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Evaluation of larval grafted queen and natural reared queen of Italian honey bees (*Apis mellifera* L.)

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Abstract
The efficacy of larval grafted queen was determined in comparison to queen reared naturally in *Apis mellifera* L. colonies. The results showed that larval grafted queens were significantly superior (p>0.05) over naturally produced queen. The maximum brood area (9525.50 cm^2), pollen area (358.40 cm^2), honey store (2.70 kg), cleanliness (3.55 g), queen weight (189.80 mg) and egg laying (1835.00 eggs per day) were recorded in larval grafting method (T1) and also showed highly significant difference over natural reared queen. The minimum brood area (6445.30 cm^2), pollen area (210.20 cm^2), honey store (1.85 kg), cleanliness (1.00 g) and egg laying (1250.00 per day) was recorded in emergency impulse reared queen (T4) Significant (p>0.05) differences were observed among all the treatments.

Keywords: Artificial larval grafting, swarming queen, supersedure queen, emergency queen, queen weight

Introduction
Queen rearing is a highly specialized process and is an essential component of progressive beekeeping. Different practices of queen rearing are followed by beekeepers which are based on bee biology that the nurse bees in a queenless hive can produce some queens from young female larvae. Queen bee is an essential working part of the honey bee colony and she must ensure that the hive stays populated by controlling the gender of the eggs laid in order to keep balance in the hive. To optimize production of honey, egg and bees' behaviour, replacement of queen is essential. Queens are raised by worker bees in specially constructed queen cells (Laidlaw and Page, 1997). A well mated and well fed queen can lay about 2000 egg/day during the spring build up (Root and Root, 1980). Queen lays fertilized (female) or unfertilized (male) egg according to the width of cell (Mattila and Seeley, 2007). The young queen larva develops differently because it is heavily fed with royal jelly, a protein rich secretion from gland on the nurse bees. If not heavily fed the larva becomes regular worker bee (Jensen, 2000). 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 from which virgin queen emerged subsequently. In natural conditions, during swarming season, the old queen leaves the nest with the prime swarm before the emergence of first virgin queen (Laidlaw and Page, 1997). Many techniques of rearing queen bees have been developed to allow beekeepers to reproduce good stock to replace old or undesirable queens in their colonies, or to start new colonies. Successful queen rearing demands suitable conditions. The quality of the queen bee determines the benefits received from bee colony as through the queen, via its progeny, the productivity, temperament and behavior of the colony can be manipulated by the beekeeper. Various environmental factors affect the quality of the queen and rate of queen development (Mahbobi, A., Farshineh, A.M., Woyke, J. and Abbasi, S. (2012).

Materials and Methods
The comparative study of queen reared under artificial method and natural method were evaluated on the basis of brood area, pollen area, honey store, cleanliness, queen weight and eggs laid per day at University Apiary, RPCAU, Pusa, Samastipur (Bihar) during two consecutive years 2014-15 and 2015-16. Twenty bee colonies having nearly equal bee strength were selected for the experiment. The experiment comprised of four treatments having five replications in RBD. The treatments were:
T1 (Larval grafting reared queen)
T2 (Swarming reared queen)
T3 (Supersedure reared queen)
T4 (Emergency reared queen)

T1 (Larval grafting reared queen): For grafting purpose young 24 hours old larvae were placed with the help of grafting needle in primed wax cups (size 9 mm) fitted on mass queen rearing frames, these frames then placed in 24 hour old colonies made queenless. The material required for larval grafting method was grafting needle, wax cups, mass queen rearing frames, queen cages, breeder colonies, colonies having young brood and bees used as raiser colonies were utilized as materials for the experiment. The queen cells were transplanted at 10th day to study the newly emerged queen bee performance in 5 frame queenless colonies.

T2 (Swarming reared queen): Under the swarming impulse, several queen cells are constructed at the sides of the comb (in a top-bar hive) or at the base of the comb (in a frame hive). The queen cells formed under swarming impulse were transplanted for study their performance in 5 frame queenless colonies.

