

## 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 inhaled by worker bee (*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 box with 4 of the frame. 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 ortogonal 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 types of pollen collected by worker bees (*Apis mellifera*) consisted of *Hibiscus rosa-sinensis L.*, *Adiantum trapeziforme* and *Tagetes erecta L.*

**KEYWORDS:** Honey bees (*Apis mellifera*), *Octopus sp.* waste flour, pollen.

### I. INTRODUCTION

Livestock and octopus waste is one example of waste/disposal in the form of liquids, solids and gases that are present at one time and place can reduce environmental quality. Waste in the form of a lot of liquid contains hazardous and toxic materials due to the nature and concentration or amount, either directly or indirectly can contaminate or damage the environment. Octopus processing waste is treated biologically using bacteria using the activated sludge method and the results are not optimal because the red dye from the octopus processing is difficult to degrade naturally biological. Wastewater from octopus processing has not been able to meet the required quality standards set by the government. In this regard, it is necessary to develop other methods namely by utilizing octopus waste into honey bee-made feed. Honey bees need food in the form of nectar and pollen. In addition to nectar and pollen, worker honey bees also collect water to protect colony survival, regulate hive temperature humidity and dissolve honey which will be consumed by honey bee chicks. Nectar is a compound complex produced by the nectaring glands of plants in the form of a solution. Nectar is needed by bees as a source of carbohydrates, a source of vitamins and minerals (Rompas, 2011). Quality and quantity of plant flowering in an area are influenced by the season, so the amount of flower availability as a food source for bees become 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, Fluri and Imdorf, 2005).

Honey bee production in *Apis mellifera* colonies will be obtained during the season drought and the breeder will bring his bee colony to a moderately planted area flowering. During the prolonged rainy season, the honeybee colony is in trouble getting food because nectar and pollen are damaged so this period the number of bee populations will shrink due to lack of feed. If things continue for a long time will result in various developmental disorders and health of the honeybee colony, production decreases, weak to various disease and predators, population declines and even honeybee colonies it leaves the nest. To optimize production in the season it 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 cause 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 bees. Protein is needed to produce royal jelly as feed 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 too conducted by Agil (2008) who used golden snail flour as pollen artificial. One of the ingredients that have not been studied and have the potential as pollen made for honey bees is octopus waste flour (*Octopus sp.*) high protein value. Based on this, it is necessary to conduct a study to evaluate the use of waste octopus flour (*Octopus sp.*) as an alternative source protein, also evaluate the morphology of the pollen.

## II. MATERIALS AND METHODS

**Animal Experiment Prepared :** 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 (frame). 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 include colony boxes (stup), comb (frame), feeder (feeder frame). Tools for manufacturing substitute feed is plastic trays and scales. Equipment used for testing include: 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.

**Pollen Morphology :** This research was conducted by survey, using the *Random Purposive Sampling (RPS)* method. Samples that have been obtained randomly are then entered into 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.

**Data Analysis :** The results of observations during the study were tabulated and analyzed using the assumption test, namely the homogeneity test and independent normal distribution. If the values obtained are significantly different, then proceed with the test orthogonal polynomials.

## III. RESULTS AND DISCUSSIONS

**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 in treatment 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 ( $P < 0.05$ ).

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

Treatment	Weight of Worker Bees (mg/ekor)
P <sub>0</sub>	92,6 ± 0,55
P <sub>1</sub>	94,4 ± 0,55
P <sub>2</sub>	85,6 ± 0,55
P <sub>3</sub>	84,4 ± 0,55

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

According to Winston (1987) that the body-weight 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). Some of the factors can affect the weight of day-old worker bees include cell size, nurse bee size and age, colony population, disease, nectar and pollen obtained as well as the season. In research by Roulston and Cane (2002), it is stated that the body weight 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). In honey bees, the weight of the bees, in this case the weight of the day-old worker bees, will be determined the development of the colony. 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 whose body weight is below the normal range. In addition, one of the factors that can affect bee body weight is also influenced by the location of the farm, where according to Begon *et al.* (1986) that the larger endothermic animals in areas with cooler temperatures than endothermic animals in hot regions. Worker bees (*Apis mellifera*) also have different body sizes from one location to another other location (Hadisoesilo, 2001). With the body weight of a large worker bee, the worker bee can transport nectar and pollen in large quantities and are capable of sucking nectar faster than worker bees which have body weight lower/smaller (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 in 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 ( $P < 0.05$ ).

