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Evaluation of priming media and queen cup material on larval graft acceptance and queen emergence in *Apis mellifera* L.

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Abstract

The aim of this study was to evaluate the effects of seven different priming media viz., Apple Juice, Coconut Water, Royal Jelly, Diluted Royal Jelly, Honey Solution, Sugar Syrup, Distilled Water and dry grafting (Control) and three queen cup material viz., old comb wax, new capping wax and plastic, on larval graft acceptance and queen emergence during mass queen rearing and the variations between Autumn and Spring season under mid-hills of Himachal Pradesh. The larval graft acceptance as well as queen emergence (85.42% and 77.08%; 89.58% and 81.25%, respectively) during Autumn and Spring season, respectively was recorded significantly highest in royal jelly primed cups whereas lowest in non-primed cups. In queen cell cup material, the larval graft acceptance and queen emergence (62.50% and 57.03%; 67.97% and 63.28%, respectively) were observed maximum in old comb wax cups followed by new capping wax and plastic cups. No significant variations were recorded between spring and autumn season. The combined effect of cup material and priming media was also found non-significant. So, royal jelly as priming medium and old comb bees wax as artificial queen cell cup material are best for larval graft acceptance and queen emergence in *Apis mellifera* L. mass queen production in Spring and Autumn.

Keywords: Autumn, cup material, honey bee, priming media, queen rearing, spring

1. Introduction

Queen bee rearing is the most important beekeeping practice for rapid multiplication of honey bee colonies, to replace old queens every year before honey flow season to increase honey production besides introducing new queens in case of sudden loss during manipulation of colonies, transportation and due to the attack of honey bee enemies or diseases. It is good to replace the queen every year, as a young queen is more prolific and lays more number of eggs for a longer duration, and colonies headed by such queens have less swarming tendency (Gatoria *et al.*, 2004) [1]. The responses of colonies towards different queen rearing techniques due to the differences in environmental, behavioral, and biological factors have been reported by many workers (Morse, 1994 [2]; Nuru and Dereje, 1999 [3]; Nuru, 2012 [4] and Crailsheim *et al.*, 2013 [5]). Some important factors in determining the acceptance and quality of artificially reared queens were reported to be climatic conditions like temperature, relative humidity, and pollen source (Zhadanova, 1967; Koc and Karacaoglu, 2004 and Cengiz *et al.*, 2009) [6, 7, 8]. Under temperate conditions, the colony brood rearing cycle is characterized by complete cessation of brood rearing in the late fall and reduction of colony size during the winter (Avitabile, 1978) [9]. Brood rearing leading to colony expansion is initiated when nectar and pollen become available (Seeley, 1978) [10]. The highest number of queen cells is achieved by using royal jelly in July and August (Genc *et al.*, 2005) [11]. Queen bees can be reared from the end of March to September, but better quality of queens is obtained from the end of March until the end of April, (Koc and Karacaoglu, 2004) [7]. This indicated that the acceptance and emergence of queens are affected by the rearing period or season of queen production. Grafting with different approaches such as wet grafting with mainly the royal jelly as priming substrate or dry grafting was performed and reported variations in the rate of acceptance and other queen quality parameters (Ratnieks and Nowogrodzki, 1988; El-Din, 1999; Buchler *et al.*, 2013 and Kamel *et al.*, 2013) [12, 13, 14, 15]. Now days some commercial queen producers are using queen rearing kits equipped with brown coloured plastic cups. Therefore, it is imperative to know if any variations exist in the acceptance of grafted larvae in different queen cell cups material primed with different priming substrates.

Keeping all this in view, the present work was undertaken to study variations in rate of larval grafts acceptance and emergence of queens reared in different queen cell cup materials primed with different substrates for *A. mellifera* queen production between Spring and Autumn season in mid-hill conditions of Himachal Pradesh, India.

2. Materials and Methods

The present investigations were carried out during Autumn and Spring season of 2018 - 2019 in the university apiary at experimental farm of the Department of Entomology, Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. The apiary is situated at 33°51.607" N latitude, 077°09.95" E longitude and 1262 m amsl.

