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THE EFFECT OF OCTOPUS SP. WASTE AS ARTIFICIAL BEE FEED ON CHARACTERISTICS WORKER BEE (APIS MELLIFERA) AND POLLEN MORPHOLOGY

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ABSTRACT
The research aimed to evaluate the use of Octopus sp. waste as artificial bee feed and evaluate pollen collected by worker bees (*Apis mellifera*). This research using 4 treatments (P0: cornflour pollen 100% + *Octopus sp*. waste flour 0%; P1: cornflour pollen 87,5% + *Octopus sp*. waste flour 12,5%; P2: cornflour pollen 75% + *Octopus sp*. waste flour 25%; P3: cornflour pollen 65,5% + *Octopus sp*. waste flour 37,5%). This research used honey bees (*Apis mellifera*) 20 boxes with 4 of the frames. The variable observed were worker bees' body weight, worker bees' body length, and worker bees' wing length. The data were analyzed by analysis of variance, continued by polynomial orthogonal test. The results showed that the worker bees' body weight, worker bees' body length, and worker bees' wing length were significantly different (P<0,05) between the group. Characteristics of worker bees (*Apis mellifera*) day old in treatment P1 gave an average body weight of 94.4 mg, an average body length of 12.7 mm, and an average wing length of 9.7 mm. The provision of 87.5% corn pollen flour + 12.5% octopus waste flour in treatment P1 gave the best results among other treatments. The pollen collected by worker bees (*Apis mellifera*) consisted of *Hibiscus rosa-sinensis L., Adiantum trapeziforme,* and *Tagetes erecta L*.

KEY WORDS
Honey bees (*Apis mellifera*), *Octopus sp.* waste flour, pollen.

One example of waste/disposal in the form of liquids, solids, and gases present at one time and place that might impact environmental quality is livestock and octopus waste. Because of the physical and chemical characteristics or amount, waste in the form of a significant amount of liquid contains dangerous and poisonous components that can contaminate or harm the environment either directly or indirectly. *Octopus sp.* processing waste is treated biologically using bacteria using the activated sludge method. The results are not optimal because the red dye from the octopus processing is difficult to degrade naturally biological. Wastewater from *octopus sp.* processing has not been able to meet the required quality standards set by the government. In this case, it is necessary to develop other methods by utilizing octopus waste into honey bee-made feed.

Honey bees need food in the form of nectar and pollen. Besides nectar and pollen, worker honey bees collect water to ensure colony survival, manage hive temperature and humidity, and dissolve honey for honey bee chick consumption. Nectar is a compound complex produced in the form of a solution by the neoterizing glands of plants. Bees use nectar as a source of carbohydrates as well as vitamins and minerals (Rompas, 2011). The season has an impact on the quality and quantity of plant flowering in a particular area. So the amount of flower availability as a food source for bees becomes erratic. Pollen is the male flower seed of a plant that is used for the fertilization of female flowers. Pollen is the main source of protein, lipids, minerals, and vitamins required for honeybee nutrition (Keller, Fluriand Imdorf, 2005).

Honey bee production in *Apis mellifera* colonies will be obtained during the season lack and the breeder will bring his bee colony to a moderately planted are flowering. The honeybee colony has problems acquiring food during the prolonged rainy season because nectar and pollen are damaged. Hence the number of bee populations will decrease during this time, related to a shortage of food. If things continue for a long time will result in various developmental disorders and health of the honeybee colony, production decreases, weak to various diseases and predators, population declines, and even honeybee colonies leaves the nest. To optimize production in the seasonal rains, farmers provide sugar water or sugar syrup as a substitute for nectar natural. But it is rare for breeders to provide a substitute for pollen as a source of protein, fat, and minerals. The absence of protein intake in honey bee colonies caused the productivity and health of the colony to decrease.

Proteins are involved in the system immunity (immune) as antibodies, the control system in the form of hormones, as storage component (in seeds), and also in nutrient transport as a source of nutrition, protein acts as a source of amino acids for living organisms unable to form amino acids (Rijal, 2011). Protein by honey bees is used to build muscles, glands, and body tissues of larvae as well as a young bee. Protein is required for the production of royal jelly, which is used as a food source for queen bees and larvae (Somerville, 2000).

