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Quantitative assessment of royal jelly collected at different time interval in honey bees *Apis mellifera*

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Abstract
The trial has been conducted at research farm of Bee Keeping and Hill Fruit Pest Research Station Rawalpindi from mid-February to April with optimum temperature 20-30 ^{0}C during the period of 2012-2014. Three treatment time intervals 48, 72 and 96 hrs were taken for study purpose. Twenty (20) artificially made queen cups from beeswax with a special cell forming dye were selected and fitted in a wooden hive frame. In each queen cup a twenty four hours old young larvae of worker bee from a best performing colony was grafted with the help of grafting needle for the purpose of royal jelly extraction. The results showed that extraction of royal jelly has significant (P>0.05) results in treatment having 72 hrs time interval over other treatments 48 hrs and treatment 96 hrs time intervals respectively. Analysis of variance indicated significantly different effects for different treatments (F>215.09, P>F=0.0005). Least square distance (LSD) for each treatment showed that all treatments had significantly different effects from each other. This protocol concluded that the queen cups took time interval of 72 hrs extracted maximum royal jelly after larval graft results high production proved that this time interval provide optimum condition for royal jelly production, while increasing the time to 96 hrs reduced the royal jelly extraction.

Keywords: Quantitative, Assessment, Royal Jelly, Different, time intervals, Hours

1. Introduction
Royal jelly is a milky white colored secretion produced by the highly specialized set of hypo pharyngeal and mandibular glands (salivary glands) located in the head of young worker honeybee aged between 5 and 15 days to feed the worker larvae until the third day, drone larvae until pupation, and the queen throughout larval and adult stages Lercker *et al*, (1993) [6]. This fluid is homogeneous, creamy, milky white coloured, with a slightly acid flavour and a strong and pungent odour and frequently used to boost growth of larvae in the colony Munstedt K, Georgi R (2003) [8].

Royal Jelly is a nutritive mixture, designed for bee growth and Queen Bee sustenance, it contains. a complex composition of proteins, amino acids, organic acids, sterols, phenols, sugars, minerals, and other unknown substances Viuda *et al*,(2008) [12]. It also contains about 60% to 70% water, 12% to 15% proteins, 10% to 16% sugar, 3% to 6% fats, and 2% to 3% vitamins, salts, and amino acids Mohamed *et al*, (2012) [9]. Dietary Fatty acids, such as 10-hydroxy-2-decanoic acid (10-HDA unique to Royal Jelly at 32% of fatty acids) and others, such as gluconic acid (24%) 10-hydroxydecanoic acid (22%) and 5% other dicarboxylic acids Vocalic *et al*, (2007) [11]. Its composition varies depending on geography and climate.

Royal jelly is a product solely of glandular secretion; therefore, artificial supplementation may not affect its physicochemical composition keeping the final characteristics of the products intact Haydak MH (1970) [5]. As royal jelly is a viscous jelly substance. It is partially soluble in water with a density of 1.1 g/mL. Its colour is whitish to yellow, the yellow colour increasing upon storage. Its odour is sour and pungent, the taste being sour and sweet. The sensory characteristics are important quality criteria. Old royal jelly, which has not been properly stored, tends to be darker and a rancid taste can develop. For optimum quality it should be stored in frozen state Jeans *et al*, (2003) [2]. The viscosity varies according to water content and age - it slowly becomes more viscous when stored at room temperature or in a refrigerator at 5 ^{0}C.

Royal jelly has been demonstrated to possess numerous functional properties such as antibacterial, anti-inflammatory, antioxidant, antihypercholesterolemic and antitumor activities Graham, J (1992) [3]. Biological activities of honey, propolis, and royal jelly are mainly attributed to the phenolic compounds such as flavonoids. Flavonoids have been reported to exhibit a wide range of biological activities including antibacterial, antiviral, anti-inflammatory, antiallergic, and vasodilatory actions. In addition, flavonoids inhibit lipid peroxidation; plate let aggregation, capillary permeability and fragility Viuda *et al*, (2008) [12]. Royal jelly is used for asthma, hay fever, liver disease, pancreatitis, sleep troubles (insomnia), premenstrual syndrome (PMS), stomach ulcers, kidney disease, bone fractures, menopausal symptoms, skin disorders, and high cholesterol Louis ST, Wolters MO (2011) [14]. It is also used as a general health tonic, for fighting the effects of aging, and for boosting the immune system. Some people apply royal jelly directly to the skin as a tonic or to the scalp to encourage hair growth Yamanaka N, O'Connor MB (2011). (Web Md, 2014). Given the exceptional biological properties attributed to it, royal jelly (RJ) has considerable commercial appeal and is today utilized in many sectors, ranging from the pharmaceutical and food industries to the cosmetic and manufacturing sectors Anna *et al*, (2009) [1]. Keeping in view the importance of royal jelly and its multifunctional properties the study was carried out to determine the quantities of royal jelly collected at different time intervals in honey bees *Apis mellifera*