2.1 Selection and preparation of cell builder and breeder colonies

The preparation of cell builder colony required a great deal of nurse bees, sealed and young brood, pollen and honey stores. The selection of good colonies was carried out on the 5point-scale basis based on their biological and economical characteristics like colony strength, brood area, prolificness, honey stores, pollen stores, etc as per Sharma *et al.*, 2020 [16]. Top 4 strong colonies were selected as cell builder colony. The arrangement of the combs in these colonies was as follows:

HSEYCPESH

Where, H-Honey comb, S-Sealed brood comb, E-Emerging brood comb, Y-Young larvae brood comb, C-Grafted larvae frame, P-Pollen comb as per Gatoria *et al.*, 2000 [17]. The grafting was done in queen less cell builder colonies by using the Doolittle (larval grafting) method of queen rearing. The larvae of desired age for grafting were obtained from selected mother (breeder) colonies. Among top selected strong colonies (9-10 frames strength), one colony having high prolificness and good brood rearing ability was used as a breeder colony. The colony was maintained regularly by feeding them with sugar syrup, pollen substitute, and addition of sealed healthy worker brood from other colonies of apiary.

2.2 Queen cell cup material

Old comb bee wax and new capping bee wax (light coloured) were used to prepare artificial queen cell cups of 9 mm diameter. The plastic (brown coloured) queen cell cups of the same diameter, procured from the Global Apiaries village Fatehpur, post office Rampur Sainia, Tehsil Derabassi, Distt. Mohali, Punjab India were also evaluated. Standard method was used to prepare wax cups. The cups were removed by twisting them with the help of thumb and index finger gently (Fig. 1). These cups were mounted on a wooden cell bar with the help of molten bees wax on queen rearing frame (Fig. 2). The frame had two removable wooden cell bars and the wooden bar had sixteen queen cell cups affixed.

An empty comb was placed at the centre of the selected breeder colony for egg laying. The worker cells with freshly laid eggs were marked by tachni pins. After 24 hours of hatching, larvae were grafted with the help of grafting needle into the bottom of primed artificial queen cell cups affixed on queen rearing frame. Then, it was placed in the centre of cell builder colony (Fig.4 a and b).

2.3 Priming media

In present studies seven priming media viz., Apple Juice, Coconut Water, Royal Jelly, Diluted Royal Jelly (1:1 with

distilled water), Honey Solution (1:1 with distilled water), Sugar Syrup (1:1 with distilled water), Distilled Water and dry grafting (Control) were evaluated and compared for their influence on acceptance of grafted larvae and emergence of queen (Fig. 3). For the collection of royal jelly a strong colony was dequeened and allowed to raise queen cells. Before the sealing of raised queen cells, larvae were removed and collect the royal jelly into a sterilized eppendorf tube with a fine sterilized camel hair brush. Apple Juice and coconut water were purchased from the grocery shop. Honey was procured from the Department of Entomology, UHF, Nauni and diluted with distilled water to form honey solution. (1:1). Sugar purchased from grocery shop was dissolved in distilled water to form sugar solution (1:1).

2.4 Effect of season on queen production

The above experiments were carried out in Spring and Autumn season with all the above treatments and the data recorded were analysed for both seasons. For the analysis Two-Sample t-Test (one-tailed test) was applied by SPSS.

2.5 Observations were recorded

Three days after grafting, the graft frame is checked to assess cell acceptance. It is always handled gently without shaking or jarring. Normally the bees have further extended the walls of accepted cells with beeswax, and each accepted larva is floating on a deep bed of royal jelly. The number of grafted larvae and accepted larvae were recorded for each treatment. The queen cell protectors were applied at Ten to Eleven days, after grafting – about one day before the queens emerge. The number of emerged queen bees was recorded.

