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Curing Methods of Laying Workers in New Honeybee Divisions

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ABSTRACT

Laying workers is one of the most important problems facing the beekeepers all over the world. The aim of this work is to evaluate some curing methods of the laying workers include introducing, new mated queens (less than 4 weeks old), new virgin queens (72 hr. old), ripe sealed queen cell (48 hr. before emergency) and unsealed brood frame in queenless colonies. Data revealed that after the first, the second and the third week there is a significant difference between all treatment and the positive control. Whereas after the fourth week there is no significant differences between the first treatment, (colonies treated with introduction mated queens) and the positive control (without any laying worker egg cells.) which indicated these colonies cured from laying worker eggs, followed significantly by the virgin queens, ripe sealed queen cells and unsealed brood treatments with the means 443, 512, 564 and 558.7 laying workers egg cells/ colony, respectively. There is no significant differences between the colonies treated with introduction of unsealed brood and the negative control. Also, the means of the drone sealed brood cell\ colony after two and four weeks were discussed

Keywords: Honey bee, laying workers, curing methods, introducing queen.

1. Introduction

Honeybees are considered one of the most important economic insects, as they are the wings of agriculture. Without honeybees, pollination of crops decreases rates of up to 100% in some crops. Honeybees are responsible for 30% of the global production of crops, and perhaps more than that.

It has indispensable products such as honey, royal jelly, and bee venom, also it collects many materials of high value such as pollen and propolis.

When a honeybee colony loses its queen, workers activate their ovaries and begin to lay eggs, this is accompanied by a shift in their pheromone bouquet, which becomes more queen like (Muerrle, 2008) (After queen loss, workers can lay eggs, but are unable to mate). They produce haploid male offspring (drones) from unfertilized eggs via arrhenotokous parthenogenesis. In a queenright colony worker-laid eggs are normally eaten by other workers, Rojek *et al.,* (2019). Thapa *et al.,* (2016) suggests that a laying worker may lay from one to four eggs per oviposition.

The appearance of laying workers in the colonies is considered one of the problems facing the beekeepers either specially after colonies loss the queen normally by death or accidentally by disease or predators attack, also introducing a queen into dequeened colonies with laying workers is very difficult (Khodairy, 1990). The presence of laying workers decreased significantly the number of either accepted new queens introduced into queenless colonies (DeGrandi and Martin, 1993) or queen larvae (queen cells) in queen rearing colonies (Woyciechowski and Radwan, 1988).

Also, in preparing swarm boxes, when the beekeepers failed to introduce the queens into the nuclei until the two days the workers with activate ovaries appears, (Elbassiouny, 2005). Little researches were conducted to find a solution to the problem of laying workers.

For this reason, this research was conducted to be an applied research to treat the problem of laying workers. The present study was aimed to evaluate curing the laying workers in new honey bee

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divisions by introducing, new mated queens, new virgin queens, ripe sealed queen cell and unsealed brood frame.

2. Materials and Methods

The present experiments were carried out in an apiary in Sohag Governorate during spring, 2022 to study the following aims:-

Eighteen new honey bee divisions, of *Apis mellifera* L., Carniolian hybrid, with the same strength, 5 frames in the hive were used for this study. The tested divisions were classified into six sections, 3 divisions each. The first, the second and the third section were treated by introducing new mated queens (less than 4 weeks old), new virgin queens (72 hr. old) and a ripe sealed queen cell (48 hr. before emergency) respectively, under have-ball cage with a hole filled with candy in the frame, but the fourth section of divisions were treated by introducing unsealed brood frame, while the fifth groups were the positive control (colonies with mated queen) and the six group were the negative control (colonies queenless) without any treating.

Before the beginning of treatments, the mated queens and the unsealed brood combs were removed from all the tested divisions except the positive control divisions and after the appearance of the first eggs of the laying worker in a week the treatments started, then the Hexagonal cell that contain the eggs of the laying worker (more than one egg in the cell or on the walls of it) and the drone sealed brood cells are counted weekly for a month, this method modified from the method used by Thapa, (2016).