2. Materials and Methods
The trial was conducted in Bee Keeping Research farm and Hill Fruit Pests Research Station Rawalpindi during the period of

2012-2014. Three treatments time intervals i.e.48, 72 and 96 hrs were taken. Twenty (20) artificially made queen cups from beeswax with a special cell forming dye were selected and fitted in a wooden hive frame. In each queen cup a twenty four hours old young larvae of worker bee from a best performing colony was grafted with grafting needle and the frames were fitted gently in a strong bee colony, dequeened twenty four hours before the introduction of frames. Accepted cups were removed for three treatments time intervals 48, 72 and 96 hours after grafting. The queen cups were cut open a few millimeters above the larvae present in the cell. The larvae removed gently and discarded. Royal jelly was extracted with the help of specially made small plastic spatula and weighed an electronic balance at different time intervals. The data were subjected to statistical analysis (Steel *et al*., 1997) [10]. The recorded data were examined for variance by STATISTIX version 8.1 and LSD at 5% level of confidence interval used to relate the means of treatments.

3. Results

The results showed that extraction of royal jelly has significant (P>0.05) results in second treatment (72 hrs) over treatment fist (48 hrs) and treatment third (96 hrs) respectively. Analysis of variance (Table 1) indicated that (F>215.09, P>F=0.0001) is significantly different. Least square distance (LSD) for each treatment showed that all treatments are significantly different from each other (Table 2). The Graph showed that the queen cups took the time interval of 72 hrs resulted the maximum extraction of royal jelly mean three day time interval after larval graft results high production, while increasing the time 96 hrs (T_3) the royal jelly extraction reduced. The results clearly indicated that three days interval is the best for maximum royal jelly production.

4. Discussions

The results indicated that bee queen larvae at the age of 72 hours (three days) produced maximum quantity of royal jelly and young workers bee produce this quantity for queen larval growth and stored in queen cup that can be collected, while increasing the time interval to 96 days (4 days), the quantity of royal jelly decrease, similarly at time interval 48 hours, the quantity of royal jelly collected is in small quantity. Three days interval for maximum production of royal jelly was the best due to the proper time availability of every step hat involved in royal jelly production. Gisele and Vagner (2004) [4] indicated same views that is reported in this paper. Further research will be conducted on the quality of royal jelly with time interval.

5. Conclusion

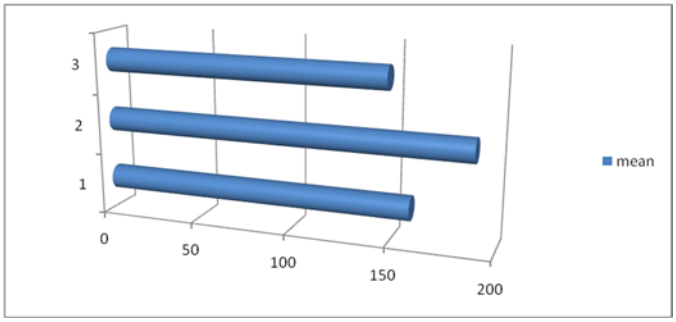
This experiment concluded that 72 hrs time interval produced the maximum royal jelly as comparing to other time intervals therefore this three days time interval (72 hrs) recommended to for maximum collection of royal jelly.

Table 1: ANOVA for Royal Jelly Quantity Assessment at Different Time Intervals

Source	DF (Degree of Freedom)	SS (Sum of Square)	MS (Mean Square)	F (F Value)	P (P Value)
Replications	2	172.22	86.11		
Treatments	2	2772.22	1386.11	215.09	0.0001
ERROR	4	25.78	6.44		
TOTAL	8	2970.22			

Table 2: LSD for Different Time Intervals

Average Royal Jelly extracted(hrs)	Milligrams
48	159.33 B
72	189.33 A
96	147.67 C



Graph # 1: Effect of Time Intervals on the Quantitative Value of Royal Jelly

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