Percentage of larval grafts acceptance and queen emergence was calculated as per following formulae:

$$\text{Per cent Acceptance} = \frac{\text{Number of larvae accepted}}{\text{Total number of larvae grafted}} \times 100$$

$$\text{Per cent Emergence} = \frac{\text{Number of queens emerged}}{\text{Total number of larvae grafted}} \times 100$$

2.6 Statistical analysis

Data recorded were analyzed by using MS-Excel, OPSTAT and SPSS. The mean value of data was subjected to statistical analysis as described by Gomez and Gomez (1986) [18] by using Completely Randomized Design and t-test.



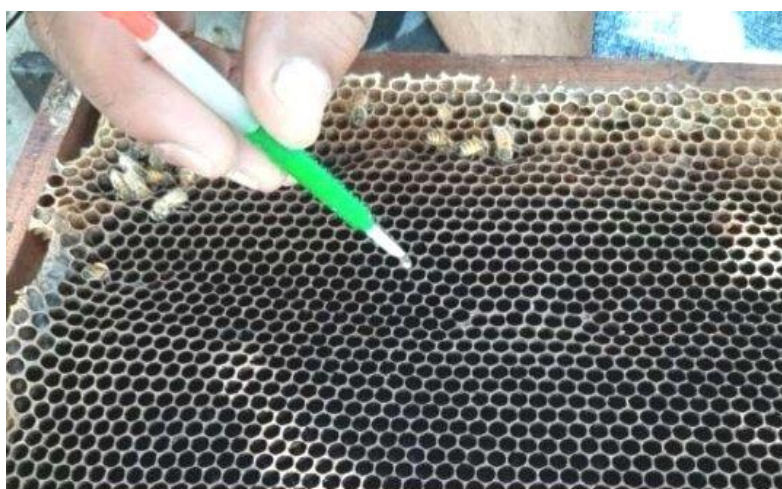
Fig 1: Preparation of artificial queen cell cups



Fig 2: Queen rearing frames with fixed artificial queen cell cups of different



Fig 3: (i) Grafting needle (ii) Priming media (iii) Micro pipette



a. Lifting of larvae



b. Grafting of larva

Fig 4: Larvae Grafting

3. Results and Discussion

3.1 Effect of priming media and cup material on queen rearing during autumn season

3.1.1 Larval acceptance rate of *A. mellifera* L. queen

The data recorded on the acceptance of the larvae in different priming media and artificial queen cell cups revealed that irrespective of effect of priming media, the larval acceptance rate was recorded statistically highest (62.50%) in old comb wax cups as compared to new capping wax cups (52.34%) and plastic cups (42.97%). Irrespective of effect of cup

material, statistically maximum larval acceptance rate was recorded in the royal jelly primed cups (85.42%) followed by diluted royal jelly primed cups (75.00%). Significantly ($\alpha=0.05$) minimum larval acceptance rate (18.75%) was recorded in dry queen cell cups (control). The interaction between effect of cup material and priming media on larval acceptance was found non-significant. Although the 100 per cent larval acceptance was found in royal jelly primed old comb wax cups whereas 12.50 per cent in plastic non-primed cups (Tab. 1 and Fig. 5).

Table 1: Effect of priming media and cup material on larval acceptance of *A. mellifera* L. queens in autumn season