Researchers seek alternative artificial pollen to overcome the shortcomings of natural protein source that occurs in the rainy season. Protein source material animals can be used as pollen made by honey bees, as can bees to conducted by Agil (2008) who used golden snail flour as pollen artificial. The high protein value of octopus waste flour (*Octopus sp.*) is one of the ingredients that has not been examined but has the potential to be used as pollen for honey bees. As a result, this research evaluating the usage of *Octopus sp*. flour as an alternative source of protein as well as the shape of the pollen is required.

MATERIALS AND METHODS OF RESEARCH

The experimental animal used in this study was Honey bee (*Apis mellifera*) as many as 20 colony boxes (stup) with a total of 4 nest combs. Alternative feed, namely the artificial pollen used comes from octopus waste (*Octopus sp*.) which is then made into octopus waste flour (*Octopus sp.). Octopus sp*. waste was obtained from PT. Istana Cipta Sembada Banyuwangi-East Java, Indonesia. Equipment used for beekeeping includes colony boxes (stup), comb, feeder frame. Tools for manufacturing substitute feed is plastic trays and scales. Equipment used for testing includes analytical scales, calipers, tweezers, stationery, and camera.

This research using 4 treatments (P0: cornflour pollen 100% + *Octopus sp.* waste flour 0%; P1: cornflour pollen 87,5% + *Octopus sp.* waste flour 12,5%; P2: cornflour pollen 75% + *Octopus sp.* waste flour 25%; P3: cornflour pollen 65,5% + *Octopus sp.* waste flour 37,5%). This research used honey bees (*Apis mellifera*) 20 boxes with 4 of the frame. The variable observed were worker bees body weight, worker bees body length and worker bees wing length.

This research was conducted by survey, using the *Random Purposive Sampling (*RPS) method. Samples that have been obtained randomly are then enteredinto the sample pot. Pollen that has been prepared previously, then observed pollen ultrastructure using *Scanning Electron Microscope* (SEM).Furthermore, identification is carried out based on the supporting literature.

The results of observations during the study were tabulated and analyzed using the assumption test, namely the homogenity test and independent normal distribution. If the values obtained are significantly different, then proceed with the testorthogonal polynomials.

RESULTS AND DISCUSSION

Evaluating the Provision of Octopus sp. Waste Flour on the Body-weight of Worker Bees (Apis mellifera) One Day Old. The average weight of worker bees (*Apis mellifera*) of one day old obtained intreatment ranged from 84.4 – 94.4 mg (Table 1). The results of the analysis provide a significant effect on the weight of worker bees (*Apis mellifera*) day old (P<0.05).

According to Winston (1987) that the bodyweight of worker bees that just came out of the cell ranged from 81 to 151 mg. This can be used as an indicator that if the weight of the body of a day-old worker bee is below the normal range, worker bees are not will be able to bring nectar and pollen into the nest which will affect the nutritional adequacy of the bee colony (Hapsari, 2007). Cell size, nursing bee size, age, colony population, disease, nectar and pollen produced, and season are all factors that might influence the weight of day-old worker bees.

Table 1 – The average body-weight of worker bees (*Apis mellifera*) of one day old

Treatment	Weight of Worker Bees (mg/ekor)
P_0	92,6 ± 0,55
P_1	94,4 ± 0,55
P_2	85,6 ± 0,55
P_3	84,4 ± 0,55

Description: Showed significant differences between treatment groups (P<0.05).

In research by Roulston and Cane (2002), it is stated that the bodyweight of day-old worker bees will increase if the feed consumed during the phase larvae are fulfilled and able to be absorbed properly. During the larval stage, the feed is in the form of royal jelly derived from young working bees and their secretions are influenced by the amount of protein consumption (Standifer, 1973).

The weight of the bees, in this case, the weight of the day-old worker bees, determines the colony's development in honey bees. This is because the weight of the bee day-old workers is directly proportional to the ability of worker bees to perform activities that are useful in meeting the feed needs of the colony. With the ideal body weight of worker bees, worker bees will be able to transport nectar and pollen more efficiently than worker bees' body weight below the normal range.

Furthermore, the location of the farm has an impact on bee body weight, with larger endothermic animals in colder climates weighing more than endothermic animals in hotter climates, according to Begon et al. (1986). Worker bees (*Apis mellifera*) also have different body sizes from one location to another other location (Hadisoesilo, 2001). A large worker bee's bodyweight allows it to transfer huge amounts of nectar and pollen, as well as consuming nectar faster than worker bees with a lower/smaller body weight (Free, 1982).