Statically analysis

Data obtained were statically analysis using one-way analysis of variance (ANOVA). The differences between treatments and materials were subjected by Duncan Multiple Range Test (Snedecor, 1956).

3. Results

3.1. Effect of curing methods in the mean number of laying workers eggs cells / colony

Data illustrated in Figures (1), (2), (3), (4) and (5) represented the mean number of laying workers eggs cells\ colony after introduction of mated queen, virgin queen, ripe sealed queen cell and unsealed brood to colonies by 1,2,3 and 4 weeks, respectively.

Data obtained in Fig. (1) showed that After one week of treatments, the highest mean number of laying workers eggs cells \ colony was recorded in queenless colonies (negative control) with 563.0 cells.

For the curing treatments, the mated queen introducing method record the lowest mean number of laying workers eggs cells \ colony with 338.7 cells \ colony followed significantly by unsealed brood frame (428.0 cells \ colony), virgin queen (465.3 cells \ colony) and ripe sealed queen cell (538.7 cells \ colony).

As expected, no laying workers eggs cells were observed in normal hive (positive control).

The results illustrated in Fig. (2) showed that after the second week of treatments introduced the mated queens to colonies recorded the lowest mean number in the cells of the laying workers eggs with the mean 186.7 cells/ colony followed significantly by the introducing of virgin queens, unsealed brood comb and ripe sealed queen cells with the means 494.3, 523.7 and 527 cells/ colony respectively.

The highest mean number of laying workers eggs cells was recorded in the negative control with the mean 606.7 cells/ colony. Whereas, there is no laying workers eggs cells in the positive control.

Data illustrated in Fig. (3) showed that the introduction of the mated queens to colonies recorded the highest reduction in the cells of the laying workers eggs after the 3rd week of introduced with the mean 49.3 cells/ colony followed significantly by the introducing of virgin queens with the mean 458.7 cells/ colony followed significantly by the introducing of ripe sealed queen cells with the mean 532 cells/ colony followed significantly by the introducing of unsealed brood with the mean 536 cells/ colony followed significantly by the negative control with the mean 605.3 cells/ colony, whereas, the positive control without any laying workers eggs cells.

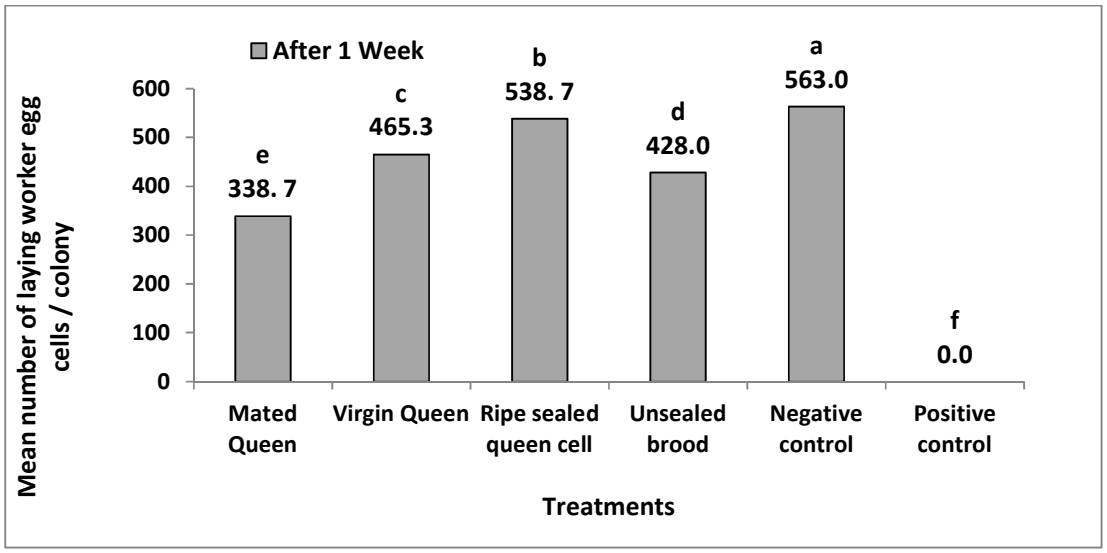

Fig. 1: Mean number of laying workers eggs cells / colony after introduction of mated queen, virgin queen, ripe sealed queen cell and unsealed brood by one week. F value= 866.10. Bars marked by different letters are significantly different (Duncan's Multiple test, $P \leq 0.05$)

