EFFECT OF SYMBIOTIC BEE-FUNGUS ON SURVIVAL OF *HETEROTRIGONA ITAMA* LARVAE (HYMENOPTERA; APIDAE; MELIPONINI)

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INTRODUCTION

- Meliponiculture (rearing stingless bee in hives) are common in Malaysia.
- Demand for stingless bees colonies increased rapidly:

  - commercialization of stingless bee products (honey, bee bread and propolis)
  - agricultural pollinators.

- An effort to multiply the number of stingless bee products must be developed in order to fulfill the public requirements in a short period of time.

PROBLEM STATEMENT

- Colony multiplication is a key part of stingless bee management.
- More colonies are needed to produce more honey or more colonies to sell.
- Multiplying colonies of stingless bees is not easy:

  - requiring knowledge & experience;

  - ability to recognize and carefully extract the brood discs at the appropriate developmental stage.

- Newly multiplied colonies are usually weak and vulnerable to infestation by pests

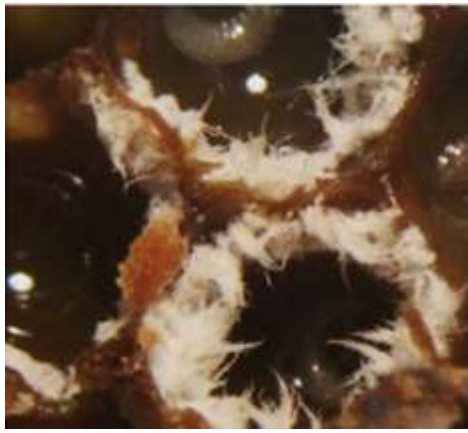
IN-VITRO REARING OF QUEEN STINGLESS BEE

- A simple yet sophisticated technique.
- Could increase the colony numbers by producing more queens rather than naturally produced queen.
- In-vitro rearing technique:

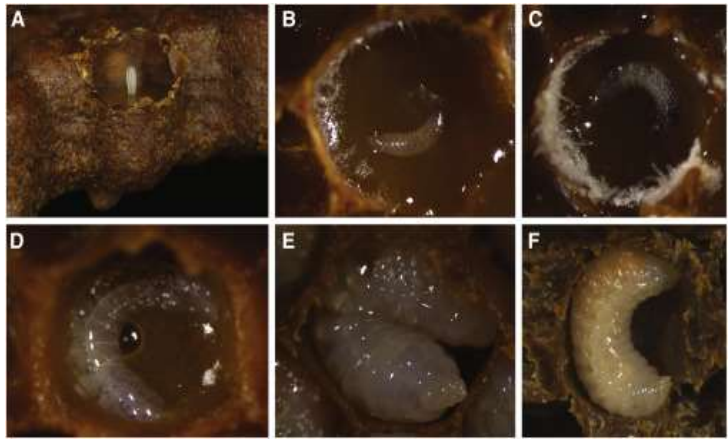
✓produce virgins queens to overcome low production of queens (Jafeé et al., 2015);

✓focused on mass provisioning of female larval.

- Menezes et al. (2013) successfully produced queen of Brazilian stingless bee, *Scaptrotrigona depilis* via in-vitro rearing.
- Larvae of stingless bee require symbiotic fungus to survive (Menezes et al., 2015).

