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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Evaluation of Methods for the Acceptance of the Artificial Inseminated Queen Bee to the Colony

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Abstract: When artificial insemination practices in honey bees are used correctly, they actively increase yield characteristics. However, producers may experience serious problems when accepting artificially inseminated queen bees to the colonies. To minimise this problem, inseminated queen bees are first accepted into small mating boxes. Small colonies are formed, supported, and developed after admission to the new colony. In addition to spending serious effort and time, this process causes maimed queen bees and even colony losses if they fail. The aim of this study is to reveal the relationship between the method and the problems encountered in the acceptance of the artificially inseminated queen bee into the colony and to present an appropriate acceptance method. In the study, 21 queen bees were used, 7 of which were queen bees in each group. Seven queen bees were naturally mated. After 7 queens were artificially inseminated, they were first given to small mating colonies using the classical method. Queen bees that accepted and laid eggs were introduced to colonies with 4-5 laths of worker bees. 7 colonies were given as queen bee thimbles 2 days before hatching. Queens that had hatched were inseminated and given to the same colonies. Whether the queen bees given to the colonies in 3 different groups were accepted into the colonies was evaluated after 10 days. The egg-laying rates of the queens admitted to the colony were checked after the egg appeared. In the controls, it was seen that the acceptance of the queen bees kept individually in their own colony was less laborious and more successful than the classical method.

Keywords: Artificial insemination, honey bee colony, queen bee.

Suni Tohumlanan Kraliçe Arının Koloniye Kabulü İçin Yöntemlerin Değerlendirilmesi

Öz: Bal arılarında suni tohumlama uygulamaları doğru kullanıldığında verim özelliklerinin arttırılmasında etkin rol oynamaktadır. Ancak suni tohumlanan kraliçe arıların kolonilere kabullendirilmesi sırasında üreticiler ciddi sorun yaşayabilmektedirler. Bu sorunu minimize etmek amacıyla tohumlanan kraliçe arılar önce küçük çiftleşme kutularına kabullendirildikten sonra küçük koloniler oluşturularak yeni koloniye kabulün ardından desteklenerek geliştirilmektedirler. Bu süreçte ciddi emek ve zaman harcamanın yanında başarısız olmaları durumunda sakatlanan kraliçe arılara hatta koloni kayıplarına sebep olmaktadır. Bu çalışmanın amacı suni tohumlanan kraliçe arının koloniye kabullendirilmesinde görülen sorunların metotla ilişkisini ortaya koymak ve uygun kabullendirme metodu sunmaktır. Çalışmada her grupta 7 adet kraliçe arı olmak üzere 21 adet kraliçe arı kullanıldı. Yedi kraliçe arı doğal çiftleştirildi. Yedi kraliçe suni tohumlandıktan sonra klasik yöntemle önce küçük çiftleşme kolonilerine verildi. Kabul edilen ve yumurtlayan kraliçe arılar 4-5 çıta işçi arı mevcudiyetine sahip kolonilere kabul ettirildi. Yedi koloni ise kraliçe arısı alınarak oluşturulan kolonilere çıkımdan 2 gün önce kraliçe arı yüksüğü olarak verildi. Çıkımı gerçekleşen kraliçeler tohumlanarak aynı kolonilere verildi. Üç farklı gruptaki kolonilere verilen kraliçe arıların kolonilere kabul edilip edilmediği 10 gün sonra kontrol edilerek değerlendirildi. Koloniye kabul edilen kraliçelerin yumurtlama oranları yumurta görülükten sonra kontrol edildi. Yapılan kontrollerde kendi kolonisinde bireysel olarak tutulan kraliçe arıların kabulünün klasik yönteme göre daha az zahmetli ve daha başarılı olduğu görüldü.

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Anahtar kelimeler: Suni tohumlama bal arısı kolonisi, kraliçe arı.