T3 (Supersedure reared queen): It is nature's method of disposing of an old, impotent or ineffective queen in bee colonies. Failure of the queen bee to distribute pheromones and lay the necessary number of eggs may lead worker bees to supersede (replace) her. For this, they build one, two or three queen cells, called supersedure or replacement cells at intervals of a few days. The queen lays an egg in each queen cell. After the first queen emerges, the remaining queen cells are destroyed. The queen cells formed under supersedure impulse were transplanted to study the newly emerged queen bee performance in 5 frame queenless colonies.

T4 (Emergency reared queen): When the queen dies or is killed, the workers reconstruct several worker cells into queen cells, normally on comb areas containing brood, around larvae younger than three days. The larvae are fed with royal jelly throughout the whole larval period. The queen cells constructed under emergency impulse were transplanted to study the newly emerged queen bee performance in 5 frame queenless colonies.

Observations: Six observations on brood area, pollen area, honey store, cleanliness and egg laying capacity were recorded at 14-days interval in *Apis mellifera* L. colonies during the period from November to February during 2014-15 and 2015-16.

Results and Discussions

Maximum brood area (pooled mean of both years of experimentation) was observed in the colonies provided with larval grafted reared queens being 9525.50 cm^2 followed by swarming reared queens (8360.20 cm^2), supersedure impulse reared queens (7745.70 cm^2) and lowest (6445.30 cm^2) being in case of emergency impulse reared queens (table-1). The maximum pollen area was recorded in the bee colonies provided with larval grafted reared queens being 358.40 cm^2 followed by swarming reared queens (285.60 cm^2), supersedure impulse reared queens (259.30 cm^2) and lowest (210.20 cm^2) in case of emergency impulse reared queens (table-1). Maximum pollen store was recorded in the bee colonies provided with larval grafted reared queens (358.30 cm^2) followed by swarming reared queens (285.60 cm^2) and minimum (210.20 cm^2) in emergency impulse reared queens (table-2).

Table 1: Comparative effect on brood area (cm^2) of queen reared under different methods in *Apis mellifera* colonies

Queen Source	2014-15*	2015-16*	Pooled Mean
T1 (Larval Grafting)	9420.50	9630. 30	9525.50
T2 (Swarming)	8300.10	8420.30	8360.20
T3 (Supersedure)	7650.60	7840.80	7745.70
T3 (Emergency)	6350.20	6540.40	6445.30
SEm (±)	77.32	78.41	75.13
CD (P=0.05)	224.22	227.38	217.87

* Data are mean of six observations at 14-days interval

Table 2: Comparative effect on pollen area (cm^2) of queen reared under different methods in *Apis mellifera* colonies

Queen Source	2014-15*	2015-16*	Pooled Mean
T1 (Larval Grafting)	352.50	364.30	358.40
T2 (Swarming)	278.80	292.40	285.60
T3 (Supersedure)	258.20	260.40	259.30
T3 (Emergency)	205.10	215.30	210.20
SEm (±)	14.23	15.12	13.82
CD (P=0.05)	41.26	43.84	40.07

* Data are mean of six observations at 14-days interval

Table 3: Comparative effect on honey store (kg) of queen reared under different methods in *Apis mellifera* colonies

Queen Source	2014-15*	2015-16*	Pooled Mean
T1 (Larval Grafting)	2.60	2.80	2.70
T2 (Swarming)	2.30	2.50	2.40
T3 (Supersedure)	2.10	2.20	2.15
T3 (Emergency)	1.80	1.90	1.85
SEm (±)	0.18	0.19	0.17
CD (P=0.05)	0.52	0.55	0.49

* Data are mean of six observations at 14-days interval

Table 4: Comparative effect on cleanliness (g) in hive of queen reared under different methods in *Apis mellifera*