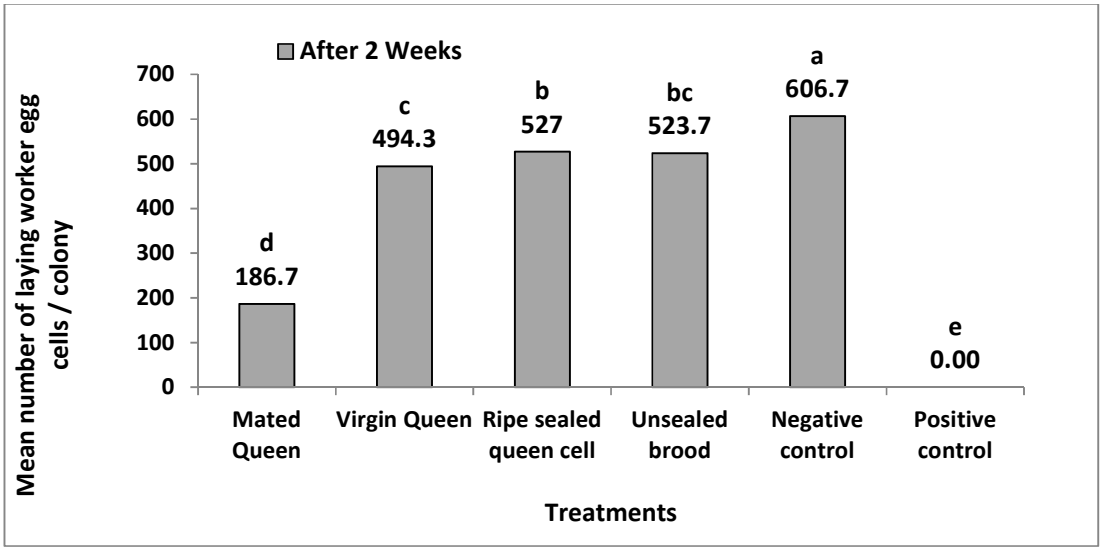

Fig. 2: Mean number of laying workers eggs cells / colony after introduction of mated queen, virgin queen, ripe sealed queen cell and unsealed brood by two weeks . F value= 601.79. Bars marked by different letters are significantly different (Duncan's Multiple test, $P \leq 0.05$)

