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Short Communication

Effects of Rearing Interlude and Grafting Technique on Honeybee *Apis mellifera* L. Queen under Field Conditions

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ABSTRACT

The proposed research work was conducted at Honeybee Research Institute of National Agricultural Research Centre, Islamabad on *Apis mellifera* Lingustica honeybee colonies during the spring months of March-April 2017. The effects of four larval grafting techniques, addition of royal jelly (A), dry grafting (B), grafting with addition of one drop of distilled water (C) Royal jelly plus distilled water (D) on queen bee rearing were investigated under field conditions. A considerable variation in queen rearing success was observed as calculated by percentage of secluded queen cells vs. grafted larvae over the study interlude. The highest larval acceptance 80.85 ± 0.64 and 81.25 ± 2.05 was observed by addition of royal jelly in March and April and lowest 42.05 ± 0.69 and 48.21 ± 2.02. The shortest pre oviposition period by addition of royal jelly in March and highest was by addition of distilled water droplet during March and April. The length of queen cell was highest in royal jelly added treatment followed by dry grafting, royal jelly plus distilled water and distilled water respectively. The queen emergence weight was slightly higher in the royal jelly added grafting than other treatments. The queen spermatheca diameter, emergence rate and mating rate were not found to be affected by the treatments and rearing interludes. Additional research is suggested and needed for clear recommendation.

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Authors' Contribution
MKR was the main investigator, he designed the study, collected data and wrote manuscript. RM compiled and analyzed the data. ZAQ and IB helped in all research activities. FAS provided guidelines in collecting data.

Key words
Honey bee (*Apis mellifera*), Queen bee rearing, Characteristics of queen, Grafting method, Royal jelly.

An Italian strain of honeybee *Apis mellifera* Lingustica as package bee was imported from Australia in 1977-78 and successfully established in Honeybee Research Institute, National Agricultural Research Centre, Islamabad (Muzaffar, 1982). As a result, beekeeping has emerged as a profitable business in Pakistan. It is reported that over 4,000 beekeepers in Pakistan are rearing about 400,000 colonies of A. mellifera which are annually producing 10,000 metric tons honey from which 27,000 families are being benefited from this activity (PARC, 2010-11).

The vital factors affecting quality of queen are rearing period and grafting methods. Honeybee Queen is recommended to be raised when the weather is slightly warm, drones are plentiful, nectar and pollen are rich in terms of quality and productivity and mating rate are at the optimum levels. (Kaftanoglu and Kumova, 1992; Genc, 1997). Some commercial queen producers prime cups with a small droplet of water or diluted royal jelly prior to grafting larvae (Laidlaw, 1985).

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A regular replacement of queens in bee colonies is a prerequisite for a successful beekeeping venture. A prevalent opinion is that a queen should not remain in the colony for more than two years. It means that every year the number of queens to be reared is equal to half of the number of colonies in the apiary. Quality queens are reared in the specialized apiaries that do the rearing on large scale and during the whole season. Rearing efficiency is to a large extent dependent upon ambient conditions but it is largely influenced by the choice and the preparation of the nurse colony and by rearing conditions. It is necessary to start with a nurse colony that is healthy and strong and has brood of different age (Skowronek and Skubida, 1988).

Cell cups in which larvae are grafted can be made of plastic but bee wax cups perform better (Chang, 1977). The diameter of 9mm is regarded as optimal (Skowronek and Skubida, 1988). A moot point is to introduce empty cups in advance to precondition the colony. According to some authors the precondition positively influences the acceptance of grafted larvae (Delaplane and Harbo, 1988).

When grafted in cell cups the larvae normally are not placed on the bare bottom but on diverse substrates that can serve as the food for the larvae or they just maintain

the right humidity. A small drop of royal jelly or of royal jelly diluted in plain water are best substrate (Skowronek and Skubida, 1988).

The present research work was designed to investigate the characteristics of queen bee reared in Islamabad area in different interludes of spring to determine the effects of rearing techniques used to attain quality prolific honeybee queens for higher honey yield.