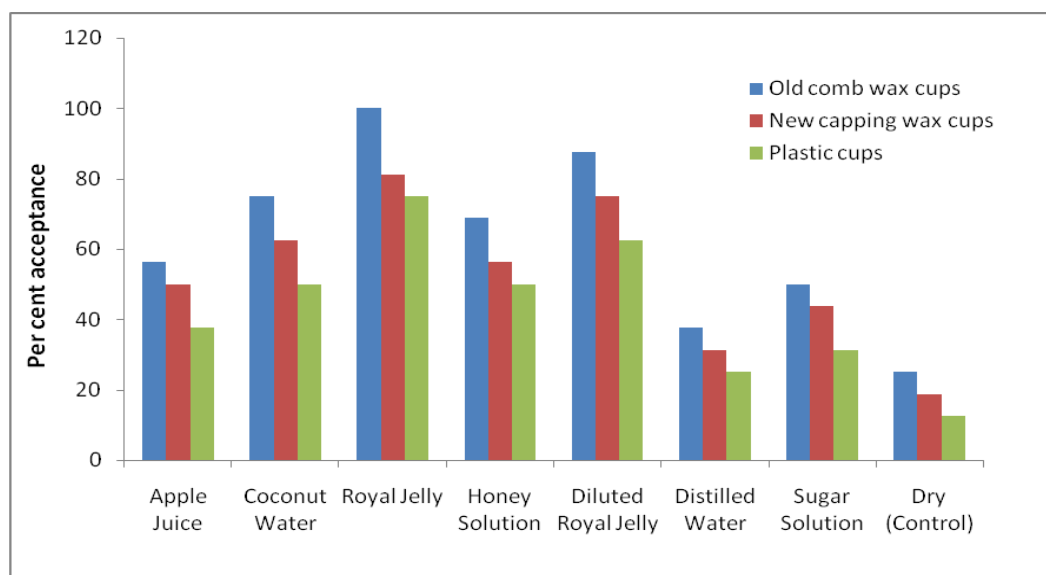
Cup Material Priming Media	Acceptance (%)			Mean
	Old comb wax cups	New capping wax cups	Plastic cups	
Apple Juice	56.25 (48.75)*	50.00 (45.00)	37.50 (37.50)	47.92 (43.75)
Coconut Water	75.00 (60.00)	62.50 (52.50)	50.00 (45.00)	62.50 (52.50)
Royal Jelly	100.00 (90.00)	81.25 (67.50)	75.00 (60.00)	85.42 (72.50)
Honey Solution (1:1)	68.75 (56.25)	56.25 (48.75)	50.00 (45.00)	58.33 (50.00)
Diluted Royal Jelly (1:1)	87.50 (75.00)	75.00 (60.00)	62.50 (52.50)	75.00 (62.50)
Distilled Water	37.50 (37.50)	31.25 (33.75)	25.00 (30.00)	31.25 (33.75)
Sugar Solution (1:1)	50.00 (45.00)	43.75 (41.25)	31.25 (33.75)	41.67 (40.00)
Dry (Control)	25.00 (30.00)	18.75 (22.50)	12.50 (15.00)	18.75 (22.50)
Mean	62.50 (55.31)	52.34 (46.41)	42.97 (39.84)	

*Values in parentheses are means of arc sine values

CD_(0.05) Cup material : 4.19

Priming media : 6.84

Cup material × Priming media : NS

**Fig 5:** Effect of priming media and cup material on larval acceptance of *A. mellifera* queens in Autumn season

3.1.2 Emergence rate of *A. mellifera* L. queen

The data in Tab. 2 clearly revealed that, irrespective of effect of priming media, the maximum queen emergence 57.03% was recorded from larvae grafted in old comb wax cups followed by new capping wax cups and plastic cups. Irrespective of effect of cup material, statistically maximum queen emergence 77.08% was found in royal jelly primed

cups followed by diluted royal jelly whereas the minimum was recorded in dry cups (control). The interaction between priming media and cup material on queen emergence was non-significant. However, the maximum 87.50% of queen emergence was recorded in old comb wax cups primed with royal jelly (Tab. 2 and Fig. 6).