Evaluating the Provision of Octopus sp. Waste Flour on the Body-length of Worker Bee (Apis mellifera) One Day Old. The average body-length of worker bees (*Apis mellifera*) of one day old obtained intreatment ranged from 11,9 – 12,7 mg (Table 2). The results of the analysis provide a significant effect on the body-length of worker bees (*Apis mellifera*) day old (P<0.05).

Table 2 – The average body-length of worker bees (*Apis mellifera*) of one day old

Treatment	Body lenght of Worker Bees (mg/ekor)
P_0	12,5 ± 0,05
P_1	12,7 ± 0,07
P_2	12,3 ± 0,03
P_3	11,9 ± 0,08

Description: Showed significant differences between treatment groups (P<0.05).

The average body length of the worker bee (*Apis mellifera*) of the day-age obtained in the treatment ranged from 11.9 – 12.7 mg (Table 2). The results of the analysis provide a significant effect on the body length of worker bees (*Apis mellifera*) day old (P<0.05). Worker bee (*Apis mellifera*) body-length ranges from day to day. In this study, the P0, P1, P2, and P3 treatments were in the normal category where according to Febriana, Mahajoeno, and Listyawati (2003), body-length worker bees that just came out of the cell ranged from 11,5 to 14,1 mg. Worker bee body length can be used to predict the amount of production due to one of the main factors. The amount of nectar that can be collected by worker bees is the capacity of the honey bag. The pocket capacity of the honey is directly proportional to the body size of the worker bees (Novita, Saepudin, and Sutriyono,2013).

One of the factors that can affect the body-length of worker bees (*Apis mellifera*) is the adequacy of nutrition obtained, in this case the fulfillment of nutritionobtained from feed that comes from plants that are around the location of the Bee farm. According to Guslim (2007), the higher a place is, the lower is the temperature in that place.

According to Ruttner (1988), adaptation to conditions in the environment reflects changes in the body size of worker bees. Body-length worker bees are generally considered an adaptation for foraging and exploitation of plant resources (Roubik and Ackerman, 1987). The greater is the body size of the worker bee, the farther the flight distance of the worker bee is looking for a feed. This is under the statement of Visscher and Seeley (1982) that worker bees (*Apis mellifera*) regularly forage for some kilometers from the nest, with the most common distance being 2 – 3 Km.

Evaluating the Provision of Octopus sp. Waste Flour on the Wing Length of Worker Bee (Apis mellifera) One Day Old. The average wing-length of worker bees (*Apis mellifera*) of one day old obtained treatment ranged from 9,1 – 9,7 mg (Table 3). The results of the analysis provide a significant effect on the weight of worker bees (*Apis mellifera*) one day old (P<0.05). Worker bee (*Apis mellifera*) wing-length range from day to day in this study the P0, P1, P2, and P3 treatments were in the normal category, where according to Andereb *et al.* (2012) worker honey bees have wingspan 8.54 – 10.01 mm. The length of the highland bee's wings is larger. Presumably, the size of the bee's wings has adapted to the wind speed in the highlands. According to Ruttner (1998) in Putra (1994), in searching for food sources, bees in the highlands need bigger and stronger wings because wind speed in the highlands is different from the area near sea level. According to Ruttner (1987) in Heroriki (1991), with different geographical conditions, it will be related to the character of the wing, especially at growth points at the tips of the wing veins.

Table 3 – The average wing-length of worker bees (*Apis mellifera*) of one day old

Treatment	Wing length of Worker Bees (mg/ekor)
P_0	9,5 ± 0,03
P_1	9,7 ± 0,03
P_2	9,4 ± 0,04
P_3	9,1 ± 0,01

Description: Showed significant differences between treatment groups (P<0.05).

The availability or absence of food around honey bee nests has an impact on wing length as well (*Apis mellifera*). Food can be acquired in the highlands from pollen-producing plants such as coffee and tea trees, which can be used to make coffee and tea flowers. Besides from coffee and tea plants, the nest is surrounded by calliandra trees a type of bee food plant that is well-known for the nectar it produces.