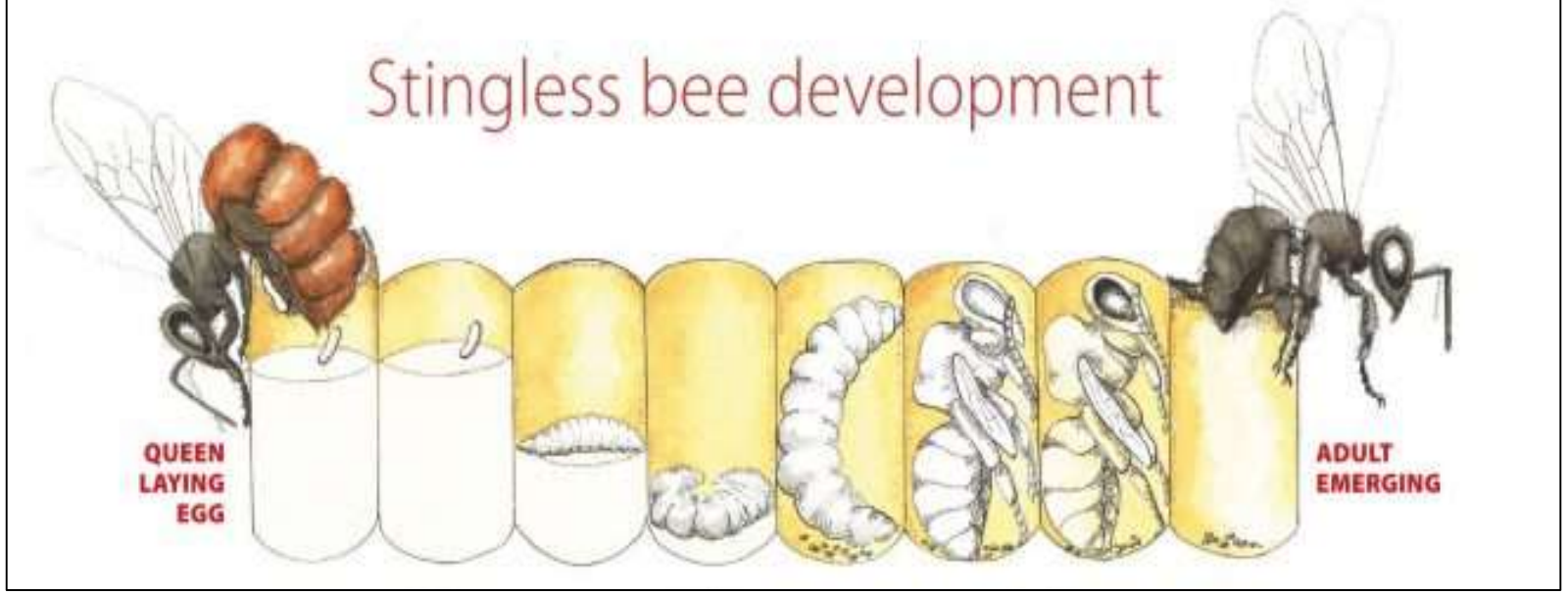


Beneficial fungus consumed by larvae (Menezes et al., 2015).



Observation growth of larvae by Menezes et al., (2015)

To date, there is lack of report on effects of symbiotic bee-fungus on the survival of our native stingless bee.



Egg: 4.20 days

Larval: 10.4 days

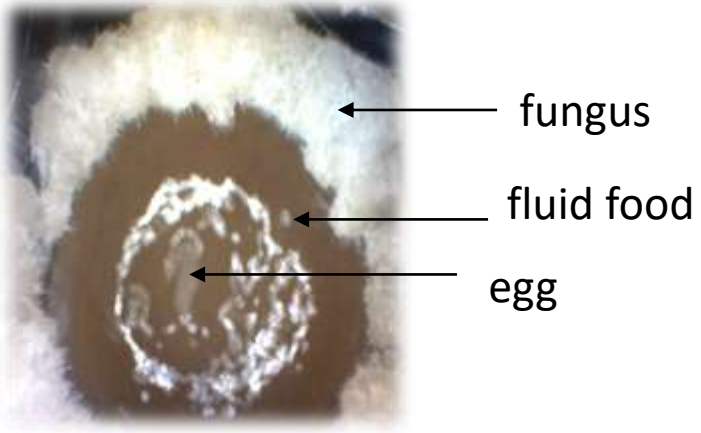
Pupa: 31.9 days

Total: 46.5 days

(Salmah et al., 1996)

- A white fungus grows from the border of the brood cell over surface of larvae food.
- Start to proliferate after 3 days the egg being laid.
- After some period of larval development, the fungus can no longer be seen.

(Menezes et al., 2015)



OBJECTIVES OF STUDY

1. To investigate the effect of symbiotic bee-fungus on survival of stingless bee, *Heterotrigona itama* via in-vitro rearing;
2. To determine the survival of stingless bee, *H. itama* reared in supplemented fungus mycelia treatment;
3. To isolate the symbiotic-bee fungus from brood cells of *H. itama.*

METHODOLOGY

Samples Collection

- Samples (brood cells) were collected at Big Bee Honey and Taman Penyelidikan Alam UMT Bukit Kor, Marang, Terengganu.
- Only one or two young combs per colony were be taken to avoid excessive damage.
- *H. itama* was used as the tested subject.