INTRODUCTION

Studies on the preservation and maintenance of desired genetic characteristics in honey bee colonies by artificial insemination of queen bees are gradually developing (Collins, 2000). It is difficult to determine the genetic transfer of drones to queen bees because drones have haploid chromosomes in honey bees, and queens mate with more than one drone (Seltzer et al., 2023). Due to the inability to control mating in the desired direction in naturally mating honey bees, the chance of success in selective breeding and genetic protection is limited (Musin et al., 2023). It has been reported that queens of honey bees have genetic immunity against various bee diseases (Lang et al., 2022). In addition to the timing of narcosis used during artificial insemination in honey bees, many factors, such as the age of the queen bees used in artificial insemination, are significant (Gillard & Oldroyd, 2020). Because the queen bee mates with more than one drone while in flight, obtaining the genetically desired yield characteristics becomes difficult. However, it is wrong to think that using artificial insemination in honey bees is sufficient. Artificial insemination can be effective when applied as part of a genetic program (Maucourt et al., 2023).

It is thought that there is no effect of CO_2 or other gasses to increase egg-laying efficiency in queen honey bees, but only due to a lack of oxygen (Gąbka, 2023). It has been observed that colony nutrition has a significant effect on the quality of honey bee queens (Dolasevic et al., 2020). It has been reported that artificial inseminated queen bees are affected by conditions such as rearing conditions, insemination age, dose of semen used in insemination, applications such as CO2 applied to queen bees before and after artificial insemination, pheromone development of the queen bee, and environmental conditions (Buescu et al., 2015).

Artificial insemination in honey bees is a reliable method for mating control. It is appropriate to inseminate between 5 and 12 days after the queen bee emerges. During this period, queen bees kept in closed special cages can be kept in small core colonies without a queen or on queen bee benches. However, when kept in this manner, the legs and tarsal joints of the queen bees can be damaged due to the behavior of the worker bees. In addition, there are various problems in the acceptance of queen bees to the colony (Cobey et al., 2013). With the queen bee bank application in honey bees, the queen bees are kept in cages one by one and placed in a colony to be looked after by the worker bees (Webb et al., 2023). Thanks to queen bee banks, large numbers of queen bees can be kept in a colony in individual cages until the time of insemination. It is less troublesome but does not provide optimum conditions for the queen bee (Cobey, 2007). It has been observed that when queen bees are kept in special cages with worker bees, they have much more spermatozoa than queens kept in cages without worker bees (Gabka & Cobey, 2018). The effect of pheromones on the acceptance of queen bees to the colony is significant. Pheromones and queen survival have a correct relationship (Cobey, 2007).

In this study, different admission methods of artificially inseminated queen bees to the colony were evaluated.

MATERIAL AND METHOD

Experimental plan: The study used 7 natural mating (control) and 14 artificially inseminated queen bees (Trial). Queen bees in the Trial-1 group were kept with the queen bee bank system until insemination from hatching (Webb et al., 2023). Queen bees kept in the queen bee bank for 5-7 days, were inseminated. 7 of the queen bees (Trial-1) in the experimental group were previously accepted to the small nucleus colony using the classical method (Alkattea, 2008). Then they were given to the nuclei colonies with 4 frame populations without queen bees. The other 7 queen bees (Trial- 2) were kept in the colony with 4 frame worker bees until insemination (5-7 days) and then inseminated and given to the same colony. To be accepted by inseminated colonies, queen bees were given by opening the cake part with certain worker bees. Colonies were observed after 10 days, and the acceptance status of the queens was noted. To evaluate the egg-laying rates of queen bees, the surviving queen bees in all groups were checked 10 days after they started to lay, and the number of eggs per 100 cells per unit area was counted.

Queen bee bank: The queen bees used in the study were produced using the Doolittle method (Wakjira et al., 2019). Queen bee cells in Trial 1 groups were caged two days before the queen bees hatch. Queens kept in cages can be injured by workers in queen banks (Cobey et al., 2013). To prevent this situation, the emerging queen bees were transferred to special wooden transport boxes and kept in the queen bank until the day of insemination.