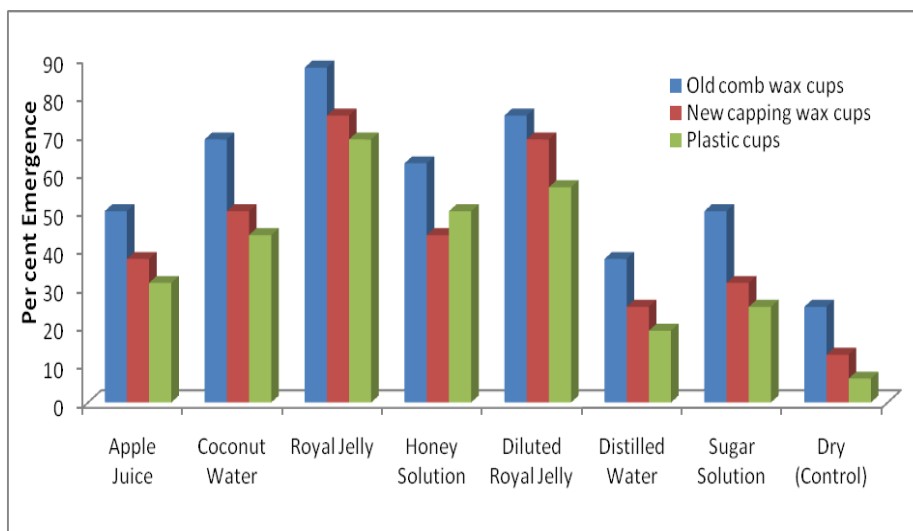


Fig 6: Effect of priming media and cup material on emergence of *A. mellifera* queens in Autumn season

Table 2: Effect of priming media and cup material on emergence of *A. mellifera* L. queens in Autumn season

Cup Material Priming Media	Emergence (%)			Mean
	Old comb wax cups	New comb wax cups	Plastic cups	
Apple Juice	50.00 (45.00) *	37.50 (37.50)	31.25 (33.75)	39.58 (38.75)
Coconut Water	68.75 (56.25)	50.00 (45.00)	43.75 (41.25)	54.17 (47.50)
Royal Jelly	87.50 (75.00)	75.00 (60.00)	68.75 (56.25)	77.08 (63.75)
Honey Solution (1:1)	62.50 (52.50)	43.75 (41.25)	50.00 (45.00)	52.08 (46.25)
Diluted Royal Jelly (1:1)	75.00 (60.00)	68.75 (56.25)	56.25 (48.75)	66.67 (55.00)
Distilled Water	37.50 (37.50)	25.00 (30.00)	18.75 (22.50)	27.08 (30.00)
Sugar Solution (1:1)	50.00 (45.00)	31.25 (33.75)	25.00 (30.00)	35.42 (36.25)
Dry (Control)	25.00 (30.00)	12.50 (15.00)	6.25 (7.50)	14.58 (17.50)
Mean	57.03 (50.16)	42.97 (39.84)	37.50 (35.63)	

*Values in parentheses are means of arc sine values

CD_(0.05) Cup material : 4.41
 Priming media : 7.21
 Cup material × Priming media : NS

3.2 Effect of priming media and cup material on queen rearing during spring season

3.2.1 Larval acceptance rate of *A. mellifera* L. queen

Data of Tab. 3 showed a significant difference in the acceptance of queen cups. The larval acceptance 67.97% was recorded maximum in old comb wax cups followed by new capping wax cups and plastic cups. Irrespective of the effect

of cup material, statistically highest larval acceptance (89.58%) was recorded in royal jelly primed cups followed by diluted royal jelly, coconut water, honey solution, apple juice and sugar solution. The lowest acceptance was observed in non-primed (control) queen cell cups which were statistically at par with distilled water primed cups (Fig. 7).

Table 3: Effect of priming media and cup material on larval acceptance of *A. mellifera* L. queens in Spring season

Cup Material Priming Media	Acceptance (%)			Mean
	Old comb wax cups	New comb wax cups	Plastic cups	
Apple Juice	62.50 (52.50)*	56.25 (48.75)	50.00 (45.00)	56.25 (48.75)
Coconut Water	81.25 (67.50)	68.75 (56.25)	62.50 (52.50)	70.83 (58.75)
Royal Jelly	100.00 (90.00)	87.50 (75.00)	81.25 (67.50)	89.58 (77.50)
Honey Solution (1:1)	75.00 (60.00)	62.50 (52.50)	62.50 (52.50)	66.67 (55.00)
Diluted Royal Jelly (1:1)	87.50 (75.00)	81.25 (67.50)	75.00 (60.00)	81.25 (67.50)