Newly built brood cell

Extraction of Eggs and Larval Food

Brood cells were squeezed into a centrifuge and pipetted into Elisa plates.

Then, brood cells were gently opened by using needle to remove eggs from brood cell

The eggs were transferred to Elisa plates and placed vertically floating on the fluid food.

Experimental Design on Effect of Symbiotic Bee Fungus

Treatment A

Larval food with fungus.

Treatment B

Larval food without fungus (autoclaved)

Study period: 7 weeks
Under in-vitro conditions:
a) Temperature: 28°C- 32°C
b) Humidity: 75%- 90%
c) Fully in dark condition

The symbiotic bee fungus was isolated from larval food of treatment A on potato dextrose agar (PDA) and it's morphology was identified based on Babitha et al. (2007), Ahn et al. (2008) and Manan et al. (2017)

Incubation of the larvae

- The incubator was set at 28˚C for temperature and above 90% for humidity (Menezes et al., 2013).

Growth and survival of the larvae

- The growth of larvae were examined using Dino-eye microscope camera and measurement of head capsule was taken until the larvae become pupae.
- Survival percentage of larvae were compared between the treatment A and B.

Data analysis

- Data were analysed using SPSS software. T-test was used to determine the differences of growth and survival of stingless bee larvae between treatment A and treatment B.

