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Correlation of Royal Jelly composition with swarming tendency in Honey Bees (*Apis mellifera*): A Review

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ABSTRACT

Honey bees are eusocial insects which respond to warm weather, abundant food source by increasing their population through swarming to ensure the survival of the colony. To maintain a superior colony a queen must have a nutrient-rich diet and high egg production. Royal jelly is a high-quality food which has numerous beneficial properties required for proper growth, development, survival of the queen. Factors like congestion, lack of adequate queen pheromone, abnormal queen pheromone, pathogenic infections, exposure to pesticides influence the queen quality which further promotes non-reproductive swarming behaviour. Worker bees analyse the queen condition to prepare for supersedure or emergency queen rearing. This review paper highlights the influence of royal jelly composition on the queen quality, the impact of queen quality on swarming tendency, correlation between royal jelly composition and swarming tendency.

Key words: Non-reproductive, Queen health, Quality, Royal Jelly, Swarming.

Honey bees are social insects that live in colonies with a single queen, thousands of infertile female workers and hundreds of drones. The queen bee is a well-developed female and sole member of the colony to lay eggs. The workers perform several useful tasks in the colony such as feeding the larvae, raising queen cells, guarding the hive entrance, collection of nectar, ventilation of hive, and construction of combs. The prime function of drones is to mate with a queen bee. An important function of the workers is to feed the queen bee with a highly nutritious food known as royal jelly (RJ). The partial digestion of honeydew in worker bee's stomach produces RJ. It plays a major role in caste differentiation in honey bees.

The larvae selected to become queen receive RJ, whereas, workers receive a combination of pollen, honey and water (Moselhy *et al*., 2013; Melliou and Chinou, 2005).

RJ is a nutrient-rich, viscous secretion secreted from the mandibular and hypopharyngeal glands of worker honeybees (Balkanska *et al*., 2010). It is a complex substance with unique combination of proteins (12-15%), lipids (3-7%), sugars (10-12%), fats (3-6%), water (60-70%), vitamins, amino acids and minerals (Morita *et al*., 2012). The major royal jelly proteins (MRJPs), form the majority of proteins in RJ and provide nitrogen to the larva along with antimicrobial protection. The proteins in royal jelly create a pH-dependent fibril network allowing the larva to hold on to its viscous surface for the development of queen in vertical cells (Pirk, 2018). A queen larva throughout its development in the cell receives nearly 50 mg of food per day (Crailsheim *et al*., 2013; Aupinel *et al*., 2005). This quantity of food is available for growth, feeding and glue herself to the cell (Wang *et al*., 2016). RJ is a vital bee product that is important for the health and quality of the queen thereby maintaining a strong colony. Modification or alteration in RJ can affect the queen quality, and further influence swarming behaviour. If the quality of royal jelly is poor, an inferior queen will develop, and processes like non-reproductive swarming, queen supersedure, or emergency queen rearing may take place (Fig 1).

Swarming is a complex phenomenon that occurs at a specific time of the year when plenty of resources are available and depends on the geographical location of a colony (Henneken *et al*., 2012). The time of swarm departure is an adaptive decision by the bee colony to avoid bad weather and fighting between the queen and her daughter queens (Gilley and Thom, 2018). Apart from reproductive swarming, many other factors trigger non-reproductive swarming behaviour in honey bees which include, increase in the colony size, congestion of brood, increase in the number of young worker bees, reduced queen pheromones, lack of availability of food resources, temperature and pathogenic infestations (Amiri *et al*., 2017; Harris, 2016; Grozinger *et al.,* 2013). Swarm preparation starts with the construction of cups to rear the queen, reduction in the amount of food given to the queen to facilitate smooth flight

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at the time of departure. Different communication signals like vibration, piping, buzz runs are performed by the worker bees at the time of swarming. The bees coordinate with each other to ensure all swarm together. After the workers, and the mother queen have departed the parental nest, they form a bivouac at a temporary location nearby while the scouting workers continue the process of scouting the area for a new home. In the parental nest, queen replacement usually occurs within a few days when a virgin queen takes over the colony and eliminates her rivals by stinging them in their cells or fighting with them once they have emerged (Schneider and DeGrandi-Hoffman, 2008). The signal-dependent interactions coordinate the timing of the release of virgin queens with the timing of the departure of prime swarms and afterswarms to ensure that a single queen leaves with each swarm and only one queen takes over the colony at the end of the swarming process.