Distilled Water	43.75 (41.25)	37.50 (37.50)	25.00 (30.00)	35.42 (36.25)
Sugar Solution (1:1)	62.50 (52.50)	50.00 (45.00)	43.75 (41.25)	52.08 (46.25)
Dry (Control)	31.25 (33.75)	25.00 (30.00)	18.75 (22.50)	25.00 (28.75)
Mean	67.97 (59.06)	58.59 (51.56)	52.34 (46.41)	

*Values in parentheses are means of arc sine values

CD_(0.05) Cup material : 4.81
 Priming media : 7.86
 Cup material × Priming media : NS

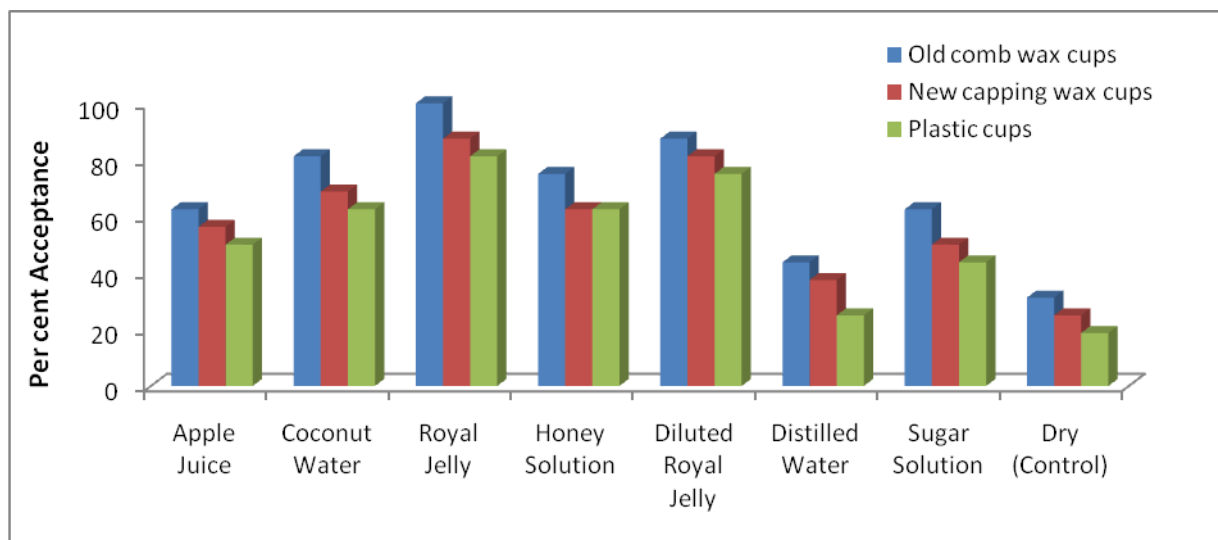


Fig 7: Effect of priming media and cup material on larval acceptance of *A. mellifera* queens in Spring season

3.2.2 Emergence rate of *A. mellifera* L. queen

Irrespective of effect of priming media, the maximum queen emergence rate was recorded from larvae grafted in old comb wax cups (63.28%) whereas the minimum queen emergence was recorded in plastic cups (42.19%). Irrespective of effect of cup material, statistically highest queen emergence i.e.

81.25% was observed in royal jelly primed cups which was statistically at par to diluted royal jelly primed cups. The minimum queen emergence (18.75%) was recorded in dry cups (control) (Tab. 4 and Fig. 8). Interaction effect of cup material and priming media for larval acceptance and queen emergence in spring season also was found non-significant.