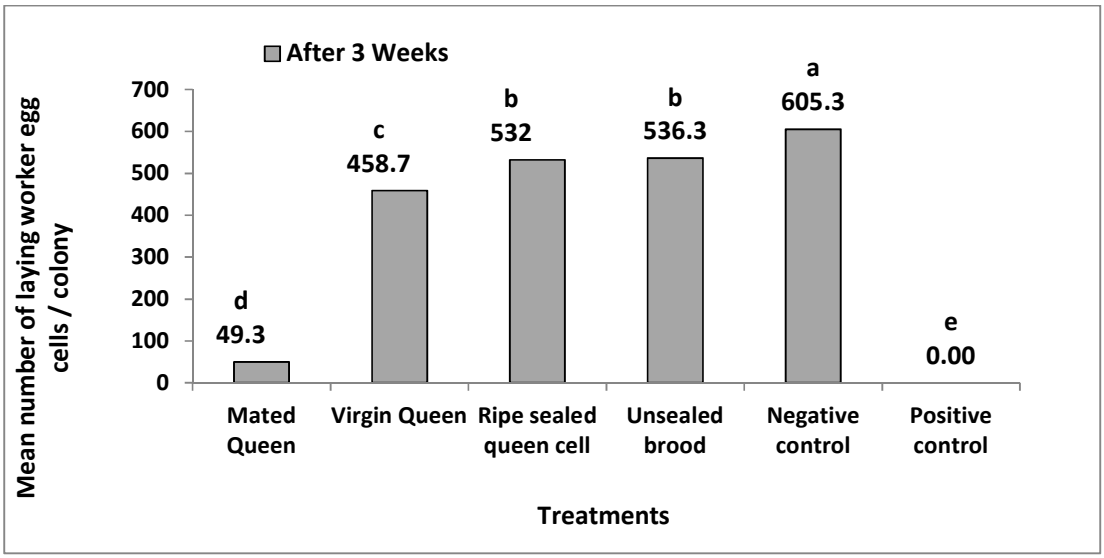

Fig. 3: Mean number of laying workers eggs cells / colony after introduction of mated queen, virgin queen, rip sealed queen cell and unsealed brood by three weeks . F value= 666.49. Bars marked by different letters are significantly different (Duncan's Multiple test, $P \leq 0.05$).

Data arranged in Fig. (4) showed that after 4 weeks there is no significant differences between the colonies treated with introduction mated queens and the positive control (without any laying workers' eggs cells.) which indicated these colonies cured from laying worker eggs.

On the other hand, the introducing of virgin queens, ripe sealed queen cells and unsealed brood varied significantly with means 443, 512, 564 cells\ colony, respectively. Whereas, there is no significant differences between the colonies treated with introduction unsealed brood and the negative control with the means, 564 and 558.7 cells\colony respectively.

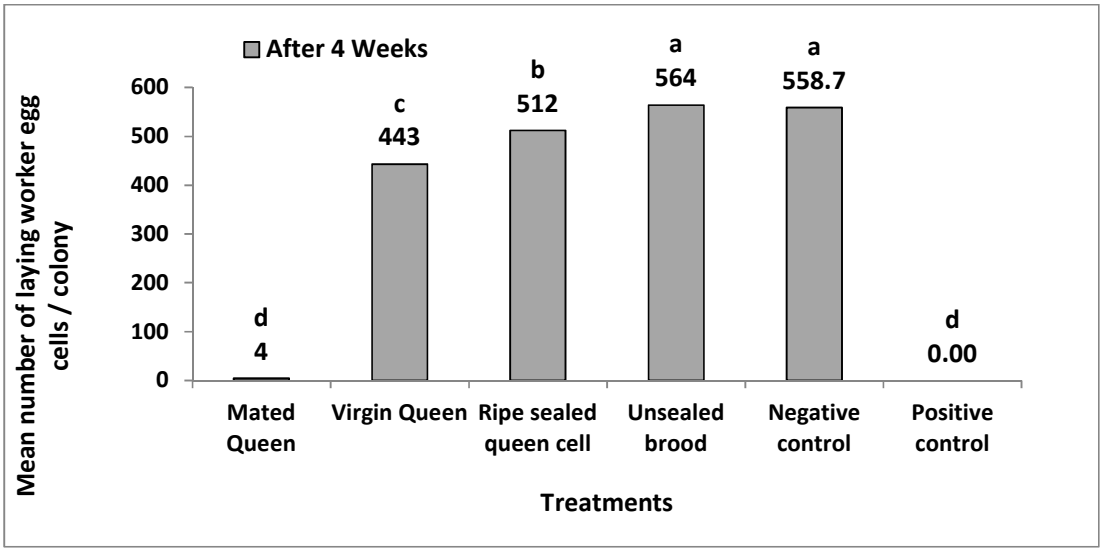

Fig. 4: Mean number of laying workers eggs cells / colony after introduction of mated queen, virgin queen, ripe sealed queen cell and unsealed brood by four weeks. F value= 2375.16. Bars marked by different letters are significantly different (Duncan's Multiple test, $P \leq 0.05$)

Data obtained in fig. (5) showed that during all weeks of investigation the mated queens treatment recorded the lowest means numbers of the laying workers eggs cells with mean 338.7 cells/ colony followed significantly by the virgin queens, unsealed brood and ripe sealed queen cells with means 465.3, 428 and 538.7 cells / colony respectively.