Materials and methods

The proposed research work was conducted in the apiary of PARC Agrotech Company and Honeybee Research Institute during 2017 at National Agricultural Research Centre Islamabad Pakistan. Three queen breeding colonies were selected prior grafting on the basis of hygienic behavior, mite infestation, and honey production. It can be concluded that screen bottom board trays alone and with soft chemicals in all three seasons effectively control the *Varroa* mite and can be used without any side effect during all the three seasons (Rashid *et al*., 2017). Three mother colonies and twenty experimental colonies were selected from 110 strong colonies. Doolittle grafting method was used to rear queen bee in March-April. Four grafting techniques including grafting with addition of some royal jelly (A), dry grafting (B) grafting with addition of distilled water (C) and Diluted royal jelly with distilled water (D) were adopted. A total of 180 larvae of 24 h age were used to raise queen bees by grafting 30 larvae for each frame. Their age was estimated by size. The larvae were grafted from comb cells to bees wax queen cups 9mm in diameter mounted on frame bar using a match stick. The grafted frames were inserted into queen less nurse colony. The nurse colony contained at least four brood combs mostly at capped stage. The nurse colonies were supplied every week with two combs of brood of different age but mostly of capped brood. In a single nurse colony the rearing was done 1 to 2 replications. The numbers of replications depended on the acceptance of successive grafts.

After 2-3 days following grafting, the colonies were inspected for larval acceptance. On 10thday of grafting, the height of queen cells were measured and placed in nursery cages having some candy (1:4 honey: Icing sugar) and nurse bees that were put in the cell building colony until queens emerged out. Shortly after emergence queens were weighed via digital weighing balance, and pre-oviposition period in field condition in mating nucs. The queens were marked, tagged and placed in mating nuclei for 48 h and then permitted to be mated naturally in Honeybee Research Institute queen mating yard after fixing the mating nuclei on iron stand. The mating rate were recorded after 07 to 10 days by observing eggs in nuclei and spermatheca diameters of queen were calculated by dissecting the queen under 4x stereomicroscope.

The data were analyzed using multiple regression analysis. Student's t-test (Johnston, 2018) was used to test for a significant difference between means. One way ANOVA was used by using the software XLSTAT 2008 (Mudassar *et al*., 2011) to compare variables the grafting methods rearing efficiency against the different rearing interludes.

Results and discussion

Maximum rate of larval acceptance 80.85 ± 0.64and 81.25 ± 2.05 was recorded during the months of March and April when royal jelly was used as substrate prior to larval grafting followed by Diluted Royal Jelly, dry grafting and distilled water droplet where acceptance rate was 78.31±1.02 and 73.05 ± 0.69, 77.21 ± 2.02 and 71.05 ± 0.67, 42.05 ± 0.69 and 48.21 ± 2.02, respectively (Table I). The highest larvae acceptance rate with addition of royal jelly could be explained by higher nectar flow at this

Table I.- Queen bee characteristics as influenced by grafting method and month.

Grafting method × Rearing months interaction	Acceptance rate (%)	Sealed queen cells	Sealed cell length (mm)	Mating rate (%)	Queen size (mm)	Spermatheca diameter (mm)	Queen emergence weight (mg)	Pre-oviposition period (days)
A× 1	80.85±0.64	24.21±0.13	18.35±1.20	73.9±1.98	16 ± 0.99	0.0983 ± 0.02	163.75±2.05	11.85 ± 0.92
A× 2	81.25±2.05	24.37±0.6	17.55±1.15	71.95±2.05	16 ± 0.28	0.955 ± 0.10	159.7±1.98	12.75 ± 2.05
B× 1	78.31±1.02	23.49±0.2	16.05±1.10	69.24±1.14	15.2 ± 0.38	0.852 ± 1.4	153.4±1.25	13.15 ± 1.15
B× 2	73.05±0.69	22.27±0.23	17.18±1.70	64.9±1.38	15.10±0.98	0.796 ± 0.03	160.17±1.24	12.95 ± 0.72
C× 1	77.21±2.02	21.49±0.47	15.05±1.10	62.04±1.12	15.4 ± 0.58	0.885 ± 1.7	155.2±1.14	12.15 ± 1.16
C× 2	71.05±0.67	21.27±0.54	16.18±1.70	61.9±1.28	15.3 ± 0.75	0.746 ± 0.47	152.14±1.34	13.15 ± 0.78
D× 1	42.05±0.69	14.27±0.23	15.18±1.70	51.9±1.38	14.10±0.98	0.712 ± 0.03	149.18±1.21	12.95 ± 0.72
D× 2	48.21±2.02	13.43±0.47	15.05±1.10	48.04±1.12	13.4 ± 0.58	0.811 ± 1.7	148.20±1.11	12.15 ± 1.16

A, royal jelly; B, diluted royal jelly; C, dry grafting; D, distilled water; 1, March; 2, April.

period of time in the region. The results are in lined with then findings of Gul and Kaftanoglu (1990) who reported acceptance rate of queen bees (64.8%) grafted with addition of royal jelly and (55.2%) dry grafting but these results were lower than our findings. However, the higher acceptance rate is lower than the results with addition of royal jelly obtained by Kaftanoglu and Kumova (1992).