Table 4: Effect of priming media and cup material on emergence of *A. mellifera* L. queens in Spring season

Cup material Priming Media	Emergence (%)			Mean
	Old comb wax cups	New comb wax cups	Plastic cups	
Apple Juice	62.50 (52.50)*	50.00 (45.00)	37.50 (37.50)	50.00 (45.00)
Coconut Water	75.00 (60.00)	62.50 (52.50)	56.25 (48.75)	64.58 (53.75)
Royal Jelly	93.75 (82.50)	81.25 (67.50)	68.75 (56.25)	81.25 (68.75)
Honey Solution (1:1)	68.75 (56.25)	56.25 (48.75)	50.00 (45.00)	58.33 (50.00)
Diluted Royal Jelly (1:1)	87.50 (75.00)	75.00 (60.00)	56.25 (48.75)	72.92 (61.25)
Distilled Water	37.50 (37.50)	37.50 (37.50)	25.00 (30.00)	33.33 (35.00)
Sugar Solution (1:1)	56.25 (48.75)	43.75 (41.25)	31.25 (33.75)	43.75 (41.25)
Dry (Control)	25.00 (30.00)	18.75 (22.50)	12.50 (15.00)	18.75 (22.50)
Mean	63.28 (55.31)	53.13 (46.88)	42.19 (39.38)	

*Values in parentheses are means of arc sine values

CD_(0.05) Cup material : 4.67
 Priming media : 7.63
 Cup material × Priming media : NS

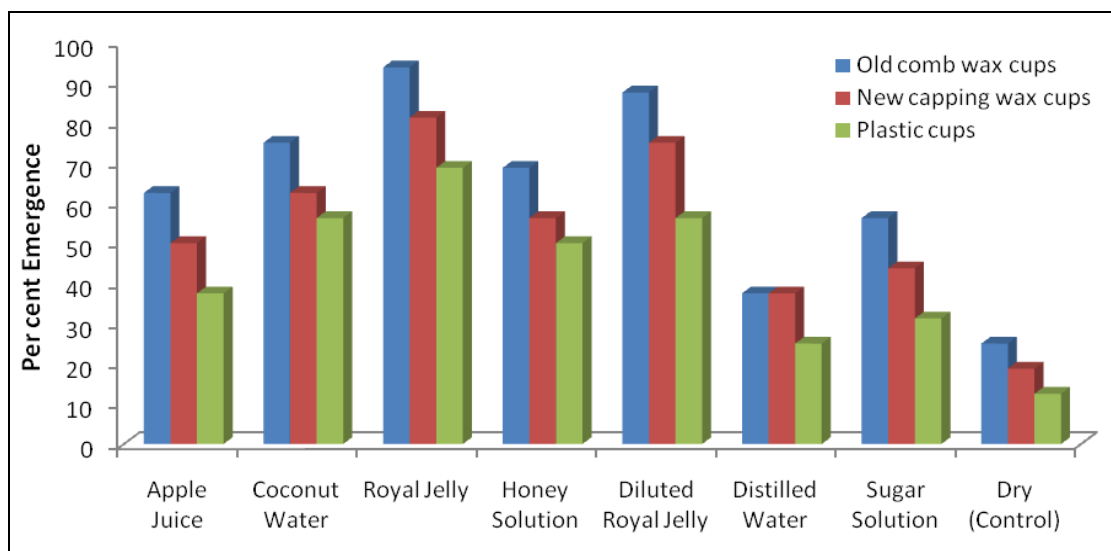


Fig 8: Effect of priming media and cup material on emergence of *A. mellifera* queens in spring season

In the present investigation, significantly highest rate of larval acceptance (62.50 and 67.97%) and queen emergence (57.03 and 63.28%) in Autumn and Spring, respectively were recorded in old comb wax cups followed by new capping wax cups and plastic cups. This indicated that the larval acceptance and queen emergence were dependent on the material used for formation of artificial queen cell cup for queen rearing. The results of present investigation are in line with observation of Laidlaw and Eckert (1962) [19] who suggested the utilization of bee wax cups for raising queens. The present findings are also corroborated by the results of Ebadi and Gray (1980) [20] and Thakur (1994) [21]. Thakur (1994) [21] used old comb wax, new capping wax and paraffin wax cups for queen rearing and reported the maximum acceptance in old comb wax cups and no acceptance in paraffin wax cups.

