social entity exhibiting a high degree of division of labor (Michener, 1974). In the colony, the food is collected by the foragers and stored by the hive bees. The latter keep a watch on the food stores of the colony and exchange of food among the hive bees is a common practice in the colony (Free, 1957). Any change in the colony condition is sensed by the hive bees, which pass this information to the foragers. The latter immediately respond to the changed condition in the colony and adjust their foraging behavior. In other words, the needs of the colony generate the foraging stimulus and the colony witnesses the resultant foraging response. However, this study was to know what patterns of responses the colony would depict in the short- and long-term to changes in its internal environment.

### Short-term responses of the honey bee colony to changes in its internal environment

Keeping all other parameters at a constant level, if one parameter of the colony is changed, then the colony shows an immediate short-term response.

### Short-term response of the honey bee colony to changes in the unsealed brood

If unsealed brood in the colony is increased, the pollen foraging increases and nectar foraging decreases (Figure

12 and Table 3). When more unsealed brood is added in the colony, more and more foragers would shift to the pollen foraging to fulfill the needs of the colony, thus the nectar foragers in the colony are proportionately reduced. Therefore, the building colonies with more unsealed brood show lesser honey storage (Sihag, 1990b). Some earlier researchers also reported that with an increase in the unsealed brood in the colony, the pollen foraging increased (Free, 1967; Cale, 1968; Al-Tikrity et al., 1972; Calderone, 1993; Dreller et al., 1999) and the nectar foraging decreased (Hoopinger and Taber, 1979).

### Short-term response of the honey bee colony to changes in the sealed brood

Contrary to the aforementioned results, increase in the sealed brood did not cause any significant change in the foraging activity of the honey bee colony (Figure 13 and Table 3). This is because, in the building colonies, addition or subtraction of sealed brood does not influence the colony need for a particular food type. Hellmich and Rothenbuhler (1986) too reported that pollen collection in the honey bee colony varied with the stage of the brood; its collection and usage were maximal at the larval stage, and when the brood became capped (sealed) pollen need in the colony decreased.

### Short-term response of the honey bee colony to changes in the colony stores

The results of this study further revealed that the addition of pollen reserve immediately discourages the pollen foraging and encourages the nectar foraging in the colony (Figure 14 and Table 3). However, the converse happens when the honey reserve is added in the colony (Figure 15 and Table 3). Earlier reports revealed that increase in pollen stores in the colony acts as the main stimulus for nectar foraging (Free, 1967; Cale, 1968; Barker, 1971; Fewell and Winston, 1992; Hoopinger and Taber, 1979). However, an increase in the honey stores of the colony stimulates its pollen foraging (Free, 1967; Cale, 1968; Barker, 1971; Fewell and Winston, 1992; Hoopinger and Taber, 1979).

From the different results on the short-term responses of this study, it became amply clear that presence of plenty of one type of food in the colony inhibits its own supply by a negative feedback and stimulates foraging for the other food type. Likewise, the scarcity of any type of food stimulates its own storage. Therefore, as suggested earlier (Cale, 1968; Antonsenko and Ermoleava, 1979), a switch on/off mechanism of a positive or negative feedback seemed to work in the honey bee colony to regulate its foraging and other activities. The colony, therefore, can be stimulated for nectar or pollen collection by simple manipulation of its unsealed brood or pollen and nectar stores and thus can be used for honey

production or pollination of crops.

### **Short-term response of the honey bee colony to changes in the colony strength**

When the colony strength was increased (keeping all other parameters at fixed levels), foraging activity of the colony (both nectar and pollen) increased significantly (Figure 16 and Table 3). The number of nectar foragers increased more than the pollen foragers (Figure 16 and Table 3). These results are in agreement with those of Barker and Jay (1974), EL-Kazafy and Al-Kahtani (2013) and Bhusal et al. (2011). The latter researchers observed that strong colonies with 20 thousand bees produced three times more honey, pollen, and brood than the weak colonies with 10 thousand bees. Hence, employing stronger colonies would be more beneficial than the weak colonies for honey production as well as pollination of crops.