Queen Source	2014-15*	2015-16*	Pooled Mean
T1 (Larval Grafting)	3.70	3.40	3.55
T2 (Swarming)	2.10	1.90	2.00
T3 (Supersedure)	1.70	1.60	1.65
T3 (Emergency)	1.10	0.90	1.00
SEm (±)	0.21	0.18	0.20
CD (P=0.05)	0.60	0.52	0.58

* Data are mean of six observations at 14-days interval

Table 5: Comparative effect on weight of queen (mg) reared under different methods in *Apis mellifera* colonies

Queen Source	2014-15*	2015-16*	Pooled Mean
T1 (Larval Grafting)	187.40	192.20	189.80
T2 (Swarming)	162.00	173.20	167.60
T3 (Supersedure)	154.00	156.60	155.30
T3 (Emergency)	144.80	148.50	146.65
SEm (±)	0.83	0.91	0.82
CD (P=0.05)	2.43	2.61	2.38

* Data are mean of six observations at 14-days interval

Table 6: Comparative effect on egg laying capacity of queen (per day) reared under different methods in *Apis mellifera* colonies

Queen Source	2014-15*	2015-16*	Pooled Mean
T1 (Larval Grafting)	1800.00	1870.00	1835.00
T2 (Swarming)	1640.00	1710.00	1675.00
T3 (Supersedure)	1420.00	1540.00	1480.00
T3 (Emergency)	1210.00	1290.00	1250.00
SEm (±)	23.82	24.43	22.11
CD (P=0.05)	69.07	70.84	64.12

* Data are mean of six observations at 14-days interval

Maximum honey store was recorded in the bee colonies provided with larval grafted reared queens (2.70 kg) followed by swarming reared queens (2.40 kg) and minimum (1.85 kg) in emergency impulse reared queens (table-3). The data recorded on cleanliness on the basis of debris collected on bottom board showed that the bee colonies provided with larval grafted reared queens were more prompt in cleaning the colonies and recorded heavier quantity of debris being 3.55 g followed by swarming reared queens (2.00 g), supersedure impulse reared queens (1.65 g) and lowest (1.00 g) in case of emergency impulse reared queens (table-4). The heaviest queens with maximum weight was observed in the colonies provided with larval grafted reared queens being 189.80 mg followed by swarming reared queens (167.60 mg) and lightest (146.65 mg) queen weight in case of emergency impulse reared queens (table-5). The maximum number of eggs laid per day by queens was observed in the colonies provided with larval grafted reared queens being 1835.00 eggs/day followed by swarming reared queens (1675.00 eggs/day) and lowest (1250.00 eggs/day) in case of emergency impulse reared queens (table-6). The results showed that bee colonies differed significantly under different treatments and almost similar trends were found in both the years of experiments in all the parameters studied.

The results were in conformity with findings of earlier workers who reported that queen bees produced by grafting method were superior in performance as compare to queen bees produced by using natural queen cells (Doolittle, 1915; Abrol, D.P., Bhagat, R.M. and Sharma, D. 2005; Abbasi, K.H., Shafiq, M., Ahmad, K.J., Razzaq, A., Saleem, M. and Ullah, M.A. 2015). Dodologlu and Emsen (2007) and Cengiz, M., Emsen, B., and Dodologlu, A., (2009) also found 93.33% and 95.00% larval acceptance rate for queenright and queenless colonies, respectively. Suryanarayanan M.C., Rao, G.M. and Rao, K.S. (1998) also reported that the quality of queens under the emergency impulse was poor, because the colony is queenless and disorganized. Kumar and Singh (2004) and Coby (2007) also found that the more number of queen cells were formed in queenless impulse but the performances of queens were better in swarming and supersedure impulse. They further reported that in *A. mellifera* colonies heavier queens were reared under swarming and supersedure impulse (185.67 mg) with egg laying capacity of 1475.33 eggs/day. The present finding indicated that queen produced through artificial larval grafting method had significant effects on honey harvest, brood development, pollen stores, cleanliness and egg laying capacity over queen reared under natural impulse, therefore, artificial reared queens should used in colonies for obtaining maximum economic return and profit.

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