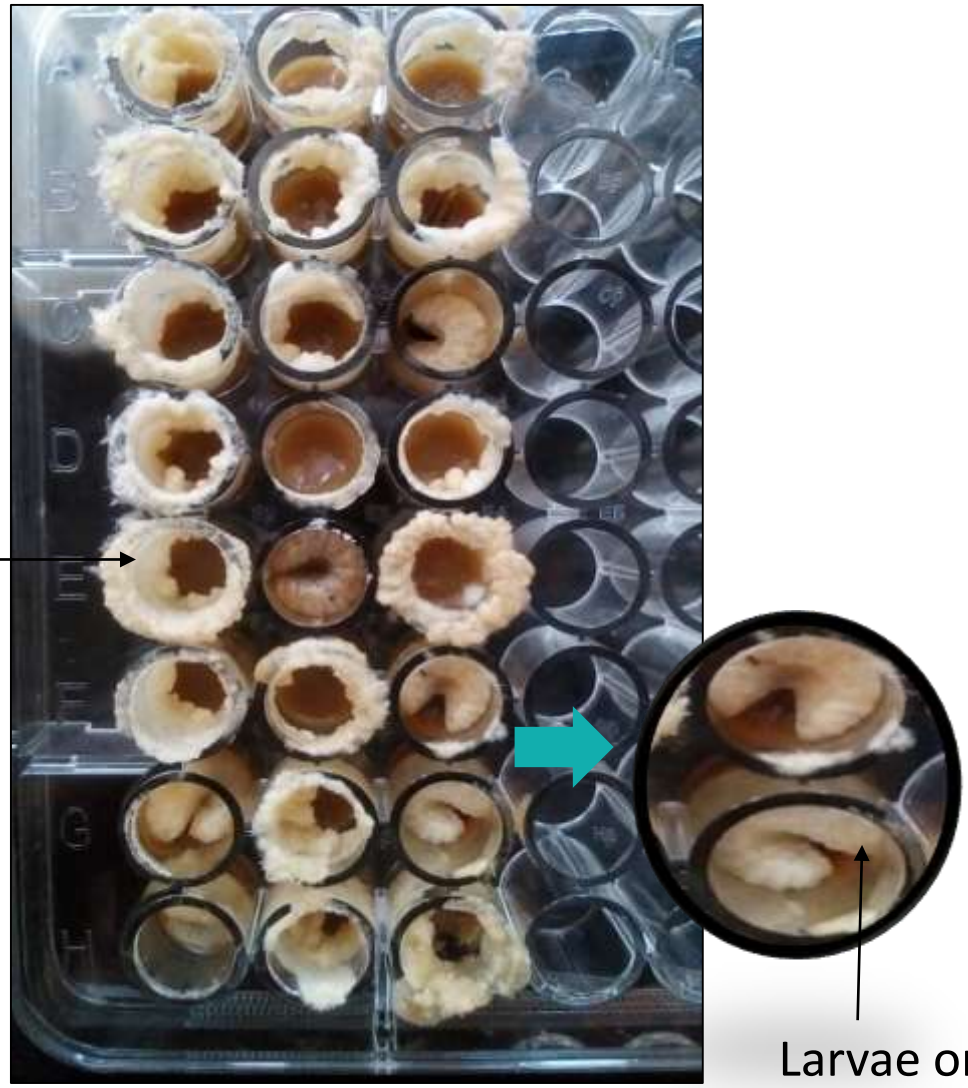
RESULTS & DISCUSSION

Objective 1: Effect of symbiotic bee-fungus on the survival of *H. itama*

Treatment	Survival (%)
Treatment A (with fungus)	78 %
Treatment B (without fungus)	0 %

N=50

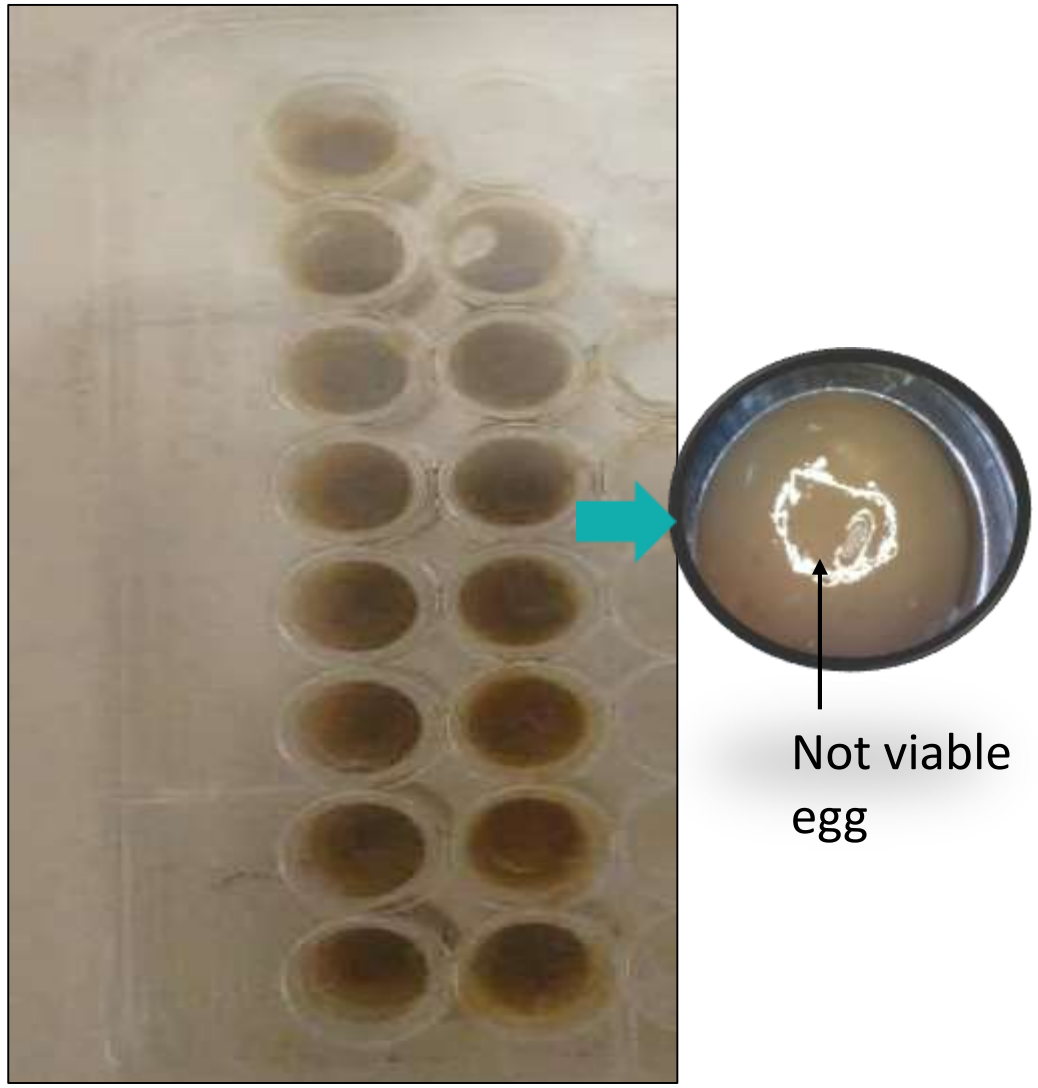
TREATMENT A (with fungus)



