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Impact of Queen Cup Materials and Substrates on Queen Rearing Performance

Mohammed Ahmed Alshogari[1,2], A. M. Raut[1]

[1]Department of Entomology, School of Agriculture, Lovely Professional University, Punjab, India

[2]Department of Plant Protection, Faculty of Agriculture, Food and Environment, Sana'a University, Sana'a, Yemen

Corresponding Author: **A. M. Raut**

Abstract

Problem -Queen bee rearing is an important process for beekeepers to facilitate the rapid multiplication of colonies. It enables the expansion of bee populations and the replacement of aging queens prior to the honey flow season, thereby enhancing overall honey production. **Approach** - This study aimed to evaluate the queen cup materials and substrates on the grafted larvae in autumn season. The substrates like apple juice, commercial royal jelly enriched with honey, sugar syrup, honey solution, and a mixture solution of each sole media were used in each queen cup materials. The cup materials included bee wax cups, paraffin wax cups and plastic cups. The young larvae (<24 h old) were selected from the mother colony and grafted into queen cups using a plastic grafting tool. **Findings** - The highest larval acceptance (43.33%) and queen emergence rates (26.67%) were recorded in control (dry grafting). There were no significant differences between the bee wax and plastic cup materials in the acceptance rate and queen emergence rate. As a substrate, commercial royal jelly enriched with honey @5µl in beewax cups had highest acceptance rate by worker (30%) and queen emergence rate (30%) while mixture substrate@5µl in plastic cups performed better than other substrates during Autumn season. **Conclusion –** Both beewax and plastic cups along with substrates performed better in the rearing of queen during the Autum season.

Keywords: Honey bee,*Apis mellifera*, substrates, queen cup materials, grafting method, queen rearing.

Introduction

Queen rearing is a pivotal practice in modern apiculture, playing a critical role in colony management, genetic improvement, and the sustainability of beekeeping enterprises (Abrol, 2013; Yi et al., 2021; Kamboj et al., 2023).Artificial queen rearing, particularly the grafting method, is widely used due to its efficiency and reliability in

producing high-quality queens (Ahmat et al., 2024; Zhong et al., 2024; Büchler et al.2024).The grafting method is an effective technique that enables the simultaneous rearing of many queen larvae of desired age ranges (Voinalovych et al.2022; Büchler et al.2024).However, below 24 hr age larvae desired for the queen development(Dhaliwal et al., 2017).Along with age of larvae, season, queen cup materials, substrates and flora also playing important role for succeed of queen rearing and development process(Contreras-Martinez et al., 2017; Dhaliwal et al. 2017; Rafique et al., 2019).Substrates provide a micro-environment and additional nutritional benefit that supports the larvae and facilitates their acceptance by nurse bees (Sharma, 2019).Natural substrates such as royal jelly are traditionally favored due to their high nutritional and chemical properties, which mimic natural conditions (Ustadi et al. 2022).

Conversely, synthetic or alternative substrates have gained attention as cost-effective and scalable options, although their efficacy varies depending on composition and application methods (Contreras-Martinez et al., 2017; Khan et al., 2021; Kamboj et al., 2023).Similarly, the material composition of queen cups significantly affects larval acceptance and queen development (Sharma et al., 2020; Khan et al., 2021; Lashari et al. 2022).However, traditional wax cups are widely used due to their compatibility with natural hive environments, but plastic and other synthetic materials have been introduced to reveal their effectiveness and impact on the acceptance and development of larvae (Abou-Shaara et al., 2024).

Comparative studies suggest that these materials can influence larval survival and the morphological traits of the resulting queens (Mattiello et al., 2022).Despite these advancements, there is a need for comprehensive evaluations to optimize these variables for improved queen-rearing outcomes. The present study aims to assess the impact of different substrates and queen cup materials on the success of artificial queen rearing using the grafting method in the Autumn season. By systematically evaluating these factors, this research seeks to provide insights that could enhance queen production efficiency and contribute to sustainable apiculture practices. Additionally, the findings will inform beekeepers on best practices for optimizing queen-rearing protocols under varying environmental and resource conditions. Therefore, the present study aimed to evaluate queen cup materials and various substrates on queen emergence process in Autumn season.