Influcence of royal jelly composion on the queen quality

The growth, productivity, and survival of a colony depends majorly on the health and reproductive capacity of its queen and the number of drones (Amiri *et al*., 2017). Worker and queen develop from fertilized egg but their phenotypical difference is due to differential larval nutrient environment (Yang *et al*., 2017). Proteins and sugars in a ratio of 1:1 make up one-third of RJ and the rest consists of water with a fair proportion of fatty acids (Buttstedt *et al*., 2016). Sugar is added to the jelly by nurse bees and originates from the honey stores. The larval nutritional state is assessed and used by workers in the selection of larvae for queen rearing and queen replacement process (Sagili *et al*., 2018). Pollen is the natural protein source for the worker bees and high-quality pollen increases the development of hypopharyngeal glands which produce high-quality royal jelly for the queen (Kumari and Kumar, 2020; Ulutas and Ozkirim, 2018).

Impact of proteins, lipids and carbohydrates on queen quality

MRJP-1 and MRJP-3 are important proteins present in royal jelly which induce growth in the queen, shorten the developmental period, increase the body size and ovarian development (Slater *et al*., 2020; Ulutas and Ozkirim, 2018; Kamakura 2011). Various metabolites, lipids, amino acids, phytochemicals, enhance honey bee nutrition and have supplemental benefaction to the antimicrobial properties and epigenetic effects of RJ. Phytosterols are a key lipid component of RJ that is essential for the production of moulting hormone and the stability of the cellular membrane. The 24- Methylene-cholesterol, b-sitosterol, D5-avenasterol, and desmosterol are also present in RJ where the increased concentration of 24- Methylene-cholesterol in diet, increase the contents of head protein and abdominal lipid content (Milone *et al*., 2020). Carbohydrates can function as phagostimulants, stimulating more food consumption in queen-destined larvae when present in high ratios. A higher level of carbohydrates and larger quantities of diets can produce more queenliness (Slater *et al*., 2020).

Inpact of sugars on the queen quality

Glucose and fructose at 12% concentration have a positive effect on the number of ovarioles and promote queen development (Kaftanoglu *et al*., 2011). In an experiment, queen larvae were fed with an artificial diet containing a mixture of RJ+ yeast, RJ + fructose, RJ + honey. It was found that the larval and pupal duration was maximum with RJ + yeast followed by RJ + fructose and least in RJ + honey. The quality and quantity of diet given to the larvae affect the number of ovarioles in bees, where 2g diet showed the best results (Aqueel *et al*., 2016). The larvae fed with 3.5–4.5 g diets with varying sugar concentrations mainly glucose and fructose for 6 days show an increase in sugar concentration that reduce the survival of queen larvae. RJ along with distilled water in a ratio of 1:1, proves to be harmful for the larvae (Kabakci and Akseniz, 2020). Larvae of 1st instar provided with a combination of sugar augments (glucose and fructose) and Juvenile Hormone (JH) produce queens of high quality while 3rd instar larvae provided with only JH treatment increase the queen quality. Mated queens treated with JH and supplemented sugars have considerably higher sperm viability and sperm count (Souza *et al*., 2019). Oral exposure of miticides to control Varroa mite can alter the glandular physiology of worker bees which further influences RJ production and impact the queen's development, survival, and reproductive health (Milone *et al*., 2020).

