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Performance of larval grafted queen vs queen produced through natural method in *Apis mellifera*

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Abstract

Trials were conducted to determine the efficacy of larval grafted queen in comparison with queen produced naturally in order to mensurate the performance of these two methods with parameters brood area (BA), mite infestation (MI), honey harvest (HH), absconding tendency (AT), and swarming tendency (ST) The results showed that larval grafting method of queen production (T2) had significant ($p>0.05$) results over natural method of queen production T2). The average brood area was 1026.7 sq. inch and 1561.7 sq. inch in natural method (T1) and in larval grafting method (T2) showed the highly significant difference. The average AT observed 0.00 and 38.66 in (T2) and (T1) again showed highly significant difference between the two methods. Similarly the average MI and ST in both types was 14.88 (T2), 64.00 (T1) and 16.00 (T2), 53.88 (T1) respectively depicting a significant difference. For HH the average values have been found 16.00 kg for (T2) is significantly higher than 8.00 kg for (T1).

Keywords: *Apis mellifera*, Artificial larval grafting, Bee queen, Comparison, Natural method

1. Introduction

The queen bee is an essential working part of the honeybee hive. She must ensure that the hive stays populated by controlling the gender of the eggs laid in order to keep balance in the hive. However, if the beekeeper is not satisfied with the way the queen bee is controlling the hive, the beekeeper can replace the queen bee to correct hive behavior and optimize honey production as well as egg production Donald E. Triesel, (2014) ^[1]. Queens are raised by worker bees in specially constructed queen cells (H. H. Laidlaw, 1997) ^[2]. Worker bees build up the queen cup once the queen has laid an egg in queen cups (Hardison, M. 1994) ^[3]. A well mated and well fed queen can lay about 2000 egg/day during the spring build up (Root and Root, 1980) ^[4]. Queen lays fertilized (female) or unfertilized (male) egg according to the width of cell (Mattila and seeley, 2007) ^[5]. The young queen larva develops differently because it is more heavily fed royal jelly, a protein rich secretion from gland on the young workers. If not heavily fed the larva becomes regular worker bee (Gensen, 2000) ^[6]. Swarm cell hang from the bottom of frame while emergency queens are generally raised in cells build out from the face of frames. When larva pupates with her head down, the workers cap the queen cell with bee wax and ready to emerge the virgin queen. In natural conditions, during swarming season, the old queen leave the nest with the prime swarm before the emergence of first virgin queen from a queen cell (Laidlaw *et al.* 1997) ^[7].

Techniques of rearing queen have been developed to allow the beekeepers to produce good stock and to replace old and undesirable queens in their colonies (Joseph Latshaw, 2011) ^[8]. Artificial rearing of queen allows the researcher and breeders to select stock economically and to explore honeybee behavior and genetics (Hamdan, K. 2010) ^[9]. It enable us to select the specific queens with desired characters such as high honey production, high brood viability, early spring build up, good temperament, clearing behavior, incidence of disease, swarming and color. The supplemental feeding rate for good quality queen production is one Kg sugar every 4-7 days (Jonestone, 2008) ^[10].

Grafting is the simple process of transferring larva from the worker cell of breeders hive to an artificial queen cell. The shape of cell along with queen-less condition of the hive receiving the newly grafting cell stimulated the workers which make them develop in to the queens. Mating yard consists primarily of queen mating nukes and hives which raise drones. Mating yard allow dozen of queens to mate and begin egg laying .Each queen mating nuclei colony required atleast 10 drones mother colonies in order to obtain best results (Richard *et al.* 2007) ^[11]. In natural conditions honeybee queens mate in flight with numerous drones from diverse

genetic resources. In natural mated queen, it is impossible to know the colonies of drone which mated with queen (Coby, 2007) [12]. The queen rearing colonies should be given sugar and water in equal parts by volume.

The study was therefore conducted to evaluate the comparative study of quality queen production through larval grafting and queen production through natural method.

2. Materials and Methods

The study was conducted to determine the comparative study of queen production by natural method and by artificial method and to mensurate the efficacy of these two methods with parameters brood area (BA), mite infestation (MI), honey harvest (HH), absconding tendency (AT), and swarming tendency (ST). at research farm of Beekeeping & Hill Fruit Pests Research Station, Rawalpindi during 2011- 2013 from February to April with available optimum temperature for larval grafting (18-27 °C with relative humidity level 60-70% and ideal weather conditions i.e. cool sunny days. The treatments applied were T1=Queen Production through natural method and T2=Queen Production through larval graft.

Six bee colonies having equal bee strength with queens produced through larval grafting (T2) and six bee colonies having equal bee strength with queen produced through natural method (T1) have been selected for this study. The (T1) were placed as such so that queen produced naturally, the data of queen produced by natural method has been calculated on the basis of brood area in sq. inch measured with the help of wire grid, honey yield in kg, mite infestation percentage, swarming and absconding tendency percentage. The material required for larval grafting method T2 is, grafting needle, wax cups, hive frame specially made to adjust queen cell blocks, Queen cages, hive frame specially made to adjust queen cell blocks with queen cages, good trait of colonies used as breeder colonies, colonies having young brood and bees used as raiser colonies, mini nukes for rearing young queen, mating yard stand to adjust mini nukes for queen mating were utilized as materials for conductance of the protocol. Six strong colonies were taken having 7-10 frames i.e. three were used as cell raiser colonies and three for breeder colonies (having good traits). For grafting purpose young 24 hours old larvae with the help of grafting needle were placed in wax cups (size 9-10 mm) fitted on wooden block, which ultimately fixed on box fixed in standard bee hive frames, these frames then placed in 24 hour old colonies without queens. Additional food was given to these colonies in the sugar water ratio 1:1 until the grafted queen emerged. The grafting cell after seven days were examined, if the walls of cells raised by bees, the cells were accepted. When cells were closed, each cell was mini caged with 5-6 young bees and placed again in the box. Emerged queens were counted. Mini nukes were prepared for the release of caged queen with some more bees. These mini nukes boxes consist of two compartments, one for food (3.50 Kg powdered sugar+1.50 Kg honey mixed in a farm of dough) and with small comb foundation frame where new queen released along with one big spoon (about 200) worker bees. These bees were taken from swarm box. Swarm box was selected from ordinary colonies having young worker bees. Mini nukes were carried to dark and cold room and shocked through hammering the box.