Material and methods

An experiment was conducted at Apiculture Area, School of Agriculture, Lovely Professional University (LPU), Punjab during autumn season. Three queen less queen cell builder colonies of *Apis mellifera* were prepared as an equal in strength

(Delaplane et al 2013). The queen cell builder colonies selected based on large numbers of nurse bees, sealed broods, pollen, and honey stores (Sharma et al., 2020). In this study, the Doolittle grafting method was used for grafting the larvae. The young larvae (<24 h old) were selected from the mother colony and grafted into queen cups using a plastic grafting tool. The polishing of queen cups was applied one day before the grafting by inserting the grafting frames into the cell builder colony (Khan et al., 2021; Büchler et al. 2024).

In the present study, six substrates were used i.e. apple juice @ 5µl (S_1), commercial royal jelly enriched with honey @ 5µl (S_2), sugar syrup @ 5µl(1:1 with distilled water) (S_3), honey solution @ 5µl(1:1 with distilled water) (S_4), mixture solution (apple juice, commercial royal jelly Enriched with Honey, sugar syrup, honey solution) (1:1:1:1) @ 5µl(S_5), and control (dry grafting without substrate) (S_6). Also used three queen cup materials i.e. bee wax, paraffin wax, and plastic cups. The bee wax and paraffin wax cups were prepared in using a silicone mold in Entomology Laboratory, LPU. The group of 60 queen cups was fixed on the 3 grafting frames at each queen cell builder colony. Observations were recorded three days after the grafting process in case of acceptance or rejection of larvae, while twelve days after recorded queen emergence rate. The acceptance and queen emergence rates were calculated according to the following formulas (Sharma et al., 2020).

$$\text{Percentage of acceptance} = \frac{\text{Number of larvae accepted}}{\text{Total number of larvae grafted}} \times 100$$

$$\text{Percentage of emergence} = \frac{\text{Number of queens emerged}}{\text{Total number of larvae grafted}} \times 100$$

The effects of the substrates and queen cup materials on the acceptance and emergence rates were performed by ANOVA using SPSS software (version 22). The significance level for all tests was set at 0.05, and Duncan's new multiple-range tests were used to rank the groups.

Result and discussion

Acceptance rate of queen larvae *A. mellifera* L.

All the substrates were performed non-significant impact on acceptance rate in bee wax queen cups ($F_{(5,45)}$ = 1.714;p>0.05) and plastic queen cups ($F_{(5,45)}$ = 3.742; p=0.006). However, none of the substrates performed well in the paraffin wax queen cups during the Autumn season (Table 1). However, the cup materials ($F_{(2,153)}$ =13.279; p=0.000) and substrates ($F_{(2,153)}$ =4.353; p=0.001)statistically significant influenced on the acceptance rate by the worker on grafted larvae. In combined effect of cup materials and substrate ($F_{(10,153)}$ =1.451; p>0.05) does not have a strong

influence beyond their individual effects during the autumn season. In present work, plastic queen cups had 31.67% accepted by the worker followed by bee wax queen cups (23.33%) (Table 1). This finding is lined with Dhaliwal et al. 2017 who observed the highest emergence rate of queens in plastic cups using the Doolittle method. This result may obtain due to unfavorable temperatures and the unavailability of food resources. Apple juice and commercial royal jelly enriched with honey as substrates accepted by the worker during the grafting process (Contreras-Martinez et al., 2017; Rafique et al., 2019; Sharma et al., 2020; Khan et al., 2021; Kamboj et al., 2023). Various type of bee wax (fresh bee wax, old bee wax and uncapping bee wax) materials used in queen cups where enhance the acceptance rate in Spring season where the ample amount of bee flora available (Lashari et al. 2022).