Artificial insemination: Queens kept in a queen bee bank (Trial 1) or distributed to queenless colonies (Trial 2) were inseminated within 5 to 7 days (Bieńkowska et al., 2008). The inseminated queens (Figure 1) were induced to laying by the application of CO_2 one day later. Subsequently, they were taken to trial colonies, and their laying process, brood pattern and survival were checked.

The acceptance of queen bees to colonies: Care was taken to ensure that the conditions in the colonies without queen bees used in the study included equal conditions for each colony. In addition, care was taken not

to have open brood combs in the colonies. Queen bees in the control group (C) were distributed to colonies without queen bees two days before hatching, with two thimbles per colony. The queens emerging from the thimbles were not interfered with during the natural mating process. Queen bees in Trial 1 (T-1) group were first taken to small core colonies after insemination, and the egg-laying process was observed (Figure 2). Egg-laying queens were distributed to non-queen colonies with 4-5 frame worker bees (Figure 3). Queens in the Trial 2 (T-2) group were inseminated within 5 to 7 days after they emerged in their colonies and were given directly to the same colonies. Queen bees in Trial 2 roamed freely in the colony until the day of insemination. A queen bee grid was placed at the entrance of the colony to prevent it from flying. After the queen bees were given to the colonies for each group, they were left alone for ten days to prevent adverse effects of stress. At the end of the process, the egg lays status and survival rates of queen bees in all groups were evaluated.

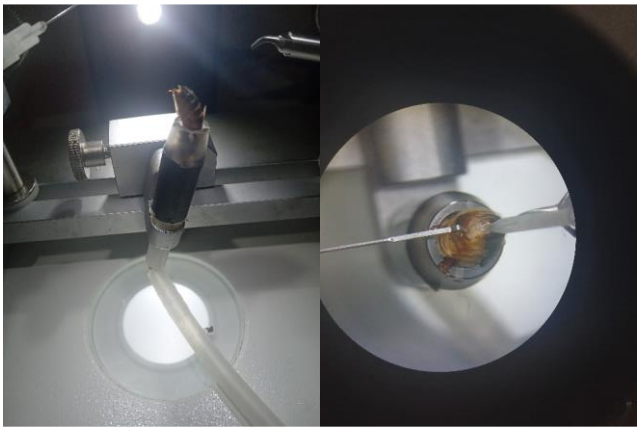

Figure 1. Artificial insemination.

Figure 2. Nücleus colony and queen control.

Statistical Analysis: Analysis of variance one-way Analysis of Variance (ANOVA) were performed using the SPSS 22.0 package programs (IBM., Corp., 2011). In addition, the number of animals used in the study was determined using the G power 3.1 program and the F test (ANOVA: Fixed) module.

Figure 3. The acceptance of queen bees to colonies.

RESULTS

As a result of the study, the survival rates of the queen bees in each group are given in Table 1. While no loss was observed in the queen bees in the control group, it was observed that the highest number of losses occurred in the T-1 group during the queen's admission to the colony. It was observed that only 1 of 7 queen bees in the T-2 group had a problem.

Table 1. Survival of queen bees among the grou**ps.**

Replications	Groups	(+/-)*
1.	C	+
2.	C	+
3.	C	+
4.	C	+
5.	C	+
6.	C	+
7.	C	+
1.	T-1	+
2.	T-1	+
3.	T-1	+
4.	T-1	-
5.	T-1	-
6.	T-1	+
7.	T-1	-
1.	T-2	+
2.	T-2	-
3.	T-2	+
4.	T-2	+
5.	T-2	+
6.	T-2	+
7.	T-2	+

*Survival: +, Dead:-
C: Natural mating (Control), T-1: By establishing a core colony (Trial-1),
T-2: Without formation of a core colony (Trial-2)

When the egg laying rates of the queen bees living in each group were evaluated statistically, no significant difference was found between the groups (Table 2).