The highest sealed queen cells were obtained when royal jelly was used in grafting during the month of March and April followed by Diluted Royal Jelly. The highest queen cells were obtained when royal jelly was used in grafting in the months of March and April but there was no significantly different results were observed between royal jelly and other treatments except in distilled water substrate where less than fifty percent sealed queen cells were recorded as compared to grafted larvae which may be due to less appealing substrate for bees (Table I). The result of queen cells was similar to the findings of Dodologlu and Genc (1997) and Emsen (2001). On the other hand, another research showed that the results of queen cell were found lower in royal jelly addition method in the month of June. The reason for lower queen cell in July with addition of royal jelly could be explained by lower swarming in that month.

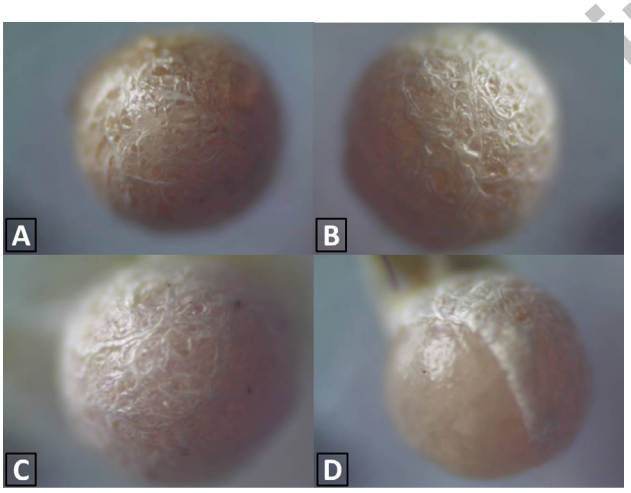

Fig. 1. Spermatheca of dissected queens reared via various grafting Methods under stereomicroscope at 4x. A, royal jelly; B, diluted royal jell; C, dry grafting; D, distilled water droplet.

The longest pre-oviposition period was obtained in March. The shortest period was observed by addition of royal jelly in July and by the other two methods in June and July. Gul and Kaftanoglu (1990) also reported similar pre-oviposition period (10.36 days) by addition of royal jelly. Grafting methods and rearing interludes had no effect on number of spermatheca diameter (Fig. 1) and queen emergence weight, which indicate characteristics of queen bees. The reason of this result could be sufficient nursing bee numbers, suitable climate and higher nectar flow in the months of March and April. It is concluded that better quality queen can be obtained by rearing with the addition of royal jelly as substrate into queen cell cups just before grafting in the month of March and April. However, more studies are needed before making definite recommendations.

Another characteristic to be analyzed was queen rearing efficiency verses the various grafting methods used. Based on the study interlude observations, it was found the larval acceptance of grafted larvae varied only slightly in royal jelly, diluted royal, and dry grafting but acceptance was at lower stage in distilled water addition treatment. As in the previous studies (Fell and Morse, 1984) the difference was not significant. In this study, the rearing efficiency was assessed as the number of capped queen cells vs. grafted larvae. An important issue to be resolved was the assessment of losses over successive rearing interludes via various grafting techniques. Rearing efficiency was recorded maximum in royal jelly and minimum in distilled water added grafting methods (Fig. 2).

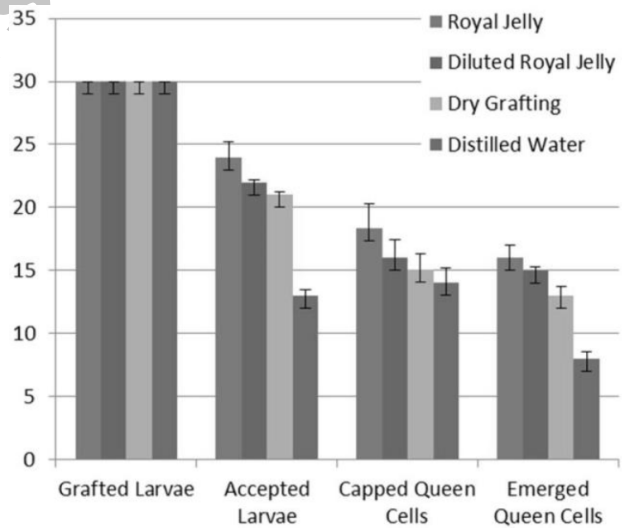

Fig. 2. Queen rearing efficiency as affected by various grafting methods (Mean ± SE).

Conclusion

It can be concluded that addition of royal jelly in queen cups prior to larval grafting during the month of March is effective for maximum larval acceptance during the queen rearing process.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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