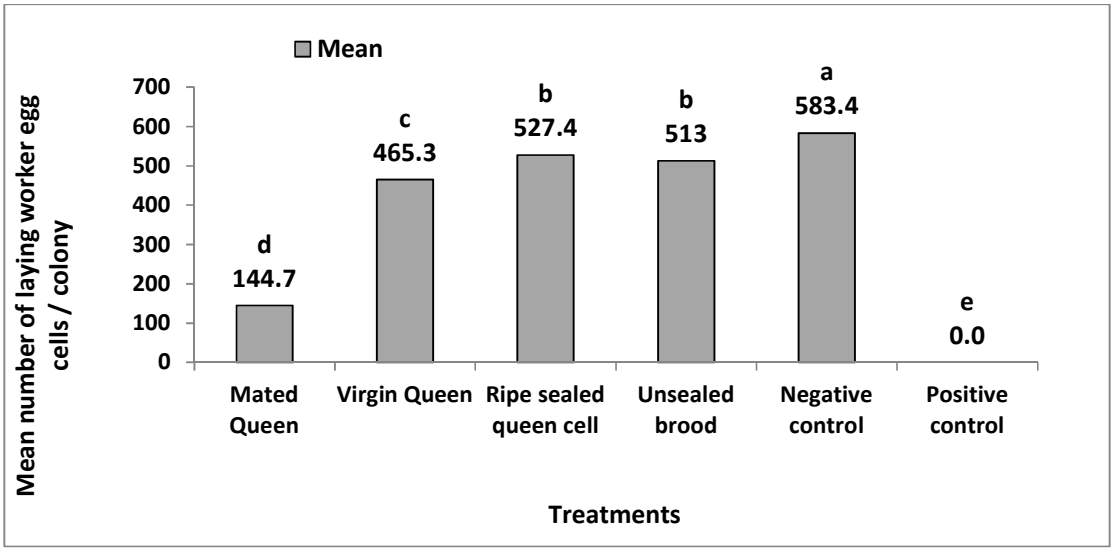

Fig. 5: Mean number of laying workers eggs cells / colony after introduction of mated queen, virgin queen, ripe sealed queen cell and unsealed brood in all weeks. F value= 2168.07. Bars marked by different letters are significantly different (Duncan's Multiple test, $P \leq 0.05$)

3.2. Effect of curing methods in the mean number of drones sealed brood cells \ colony

Data illustrated in Fig. (6) showed that after two weeks of the introduction of the mated queens, virgin queens, ripe sealed queen cells and unsealed brood no significant difference in the mean numbers of drone sealed brood cell \ colony between all treatments and the negative control with means, 187.7, 195.7, 193.3, 183.7 and 183.7 drone sealed brood cell \ colony, respectively. While there is a significant difference between it and positive control (57.67) drone sealed brood cell \ colony.