Fungus appear on the border of brood cell

Larvae on week 4

TREATMENT B (without fungus)

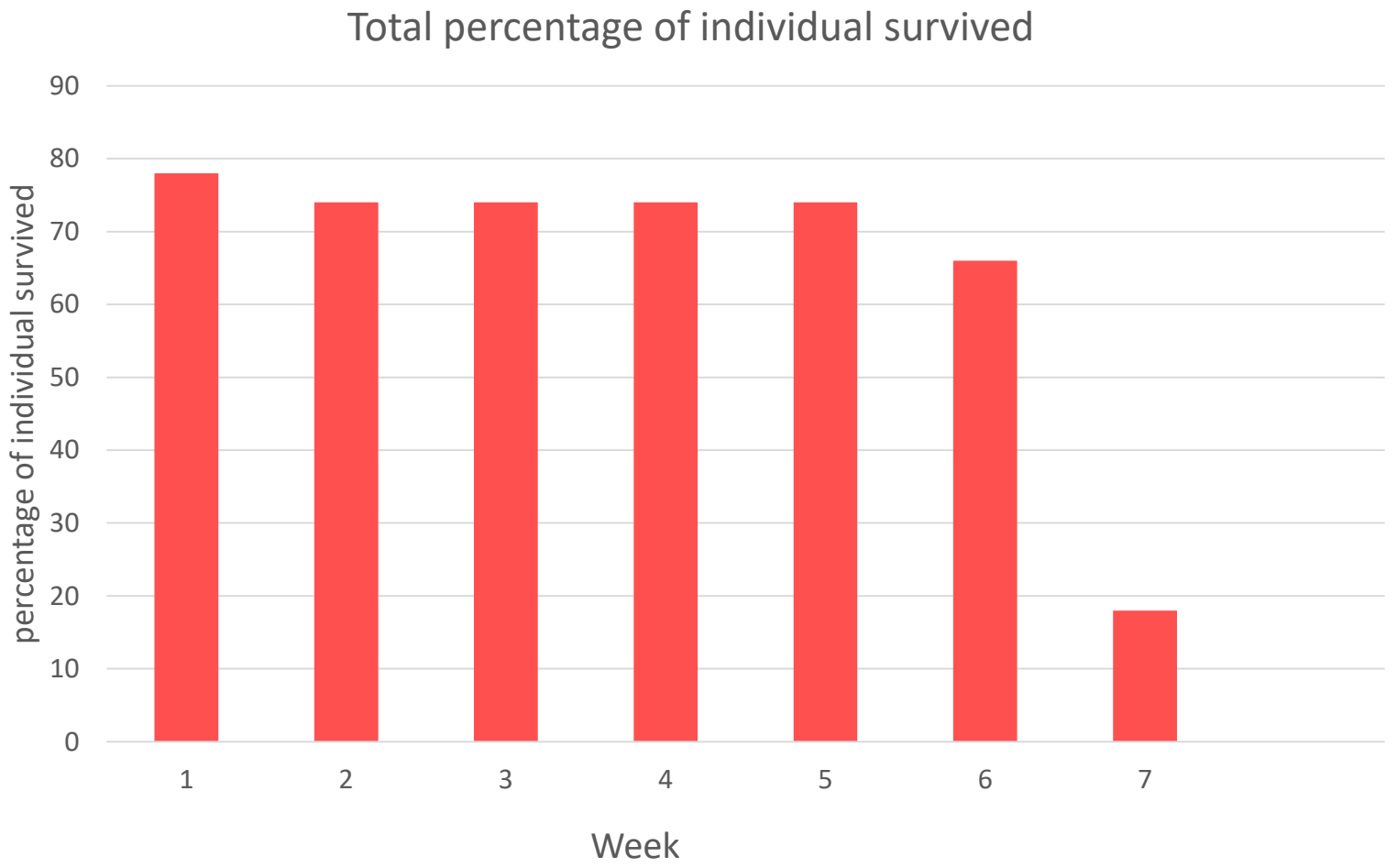


Not viable egg

H. itama larvae consume the fungus

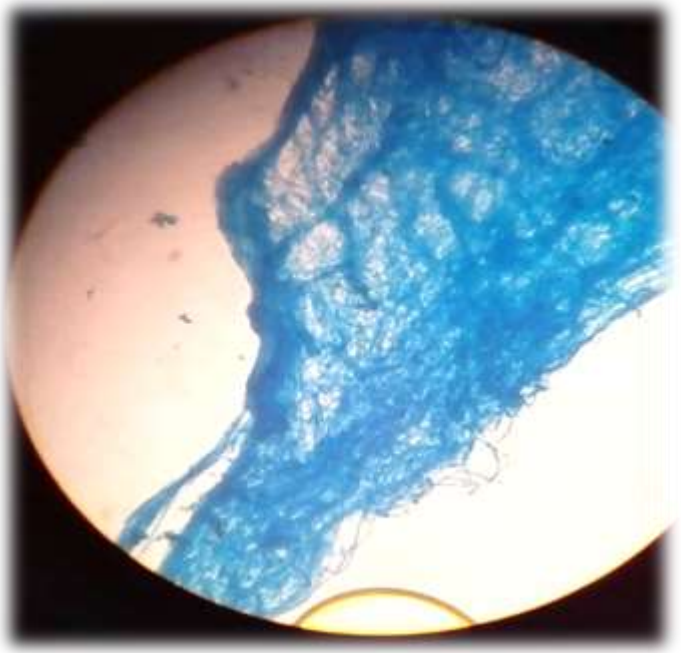
- The larvae need to consume this fungus in order to pupate (Paludo et al., 2018).
- The fungus was believed to provide steroid precursors which essential for the development of the larvae (Menezes et al., 2015).
- The fungus seems to have mutualistic relationship with the stingless bees.
- The brood cells provide stable conditions and associated with a semi liquid food which is crucial for fungus growth (Menezes et al., 2015).
- The fungus offers nutrient for the bees and this was proved when the larvae feed on the fungus mycelia.

Objective 2: Survival of *H. itama* in supplemented fungus mycelia treatment



- Larvae of *H. itama* depends on beneficial fungus in order to survive.
- Larvae of stingless bee were found to lick on mass of spores of the fungus (Roubik, 1989).
- Egg of the *H. itama* did not develop into larvae in the absence of the fungus.
- Fungus offers steroid precursors which crucial for developmental of larvae (Menezes et al., 2015 & Paludo et al., (2018).
- Most of the larvae died in the late stage of larvae.
- Larvae were affected by unknown pathogenic microbes.