Table 2. Between Groups Egg-laying Rate (%).

GROUPS	Egg-Layer Rate (%)
C	56
T-1	53
T-2	52
SEM	2.692
P	0.818

C, N=7; T-1, N=4; T-2, N=6 (N: replicate); C: Natural mating (Control),

T-1: By establishing a core colony (Trial-1), T-2: Without formation of a core colony (Trial-2)

DISCUSSION

In a study, it was stated that queen bees kept in queen bee banks can be injured by worker bees. In addition, it was predicted that there may be difficulties accepting the queens kept in the colony (Cobey et al., 2013). Because most of the wooden cages used for queen bees in our study are closed, it is thought to reduce the adverse effects on worker bees. The fact that the acceptance rate of the queen bees in the T-1 group was lower than that in the other groups in the study are consistent with the current study. It has been reported that the tarsal claws, legs, and antennae were injured or lost during the admission of queen bees to the colony. In addition, it was observed that the yield characteristics of injured bees decreased, and some of them were renewed in the colony (Gerula & Biencoswska, 2008). In our study, the causes of injury and death of queen bees were not examined. Acceptance to the colony is based. However, the study stated that some difficulties may be experienced in accepting artificial insemination queen bees to the colony compared with natural mating. In our study, although there was no loss in naturally mating queens (C), the main losses were observed in artificially inseminated colonies. In our study, to minimize these losses and troubles, the method for giving queen bees to the colony was discussed. It was observed that there was a problem in the acceptance of artificial insemination queens (T-1) imposed on a separate core colony before being introduced to the main colony. However, it was remarkable that this process developed more smoothly in queen bees (T-2) in their own colony and was inseminated and given to the same colony.

It has been emphasized that the size of the colony in which the artificially inseminated queen bees are given has a significant effect on the acceptance of the queen bee. In addition, it was mentioned in the study that although queen bee banks provide convenience in queen bee production, they do not fully provide the necessary conditions for queen bees. It has been predicted that there may be a relationship between pheromones and the survival of the queen bee. In addition, it has been reported that there is a need for a study on how the applications made during artificial insemination affect the pheromone status of the queen bee (Cobey, 2007). The fact that the colonies we used in our study have a worker density of 4-5 staves is seen as a factor that makes queen acceptance difficult. However, it is thought that the acceptance of queen bees grown in their own colony after artificial insemination is more uneventful than those transferred from the core colony, which may be related to the fact that the queen is exposed to less stress in her own colony.

It has been predicted that injuries to the legs and feet of queen bees may prevent interaction with colony worker bees, depending on the problem in the tarsal gland, a vital pheromone production site (Gerula & Biencoswska, 2008). Our study used only one side of an open wooden queen transport cage to prevent this situation. However, its effect on eliminating negativity is unknown. No injuries were observed among the accepted queens in the T-1 group. However, it has not been determined how the bees died. Damage to the pheromone secreted from the tarsal glands in the feet can be an important factor in the queens acceptance. It is thought that queen bees traveling without cages can be accepted to the colony more easily when they are inseminated. However, this situation increases time, cost, and workforce in enterprises.

CONCLUSION

The negativities experienced during the acceptance of artificially inseminated queen bees to the colony affects the success of artificial insemination. In addition to the loss of time, it can cause colony loss. The queen bees kept in queen bee banks can be injured by the worker bees, threatening the sustainability of the colony due to injury or loss of limbs. For the queen bees to be accepted into the colonies, first, the small core is given to the colony and then given to the standard colony after laying eggs. This requires a long process and effort, and some problems can be seen in the acceptance. As a result, it was seen that the free movement of the queen bee in the colony was positive before insemination. In addition, problems were observed during the introduction of queen bees, which were inseminated from core colonies to standard colonies. To prevent this situation, it is thought that the creation of smaller colonies may cause additional time and labor. Acceptance of the queen bee to the colony is one of the most important factors affecting the success of artificial insemination. Studies on this subject will contribute to the field.

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