The wing-length size of the worker bee is considered an indicator of adaptation to foraging activities and exploitation of roaming plant resources (Jasmi, 2013). The general range of worker bees in search of food is 2 – 3 Km, but most researchers report distances in looking for a shorter feed, namely 745 – 1413 m with an average distance of 534 – 1138 m depending on the hive and location of the beekeeping(Waddington et al., 1994). In worker bees (*Apis mellifera scutellata*) in Africa, the distance that worker bees can travel in the food search is 1200 m with an average distance of 420 – 620 m (Schneider and McNally, 1993). According to another study, worker bees travel an average distance of 1,073 meters in their search for food (Hall, 1997). Frisch (1967) also said that worker bees can forage for up to 13.5 kilometers.

Evaluating the Pollen. Pollen is the part of the flower that is in the form of a sac containing male gametophyte in flowering plants such as *Pinophyta* and *Magnoliophyta* (Puspaningrum, 2008), while spores are usually produced from non-plants vascular plants such as algae, fungi, mosses, and other low-level vascular plants such as *Bryophyta* and *Pteridophyta* (Suedy, 2012). Pollen can be spread by insects, wind, and water. The spread of pollen is also influenced by various factors, including air turbulence, wind direction, speed, weight and pollen form, height, and strength of the pollen source (Birk and Birk, 1980). Pollen has a diverse morphology consisting of units, polarity, symmetry, shape, aperture size, and sculpture. By identifying the pollen, then the taxonomy of the producing plant will be known.

Morphological characteristics used for pollen identification include the structure of the wall, polarity, symmetry, shape, and size of pollen. Pollen and spores come from plants that live in a certain environment so that they can be used to reconstruct the surrounding flora and vegetation. Based on the identification results3 species of bee food source plants (*Apis mellifera)* were found in Junrejo District, Batu City - East Java. Plants found include *Hibiscus rosa-sinensis* L. (Fig. 1), *Adiantum trapeziforme* (Fig.2) and *Tagetes erecta L*. (Fig. 3).

Hibiscus rosa-sinensis L. belongs to the *Malvaceae* family. *Hibiscus rosa-sinensis L.* has small flowers, pollen collected to form tubes, and have various colors (Kariman, 2014). *Hibiscus rosa-sinensis L.* flower extract functions as an agent antimalarial by absorbing ultraviolet radiation (Hidayat *et al.,* 2015). On wrong one member of the *Malvaceae*, namely *Malva*, has a pollen length of more than 6 µmand pollen diameter is more than 100 µm (Moore *et al.,* 1991). Based on these facts, it may be concluded that there is not a significant difference in size amongst members of the same family.

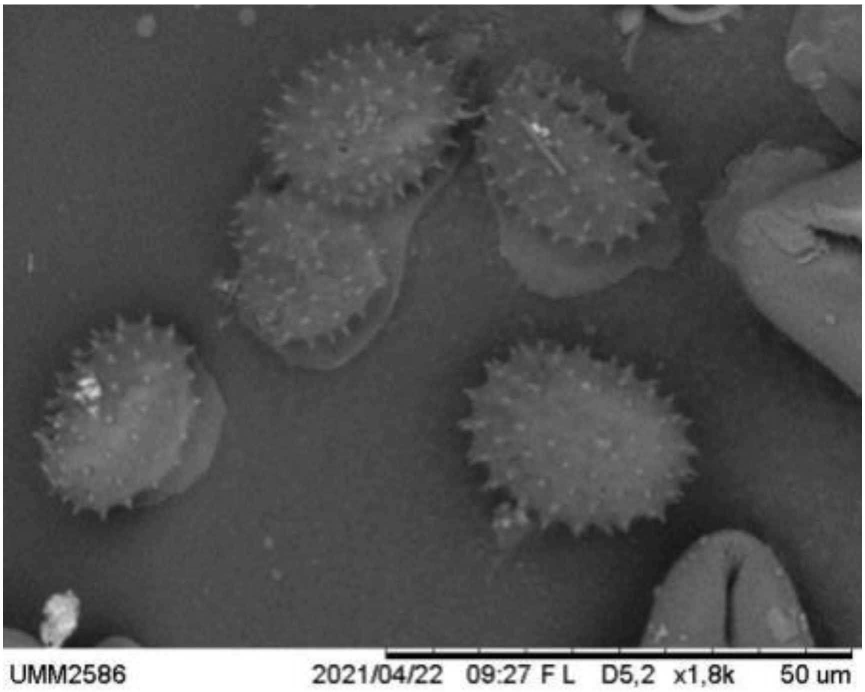

Figure 1 – Microscopic Appearance Pollen of *Hibiscus rosa-sinensis L*.