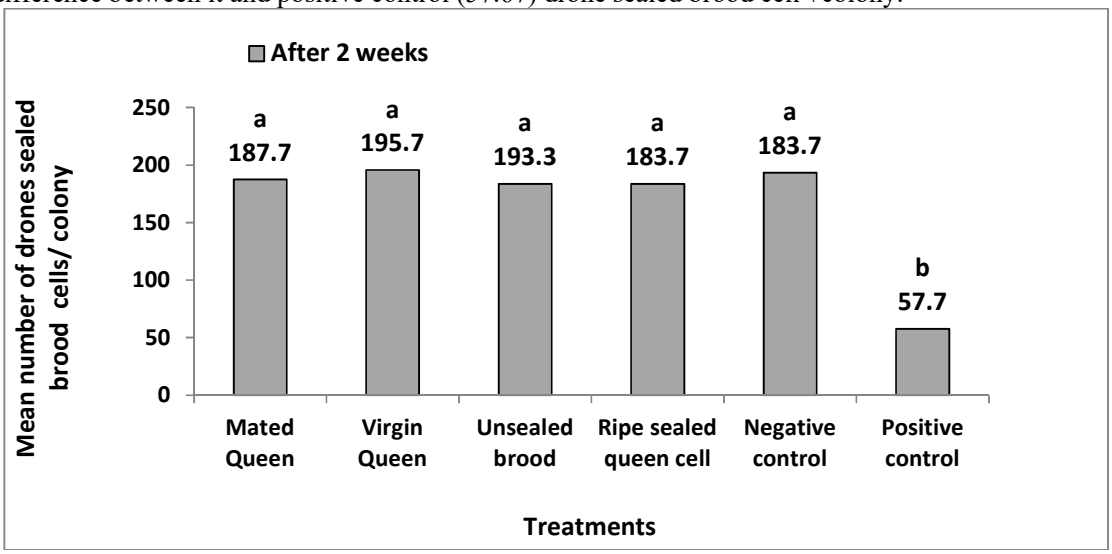

Fig. 6: Mean number of drones sealed brood cells / colony after introduction of mated queen, virgin queen, ripe sealed queen cell and unsealed brood by two weeks. F value= 87.73. Bars marked by different letters are significantly different (Duncan's Multiple test, $P \leq 0.05$).

Data illustrated in Fig. (7) showed that after four weeks of treatment, it was found that the number of the drones sealed brood cells in the first treatment (the introduction of a mated queen) decreased significantly, as there were no significant differences between them and the positive control treatment with averages, 58.7 and 61.7 drones sealed brood cells\ colony respectively, followed significantly by unsealed brood and virgin queen with the means, 443 and 512 drones sealed brood cells\ colony respectively. Finally, there are no significant differences between ripe sealed queen cells and negative control with the means, 564 and 549.7 drones sealed brood cells \ colony, respectively.

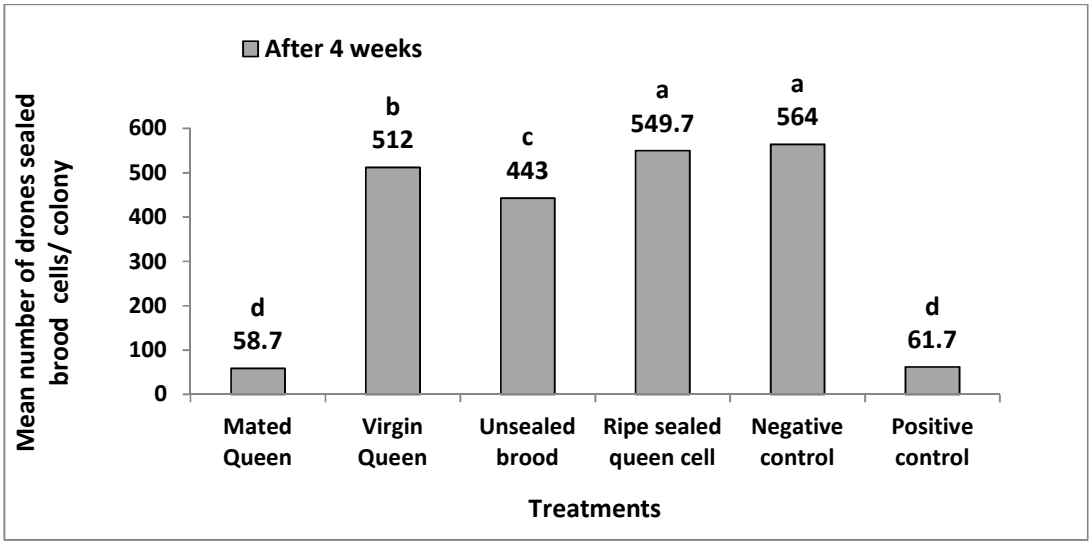

Fig. 7: Mean number of drones sealed brood cells / colony after introduction of mated queen, virgin queen, ripe sealed queen cell and unsealed brood by four weeks. F value= 2059.94. Bars marked by different letters are significantly different (Duncan's Multiple test, $P \leq 0.05$).

The results in Fig. 8 showed that there were significant differences between all treatments, where the highest number of drones sealed brood cells / colony was in negative control treatment, followed significantly by ripe sealed queen cell, virgin queen, unsealed brood, mated queen and positive control with means, 378.7, 366.7, 353.8, 313.3, 123.2 and 59.7 drones sealed brood cell / colony respectively.