Influence of queen quality on swarming tendency

Swarming is a complex process that is mediated by multiple environmental, social, physiological factors which increase the honey bee colony. Reproductive swarming normally occurs in strong colonies during specific times of the year, usually when resources are plentiful to support rapid population growth (Somerville,1999; Simpson, 1959). Generally, warm weather with a rise in temperature after the winter period stimulate a colony to swarm in spring. Queens of more than one year are likely to swarm rather than younger queens. A young and healthy queen lays good quality eggs in a uniform brood pattern. Swarms comprising of younger bees are beneficial as comb building, brood rearing is typically performed by younger bees. The amount of food provided to the queen 14-15 days before swarming is significantly reduced by worker bees to ensure a reduced weight and smooth flight with the departing swarm (Grozinger *et al*., 2013). Moreover, the vibratory signals also stimulate egg laying which helps the queen to lose weight for flight.

Effect of congestion and reduced pheromones

Congestion in a colony occurs due to the prevailing breeding situation and the restricted space provided for the colony which reduces the queen pheromones and stimulate worker bee to start construction of queen cells (Grozinger *et al*., 2013). The queen pheromones are distributed throughout the colony in high amounts which inhibits the urge to swarm.

An increase in colony size leads to the dilution of signal among the worker bee population and encourages swarming tendency (Harris, 2016). An old or a sick queen either produces less queen substance or produce an abnormal blend of chemicals. The worker bees respond to these poor chemical signals by producing supersedure queen cells (Rangel *et al*., 2016; Henneken *et al*., 2012). At any point of time when the queen is lost or fails due to poor nutrition, supersedure queen cells may be produced. The supersedure queen cells produced during poor colony nutrition are of low-grade quality as compared to the swarm cells produced under optimum nutrition conditions.

Effect of pathogenic infestations

Though the queen is less susceptible to pathogenic infections however *Acarapis woodi*, *Varroa destructor* and numerous viruses like deformed wing virus (DWV), sac brood virus (SBV), black queen cell virus (BCV), israeli acute paralysis virus (IAPV) can affect the queen's health and promote non-reproductive swarming. Deformed Wing Virus affects the drones which mate with young queens and cause transmission of disease from an infected queen to her offsprings. The black queen cell virus attacks queen larvae and has been detected mostly in the faeces, gut and ovaries of queen bees. The queens suffering from Nosemosis (microspore disease) have aberrant physiology, display changed pheromone production, and in extreme cases oocytes degeneration leading to infertility. The Arachnids consisting of Acarina (mites and ticks) are also major predators of honey bees. The *Varroa destructor* (ectoparasitic mite) prefer to parasitize drone brood due to their longer development time whereas queen infestation by *Varroa* occurs only at extremely high mite infestation in the colony (Amiri *et al*., 2020). Young queens may become infected in the mating nuclei, if the worker bees are already infected by pathogens. A diverse range of predators are also known to invade the honey bee colonies. The highest pest attacks are from the order Hymenoptera followed by Coleoptera, Lepidoptera and lest by Orthoptera, Thysanura. Other bee predators include molluscs, amphibians, reptiles, apes and mammals (Sharma *et al*., 2013).

Effect of pesticides

Exposure to pesticides used by beekeepers and agricultural chemicals influence physiological changes like decreased sperm viability, lower body weight, reduced ovariole number in the queen bee. A low survival and high queen supersedure rate can be seen in bee colonies where adequate nutrition is unavailable that results in poor brood health with abnormal egg-laying patterns (Milone *et al*., 2020; Lee *et al*., 2019).