Mating stands were made and mounted mini nukes on them in

a mating yard and opened entrance hole located at the bottom of mini nukes for free movement of bees and queen. Finally the performance of T2 method was evaluated on the basis of same parameters as mentioned in T1 i.e. the brood area in sq. inch, mite infestation, swarming and absconding tendency percentage and honey harvest in kilogram respectively.

2.1 Statistical Analysis

The data was subjected to statistical analysis (Steel *et al.*, 1997) [13]. The recorded data was examined for variance by STATISTIX version 8.1(RCBD, Two way Factorial) and LSD at 5% level of confidence used to relate the means of treatments and also study the correlation, used to measure the relationship between two or more variables.

3. Results and Discussions

The result showed that larval grafting method of queen production has significant ($p>0.05$) results overt natural method of queen production. The average brood area is 1026.7 sq. inch and 1561.7 sq. inch in T2 and T1 (Table 1) showed the highly significant difference. The average AT were observed 0.00 and 38.66 in T1 and T2 again showed highly significant difference of both methods. Similarly the average MI and ST in both types is 14.88 (T2), 16.00 (T2) and 64.00 (T1), 53.88 (T1) respectively again determine the highly significant difference. For HH the average values has been found 16.00 kg for (T2) is significantly higher than 8.00 kg for (T1).

Analysis of variance for 2-way factorial (Table. 2) for brood area indicated that ($F>700.96$, $P>F=0.0014$) was significantly different for larval grafting method and natural method of queen production. Absconding tendency (AT) were found ($F=46056.00$, $P>F=0.0208$) significantly different for larval and natural queen production method. Mite infestation effects (MI) and swarming tendency (ST) were found to be ($F=37.52$ $P>F=0.025$) and ($F=34.56$, $P>F=0.0277$), significantly different for both methods. Finally for honey production the values showed highly significant difference ($F=96.00$, $P>F=0.0000$) for larval grafting method and natural method of queen production. LSD for each parameter showed the result that all two means are significantly different from one another. The correlation study (Table 3) of different parameters of queen production by natural method (T1) and by artificial method (T2) showed that there is simple correlation between these two methods and an increase in one variable leads to the decrease in other variable mean negative correlation, while increase of one variable tend to increase other variable is positive correlation Donald E. Trisel (2014), furthermore correlation coefficient determine that there is a strong correlation between these two method. The results showed that BA is highly positively significantly differently correlated as increase of brood area in T1 lead to an increase of brood area in T2 as calculated value is greater than tabulated value at 0.05 and 0.01 level of significance. Similarly the ST and AT is highly positively significantly differently correlated, and BA and HH is negatively significantly differently correlated. The results were also in accordance to that of (A. Dollittle *et al.* 2004) [14], concluded that queen bees produced by grafting method were found to be superior as compare to queen bees produced by using natural queen cells.

Table 1: Comparative effect of different parameters on production of queen

S. No	Parameters	T1 (queen produce through natural method)	T2 (queen produced artificially)
1	AT(absconding tendency)	38.66 A	0.00 B
2	BA (brood area)	1026.7 A	1561.7 B
3	HH (honey harvest)	8.00 A	16.00 B
4	MI (mite infestation)	64.00 A	14.88 B
5	ST (swarming tendency)	53.88 A	16.00 B

Table 2: Analysis of variance (anova), for different parameters of queen production

S.N.	Source	DF	SS	MS	F	P
RCBD, 2 way Factorial for AOV for AT						
1	Rep	2	96.33	48.17		
2	T	1	2242.67	2242.67	46056	0.0208
	Error	2	96.33	48.17		
4	Total	5	2435.33			
RCBD, 2 way Factorial, AOV for BA						
1	Rep	2	15358	7679		
2	T	1	429338	429338	700.96	0.0014
3	Error	2	1225	612		
4	Total	5	445921			
RCBD, 2 way Factorial, AOV for HH						
1	Rep	2	4.00000	2.00000		
2	T	1	96.0000	96.0000	4.8	0.0000
3	Error	2	3.996	1.998		
4	Total	5	100.000			
RCBD, 2 way Factorial, AOV for MI						
1	Rep	2	112.37	56.18		
2	T	1	3618.18	3618.18	37.52	0.0256
3	Error	2	192.85	96.42		
4	Total	5	3923.40			
RCBD, 2 way Factorial, AOV for ST						
1	Rep	2	134.62	67.31		
2	T	1	2153.10	2153.10	34.56	0.0277
3	Error	2	124.62	62.31		
4	Total	5	2412.34			

[Brood area (BA), mite infestation (MI), honey harvest (HH), absconding tendency (AT), swarming tendency (ST)]

Table 3: Correlation study of different parameters of queen production

	AT	BA	HH	MI
BA	-0.916**			
HH	-0.940**	0.984		
MI	0.844**	-0.965	-0.942	
ST	0.996**	-0.905	-0.905	0.819**

4. Conclusion

Queen production through artificial larval graft method had significant effects on honey harvest, and brood development, with minimal mite infestation and swarming tendency, and no absconding tendency, than queen produce through natural method, therefore artificial larval graft method is recommended for beekeepers for obtaining maximum beneficial results

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