The present investigation also revealed that the primed cups were more preferred over non-primed cups by the workers. Among all the priming media tested, the royal jelly was found to be the best medium followed by diluted royal jelly. In rest of the treatments, coconut water showed good results, overall 62.50 per cent larval acceptance and 54.17 per cent queen emergence was recorded. Chhuneja and Gill (2014) [22] also reported that the acceptance in primed artificial queen cell cups was better over the non-primed cups. The findings are also in close agreement with Macicka, 1985 [23] who reported that the acceptance of larvae grafted with royal jelly priming was higher in comparison to dry grafting. Similar kinds of

observation were also recorded by Bobrzecki and Probucki (1975) [24], Ebadi and Gray (1980) [20], Thakur (1994) [21], Singh *et al.* (2001) [25], Arun (2011) [26] in *A. mellifera* and Verma and Sharma (1997) [27], Abrol *et al.* (2005) [28] in *A. cerana* which support present findings. Our finding clearly showing that the bee products viz. Royal jelly and wax used for queen rearing resulted in a higher rate of larvae acceptance consequently the emergence of queens. So, it can be concluded that, in queen rearing, the bees preferred bee products over other materials used.

3.3 Comparison of acceptance of larvae and emergence of queen produced between autumn and spring season

In present investigation evaluation of priming substrates and cup material was done and compared for two seasons. Data presented in Tab. 5 and 6 was compared by using two-sample t-test. The p-value was found greater than 0.05 for all the treatments (Do not reject the null hypothesis). Thus, the acceptance of larvae and the emergence of queen bee varied non-significantly during Autumn and Spring season for all the treatments. It is evident from the present studies that both seasons were equally good for mass queen rearing in *A. mellifera* L. under mid hill conditions of Himachal Pradesh. This may be because at that time weather is warm, drones are plentiful, nectar and pollen are rich in terms of quality and productivity Kaftanoglu and Kumova, 1990 [29] and Genc, 1997 [30].

Table 5: Comparison of per cent acceptance of larvae between autumn and spring season in *Apis mellifera* L.

Treatments	Acceptance (per cent)										
	Cup Material			Priming Media							
	Old comb wax cups	New capping wax cups	Plastic cups	Apple Juice	Coconut Water	Royal Jelly	Honey Solution (1:1)	Diluted Royal Jell (1:1)	Distilled Water	Sugar Solution (1:1)	Dry (Control)
Autumn	62.50	52.34	42.97	47.92	62.5	85.42	58.33	75	31.25	41.67	18.75
Spring	67.97	58.59	52.34	56.25	70.83	89.58	66.67	81.25	35.42	52.08	25.00
p-value	0.656	0.563	0.397	0.274	0.411	0.678	0.294	0.481	0.561	0.252	0.288

Table 6: Comparison of per cent emergence of queen between Autumn and Spring season in *Apis mellifera* L.

Treatments → Season ↓	Emergence (per cent)										
	Cup Material			Priming Media							
	Old comb wax cups	New capping wax cups	Plastic cups	Apple Juice	Coconut Water	Royal Jelly	Honey Solution (1:1)	Diluted Royal Jelly (1:1)	Distilled Water	Sugar Solution (1:1)	Dry (Control)
Season1	57.030	42.970	37.500	39.58	54.17	77.08	52.08	66.67	27.08	35.42	14.58
Season 2	63.281	53.125	42.188	50.00	64.58	81.25	58.33	72.92	33.33	43.75	18.75
p-value	0.579	0.345	0.644	0.315	0.326	0.670	0.468	0.588	0.417	0.468	0.561

4. Conclusion

The per cent larval acceptance and queen emergence was maximum in artificial queen cell cups made of old comb wax and primed with royal jelly among all the priming media and cup materials evaluated for queen production in present study. So, it can be concluded from present investigations that maximum number of queens can be produced by using the royal jelly as priming medium and old comb wax as artificial queen cell cup material in *Apis mellifera* L. mass queen rearing under mid-hill conditions of Himachal Pradesh in both Spring and Autumn season.

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