Table 1: Effect of queen cup materials and substrates on acceptance rate of queen larvae *A. mellifera* L. during Autumn season in2024

Cup materials (C) / Substrates (S)	**Acceptance rate (%)**			**Mean**
	Bee wax cups	Paraffin wax cups	Plastic cups	
Apple juice	20.0^{AB}	0.0	30.0^{B}	16.67^{b}
Commercial royal jelly(Enriched with Honey)	30.0^{AB}	0.0	20.0^{B}	16.67^{b}
Sugar syrup	20.0^{AB}	0.0	20.0^{B}	13.33^{b}
Honey solution	0.0^{B}	0.0	10.0^{B}	3.33^{b}
Mixture solution	20.0^{AB}	0.0	30.0^{B}	16.67^{b}
Control (dry grafting)	50.0^{A}	0.0	80.0^{A}	**43.33^{a}**
Mean	23.33^{a}	0.00^{b}	31.67^{a}	
Factors	**F_{value}**	**P_{value}**	**Df**	
Cup materials (C)	13.279	0.000**	2	
Substrates (S)	4.353	0.001**	5	
C ×S	1.451	0.163^{NS}	10	

^{A,a}Similar letter marked by common letters are not significant according to Duncan's Multiple Range Test.

Emergence rate of queen *A. mellifera* L.

All the substrates had no significant impact on successful emergence of queen in bee wax queen cups ($F_{(5,45)}$=1.202; p=0.324)and plastic queen cups($F_{(5,45)}$=1.202; p=0.324)(Table 2). Honey solution used substrates in all cups observed no emergence of queen during the Autumn season. Both bee wax and plastic queen

cups observed no significant differences in queen emergence after acceptance by the worker bees (Table 2). However, there were no influenced of cup materials and substrates ($F_{(10,153)}$=0.574; p=0.833)on queen emergence after the accepted by worker during the Autumn season (Table 2). The fresh, old and uncapping bee waxes were used for preparing the queen cups and enhanced the rate of queen emergence during the Spring season (Lashari et al. 2022). Along with bee wax queen cups, apple juice and royal jelly also impactful for successful emergence of queen after the acceptance (Contreras-Martinez et al., 2017; Rafique et al., 2019; Sharma et al., 2020; Khan et al., 2021; Kamboj et al., 2023).

Table 2: Effect of queen cup materials and substrates on queen emergence after successful grafting in Autumn season during 2024

Cup materials / Substrates	**Emergence rate (%)**			**Mean**
	Bee wax cups	Paraffin wax cups	Plastic cups	
Apple juice	20.0^{AB}	0.0	20.0^{AB}	13.33^{ab}
Commercial royal jelly(Enriched with Honey)	30.0^{AB}	0.0	20.0^{AB}	16.67^{ab}
Sugar syrup	20.0^{AB}	0.0	20.0^{AB}	13.33^{ab}
Honey solution	0.0^{B}	0.0	0.0^{B}	0.00^{b}
Mixture solution	20.0^{AB}	0.0	30.0^{AB}	16.67^{ab}
Control (dry grafting)	40.0^{A}	0.0	40.0^{A}	26.67^{a}
Mean	21.67^{a}	0.00^{b}	21.67^{a}	
Factors	**F_{value}**	**P_{value}**	**Df**	
Cup materials (C)	8.224	0.000**	2	
Substrates (S)	1.947	0.090^{NS}	5	
C ×S	0.574	0.833^{NS}	10	

^{A,a}Similar letter marked by common letters are not significant according to Duncan's Multiple Range Test.

Conclusion

The bee wax and plastic queen cups were used for artificial queen rearing along with individual or mix substrates encourage the successful emergence of queen. The paraffin wax queen cups not performed satisfactory along with individual or mix substrates. However, along with queen cups and age of larvae for grating may other factors like size of cups, season, and grafting bar level also evaluate for successful artificial rearing of queen.

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