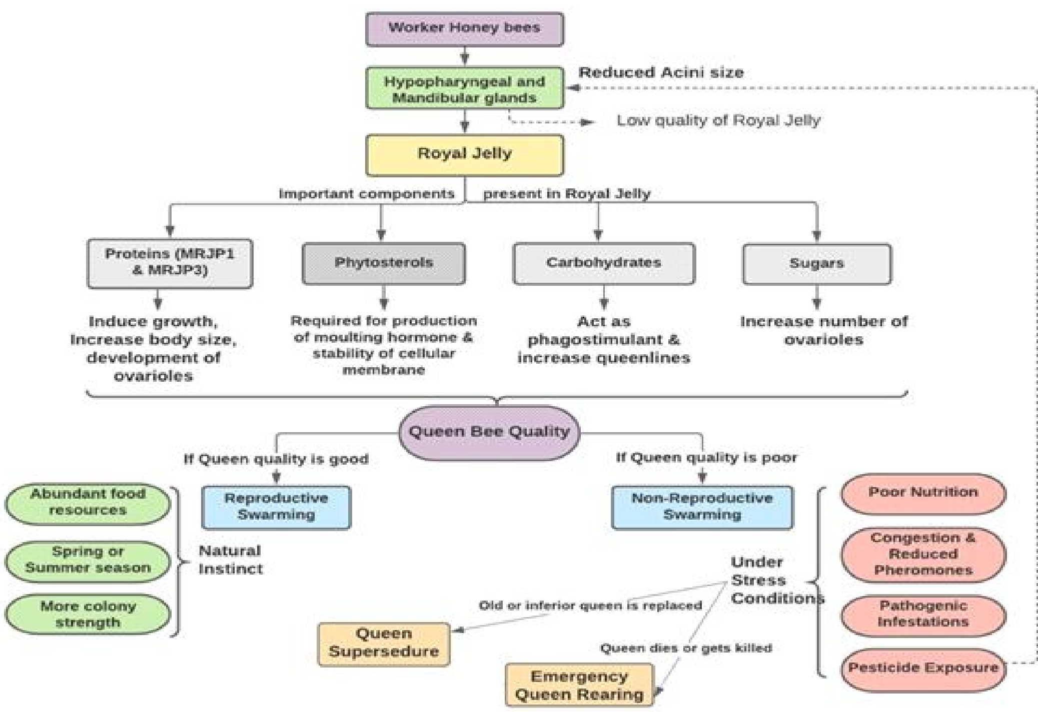

Fig 1: Worker honey bees secrete RJ from the hypopharyngeal and mandibular glands. It has important components like proteins, carbohydrates, sterols, sugars which directly influence the queen quality. When the queen quality is good, reproductive swarming takes place along with presence of more colony strength, abundant food supply during spring or summer season. This is a natural phenomenon to expand the bee colony. Conditions like poor nutrition, congestion, reduced pheromones, pesticide exposure cause non-reproductive swarming. Exposure to pesticides reduces the acini size of the hypopharyngeal glands in worker bees which further produce low quality of RJ and thereby affect the queen quality. If an old or inferior queen is present in the colony, replacement of the queen known as queen supersedure is done. If the queen is dead or killed, emergency queen rearing is done.

Neonicotinoids are neuroactive insecticides that reduce the size of the hypopharyngeal glands of nurse bees and also reduce the levels of acetylcholine. A smaller hypopharyngeal gland may lead to malnutrition as the queen and young larvae are fed on RJ (Fig 1). Changes in the lipid and carbohydrate composition in RJ can be observed when larvae are exposed to neonicotinoids (Schott *et al*., 2021).

Bee diseases, pest, predators, pesticides and poor colony management skills pose problems in efficient beekeeping (Chimdessa *et al.,* 2020). Therefore, honey bees can naturally reduce their risk of exposure to pathogens and pesticides through swarming by avoiding an infectious habitat and weeding out infected individuals. Swarming is also beneficial for finding new hive locations, nectar sources, and creating genetic diversity by enhancing the productivity and health of the colony (Diao and Hou, 2018).

CONCLUSION

The composition and quality of royal jelly are of great significance as the development of the queen is highly dependent on it. Changes in royal jelly composition can enhance or diminish the quality of the queen and may cause swarming or replacement of the queen. Though swarming is a natural method for establishing a new colony but factors such as poor nutrition, reduced pheromone production, overcrowding, exposure to pesticides, and pathogens can impact queen quality thereby increasing the chance for non-reproductive swarming. Future studies are required to understand the exact effect of royal jelly composition on the queen quality and its impact on swarming for building a strong correlation between them.

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