Hibiscus rosa-sinensis L. with color different flowers have several apertures more than 6 or many on the surface called poly, aperture type is called porat because the aperture is round (port)and the position of the aperture which is often referred to as the panto prefix because the aperture is spread out on the entire surface of the pollen grain. Aperture position in *Hibiscus rosa-sinensis L*. with different flower colors observed in the equatorial area. This is reinforced by Ertdman (1952), if the pores are few pores are only found in the are equatorial, but if the amount is large it can form all over the pollen surface. So for *Hibiscus rosa-sinensis L*. pollen in general, the aperture is often calledpolypantoporate.

The *Adiantum* clan consists of approximately 200 species, has a leaf shape that is diverse and wide distribution area. On the island of Java, this Adiantum clan spread widely from West Java - to East Java at an altitude of 250 - 2000 meters above sea level (Backerand Posthumus, 1939). Suplir *(Adiantum trapeziforme*) is a family ofAdiantaceae, class Filicopsida, order Polypodiales, genus Adiantum, and species of *Adiantum trafeziforme* (*Gifford and Foster, 1989).*

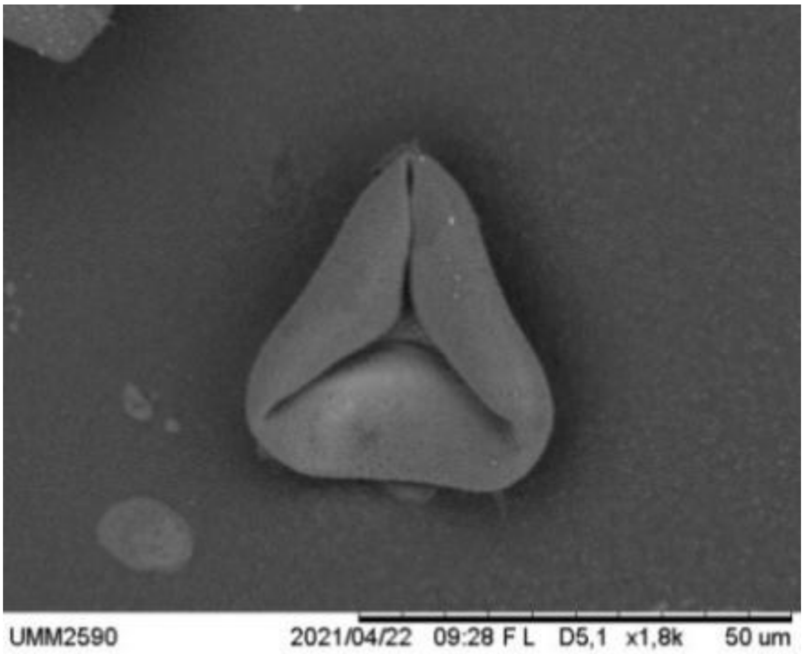

Figure 2 – Microscopic Appearance Pollen of *Adiantum trapeziforme*

Suplir pollen (*Adiantum trapeziforme)* has a polar axis length of 24.26– 43.75 µm, equatorial axis 35.62 – 56.25 µm, oblate-subspheroidal.The genus Adiantum has a wider distribution than diploid plants and at higher elevations, polyploid plants are more common(Perwati and Purnomo, 2002).