### **Long-term responses of the honey bee colony to changes in its internal environment**

Majority of the short-term responses of the honey bee colony to changes in its internal environment do not sustain for long and wear off in the long-term and the colony readjusts itself in such a way that several new patterns of relationships originate under the ongoing status of the colony. That is why the honey bee colony exhibits many expected and unexpected responses; the patterns of these responses are different from those reported under the short-term responses. For example, under the persistent pollen and nectar supply, with an increase in the colony strength, there is an increase in the pollen and nectar foraging. The latter situation results in an increase in the pollen and nectar storage in the colony; consequently, there is an increase in the brood rearing activity too (Figures 17 to 19, Table 5 to 7). However, due to the persistent brood rearing activity, the stored pollen is utilized simultaneously. Thus, the pollen area does not increase significantly but nectar storage area does so. That is why the colony parameters in the long-term grow and interact with each other in complex manners to generate 'novel expected and unexpected' responses (Tables 5 to 10).

### **Importance of knowledge on the short- and long-term colony responses in the management and use of honey bees for honey production and pollination of crops**

Honey bees are the important pollinators of several cultivated crops and help increase their seed/fruit yield (Sihag 1986; Breeze et al., 2011). Due to the massive loss of wild bee pollinators (Kremen et al., 2002; Klein et

al., 2007; Potts et al., 2010) or when wild bees do not visit agricultural fields (Garibaldi et al., 2011), managed honey bee colonies are often the only source for farmers to do pollination in the crops (Goodwin et al., 2011; Rucker et al., 2012). In recent times, beekeeping has been identified as one of the essential inputs in agriculture (Sihag, 2001). For this purpose, providing the sufficient number of pollen foraging bee force to a given crop is important. Then, it becomes important to know the ways to stimulate the colonies for pollen or nectar collection. Likewise, effect of such manipulations on the pollination of a reference crop should also become known. This study provides solutions to these problems. For example, for the quick buildup of the colonies, the latter would need a stimulus for pollen collection by providing them honey stores and unsealed brood. This manipulation will also help enhancing the pollination in the reference crop(s). Likewise, the colonies engaged in the persistent buildup activity can be stimulated for nectar collection (honey production) by providing them more pollen stores or pollen supplement. But the latter practice would discourage the bees to collect pollen; thus, inflicting a pollination loss to the reference crop. Thus, the knowledge of short-term responses would be helpful in devising the management strategy for urgent stimulation of the colony for nectar or pollen foraging. However, once generated short-term response is likely to wear off in the long-term. In that situation, to sustain the ongoing response, the colony would need a fresh stimulus after each brood cycle (21 days). For example, if the colony is in the nectar collection (honey production) mode, this will need a fresh stimulus after 21 days to stay in this mode. Similarly, a colony will need a fresh stimulus after 21 days to stay in the pollination mode. Therefore, insights into the short and long-term responses of the honey bee colony would be helpful in devising their management strategies for honey production and pollination of crops.

The colony strength plays an important role in honey production and pollination of crops (Figures 17 to 19 and Tables 5 to 7). When plenty of bee forage is available, colonies with larger bee strengths attained significantly higher strengths and collected significantly more pollen and nectar (honey) than those colonies with smaller bee strengths. The stronger colonies, therefore, seemed to bring more pollination (as these collected more pollen) and gathered more honey too. Some previous researchers also highlighted the importance of strong colonies in honey production and pollination of crops (Bhusal et al., 2011; EL-Kazafy and Al-Kahtani, 2013). Therefore, this study recommends keeping strong colonies for honey production and pollination of crops.

### **Conclusion**

If the internal parameters of the honey bee (*Apis mellifera*) colony are altered, the colony immediately changes its foraging activity according to its emergent

need, and a negative or positive feedback mechanism works to regulate the needed parameter. For example, if there is an emergent need for pollen, the colony shifts in favor of pollen foraging till the colony need is fully overcome. Likewise, if there is an oversupply of pollen, the status of the latter parameter discourages the colony for pollen foraging. However, in the long-term colony adjusts its foraging activity according to the ongoing status of the colony and its instinctive hoarding behavior. Even if the pollen or nectar is in plenty, the colony continues to forage for pollen and nectar, and the mechanism of negative or positive feedback does not work here. The short-term response wears off in the long-term (after some time) and the colony needs a fresh stimulus to stay in a particular foraging mode.

## **CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

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