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

Treatment	Body length of Worker Bees (mg/ekor)
P <sub>0</sub>	12,5 ± 0,05
P <sub>1</sub>	12,7 ± 0,07
P <sub>2</sub>	12,3 ± 0,03
P <sub>3</sub>	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 today. In this study, the P<sub>0</sub>, P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> treatments were in the normal category, where according to Febriana, Mahajoeno and Listyawati (2003) that 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 nutrition obtained 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 it is the body size of the worker bee, the farther the flight distance of the worker bees is looking for a feed. This is in accordance with 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 in 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 P<sub>0</sub>, P<sub>1</sub>, P<sub>2</sub> and P<sub>3</sub> 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 with 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 <sub>0</sub>	9,5 ± 0,03
P <sub>1</sub>	9,7 ± 0,03
P <sub>2</sub>	9,4 ± 0,04
P <sub>3</sub>	9,1 ± 0,01

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

Wing-length is also influenced by the presence or absence of feed that is around the honey bee nests (*Apis mellifera*). In the highlands there are sources of food obtained from coffee trees and tea trees which are pollen-producing plants, so that coffee flowers and tea flowers can be put to good use. Besides trees coffee and tea trees there are also calliandra trees around the nest which are a type of bee food plant that is very popular because of the nectar it has. The wing-length size of the worker bee is considered an indicator of adaptation for 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 search for food is 1200 m with an average distance of 420 – 620 m (Schneider and McNally, 1993). In another study, it was stated that the average distance of worker bees in searching for food was 1,073 m (Hall, 1997). Frisch (1967) also reported that the maximum distance of worker bees in foraging is 13.5 Km.

**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-plant 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 and 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 results 3 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 flower (*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 against malaria by absorbing ultraviolet radiation (Hidayat *et al.*, 2015). On the other hand, a member of the *Malvaceae*, namely *Malva*, has a pollen length of more than 6 μm and pollen diameter is more than 100 μm (Moore *et al.*, 1991). Based on this, it can be said that in the same family has not too big a difference in size.

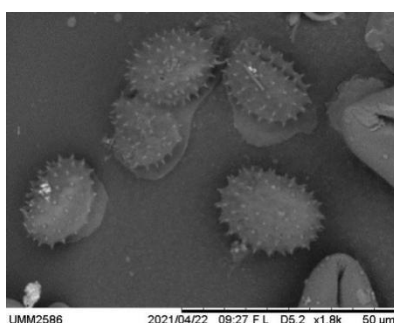


Fig. 1 Microscopic Appearance Pollen of *Hibiscus rosa-sinensis* L.

*Hibiscus rosa-sinensis* L. with different flower colors have a number of apertures more than 6 or many on the surface called poly, aperture type is called porate because the aperture is round (pore) 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 Erdman (1952), if the pores are few pores are only found in the area 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 called polyaperturate. 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 - East Java at an altitude of 250 - 2000 meters above sea level (Backer and Posthumus, 1939). *Suplir* (*Adiantum trapeziforme*) is a family of Adiantaceae, class Filicopsida, order Polypodiales, genus *Adiantum*, and species of *Adiantum trapeziforme* (Gifford and Foster, 1989).

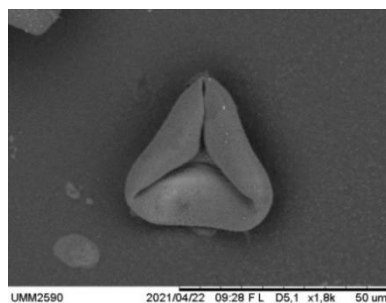


Fig 2. Microscopic Appearance Pollen of *Adiantum trapeziforme*.

*Suplir* pollen (*Adiantum trapeziforme*) has a polar axis length of 24.26–43.75  $\mu\text{m}$ , equatorial axis 35.62–56.25  $\mu\text{m}$ , 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).

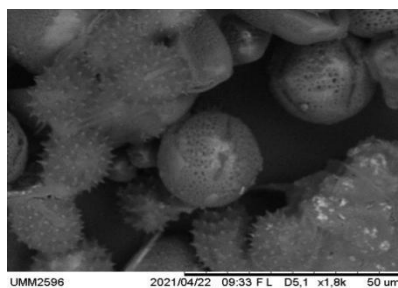


Fig 3. Microscopic Appearance Pollen of *Tagetes erecta L.*

Kenikir flower (*Tagetes erecta L.*), commonly known as the African marigold flower belongs to the plant family Asteraceae (Gopi et al., 2012). plant family Asteraceae 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 repellent 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 an aperture type called porate because the aperture is round (port) and has type ornaments called pentaporate and echinate. *Tagetes erecta L.* has a diameter is approximately between 160.7  $\mu\text{m}$  – 178.2  $\mu\text{m}$  with an exine thickness of 13  $\mu\text{m}$  (Madiha et al., 2012).

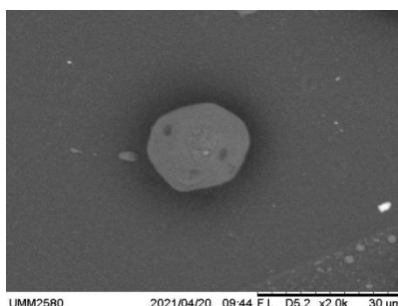


Fig 4. Microscopic Appearance of Artificial Pollen



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).

#### IV. 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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