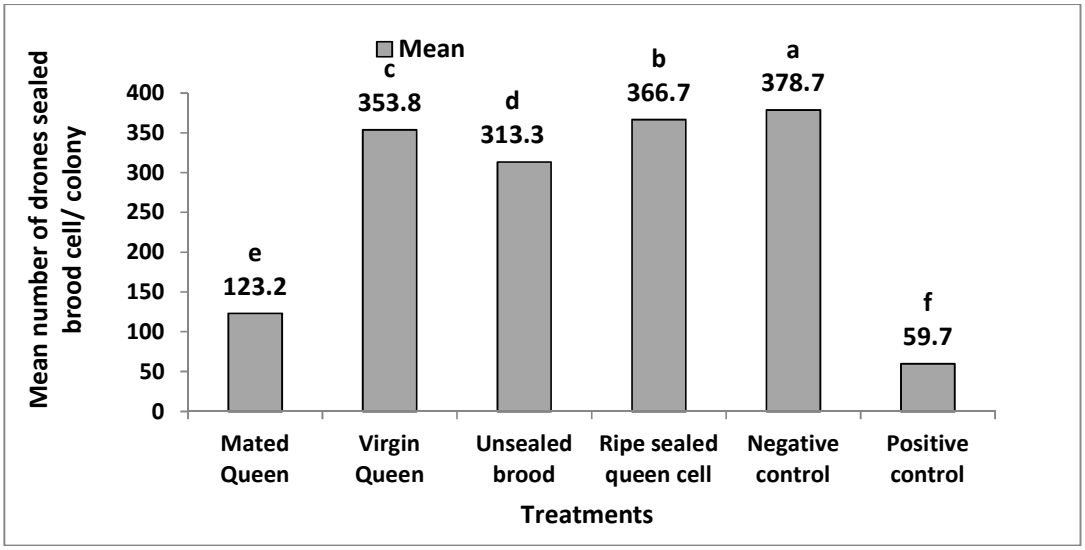

Fig. 8: Mean number of drones sealed brood cells / colony after introduction of mated queen, virgin queen, ripe sealed queen cell and unsealed brood in all weeks . F value= 2410.68. Bars marked by different letters are significantly different (Duncan's Multiple test, $P \leq 0.05$).

4. Discussion

Laying workers is a series problem facing the beekeepers. Our results demonstrate that introducing new mated queens is effective in cure the laying workers in the honeybee colonies. Whereas, introducing new virgin queens, ripe sealed queen cell and unsealed brood frame were ineffective.

These results are in agreement with Butler and Fairey (1963) who decided that older mated queens are more effective than younger virgin ones and with De Groot and Voogd (1954), Butler and Fairey, (1963), Velthuis (1970) who reported that ovary development of honeybee worker is inhibited by mated adult queen. A mixtures of pheromones produced by the queen inhibit development of worker ovaries and with Jay, (1968) who reported that a continuous supply colonies with queen larvae or pupae does not inhibit the ovary development of many worker bees of a colony and with Milojevic *et al.,* (1963) who found that the sealed queen cells (i.e. pupae) had a little inhibitory effect on the ovary development of workers in small colonies of 500 bees.

Whereas it is a partial agreement with those of El-Enany (2006) who stated that laying worker colonies could be successfully cured by introducing mated or virgin queens, unsealed, and sealed brood to these colonies. The colonies cures with virgin queens recorded the lowest mean period of curing than the colonies with mated queens. Introducing mated or virgin queens with unsealed or unsealed and sealed brood to the laying worker colonies may play an important role for curing laying workers in these colonies and with (Visscher, 1996 and Katzav *et al.,* 2003) who decided that in normal colonies, when a queen or it's worker brood is present, workers stopped laying eggs by mutual policing behavior in which any eggs laid by worker are rapidly destroyed or removed by other workers and with Jay (1972) who described that possibly a volatile brood scent was acting to inhibit ovary development of the worker. Whereas, these results are disagreeing with Kropacova and Haslbachova, (1971) who concluded that the unsealed brood had a stronger inhibiting effect than the presence of the queen.

5. Conclusion

The best method to cure the laying workers in the honeybee colonies is introducing new mated queens under have-ball cage with a hole filled with candy in the comb,(This is supported by the words of God in the Holy Quran, truth has come, and falsehood has perished verily, falsehood is bound to perish).

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