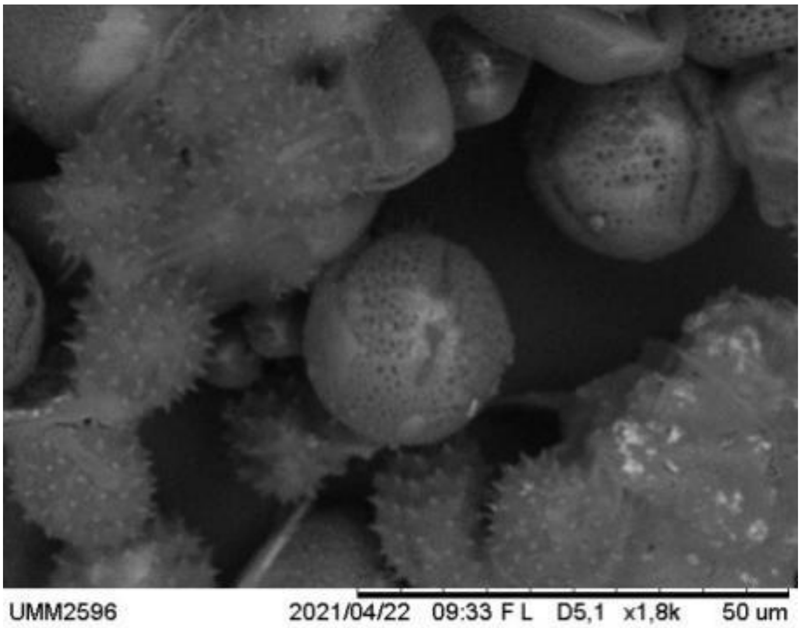

Figure 3 – Microscopic Appearance Pollen of *Tagetes erecta L.*

Tagetes erecta L. commonly known as the African marigold flower belongs to the plant family Asteraceae (*Gopi et al.,* 2012). plant familyAstarecae consists of more than 23,000 species (Moreira, 2011). *Tagetes erecta L*.is a plant that grows wild and has a colorful flower crown bright. *Tagetes erecta L.* serves as a microhabitat refugia for several species of insects because they have brightly colored flowers that can attract the insect. *Tagetes erecta L.* also functions as a repellant or repellent for insect pests. *Tagetes erecta L.*is only visited by a few insects, the wrong one is a bee.

If observed based on the morphological structure, it can be seen that pollen *Tagetes erecta L*. belongs to the class of spheroidal and tricolpate prolate pollen, has aperture type is called porate because the aperture is round (port) and has type ornaments called pentoporate and echinated. *Tagetes erecta L*. has a diameter is approximately between 160.7 µm – 178.2 µm with an exine thickness of 13 µm (Madiha *et al*., 2012).

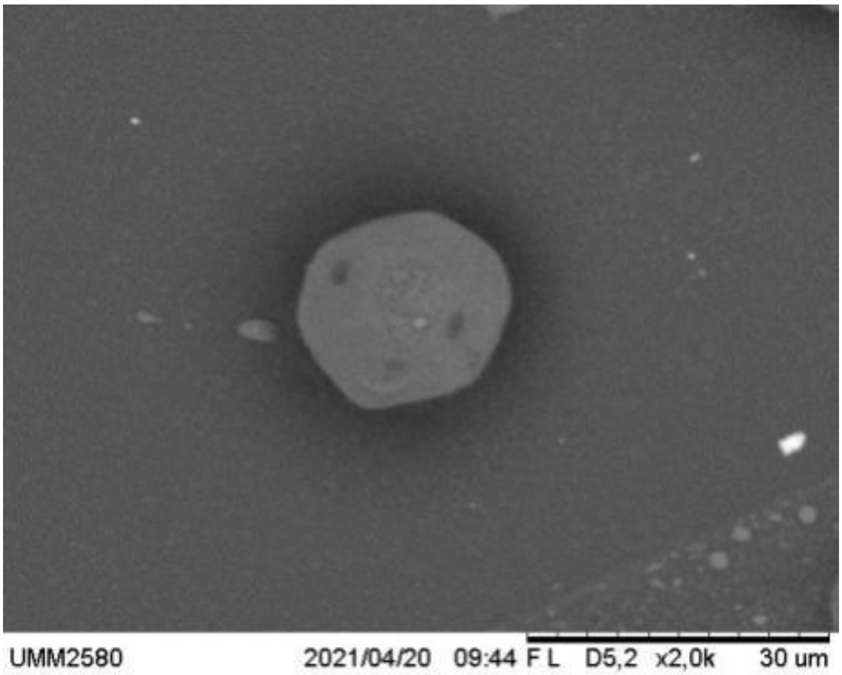

Figure 4 – Microscopic Appearance of Artificial Pollen

Erdtman (1952) mentions the shape, size, or type of pollen can also vary according to the stage of maturity. According to Faegri and Iversen (1989)shows that there are variations in size based on geographical location.

Pollen size per different individuals in one species can also be caused by differences in focus observer optics. Pollen morphology is considered an important taxonomic tool because: the use of relatively simple procedures has been used to identify pollen diversity (Bashir and Khan, 2003).

CONCLUSION

Provision of 87.5% corn pollen flour + 12.5% octopus waste flour gives the best results among other treatments and the type of pollen inhaled by worker bees (*Apis mellifera*) consists of *Hibiscus rosa-sinensis L., Adiantum trapeziforme*, and *Tagetes erecta L.*

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