Objective 3: Isolation of symbiotic-bee fungus from brood cells of *H. itama*



10x magnification

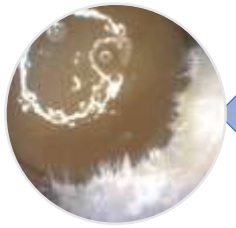
Growth of fungus isolated on PDA after 7 days observed from top view and hyphae formation of the fungus under light microscope

40x magnification

(A) Ascospores of the symbiotic bee-fungus taken by Dino-eye microscope
(B) Cell of fungus cultured on PDA
(C) Pseudomycelium of symbiotic fungus which cultured on PDA after 7 days.

The fungus is identified to be a member of genus *Zygosaccharomyces* which is a kind of yeast. This genus *Zygosaccharomyces* was first described by Barker in 1901 which previously was classified under genus *Saccharomyces* (Kurtzman & James, 2006).

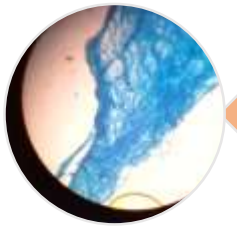
CONCLUSION



This study proves that the larvae of *H. itama* depends on the presence of the beneficial fungus in order to pupate and survive.

Higher percentage of the survived of larvae was observed from the fluid food supplemented with fungus.

The isolation of the creamy white fungus from the fluid food was identified as *Zygosaccharomyces* sp. which is a kind of yeast.



Further study need to be done for identification of the fungus until species level through DNA sequencing and molecular phylogenetics.

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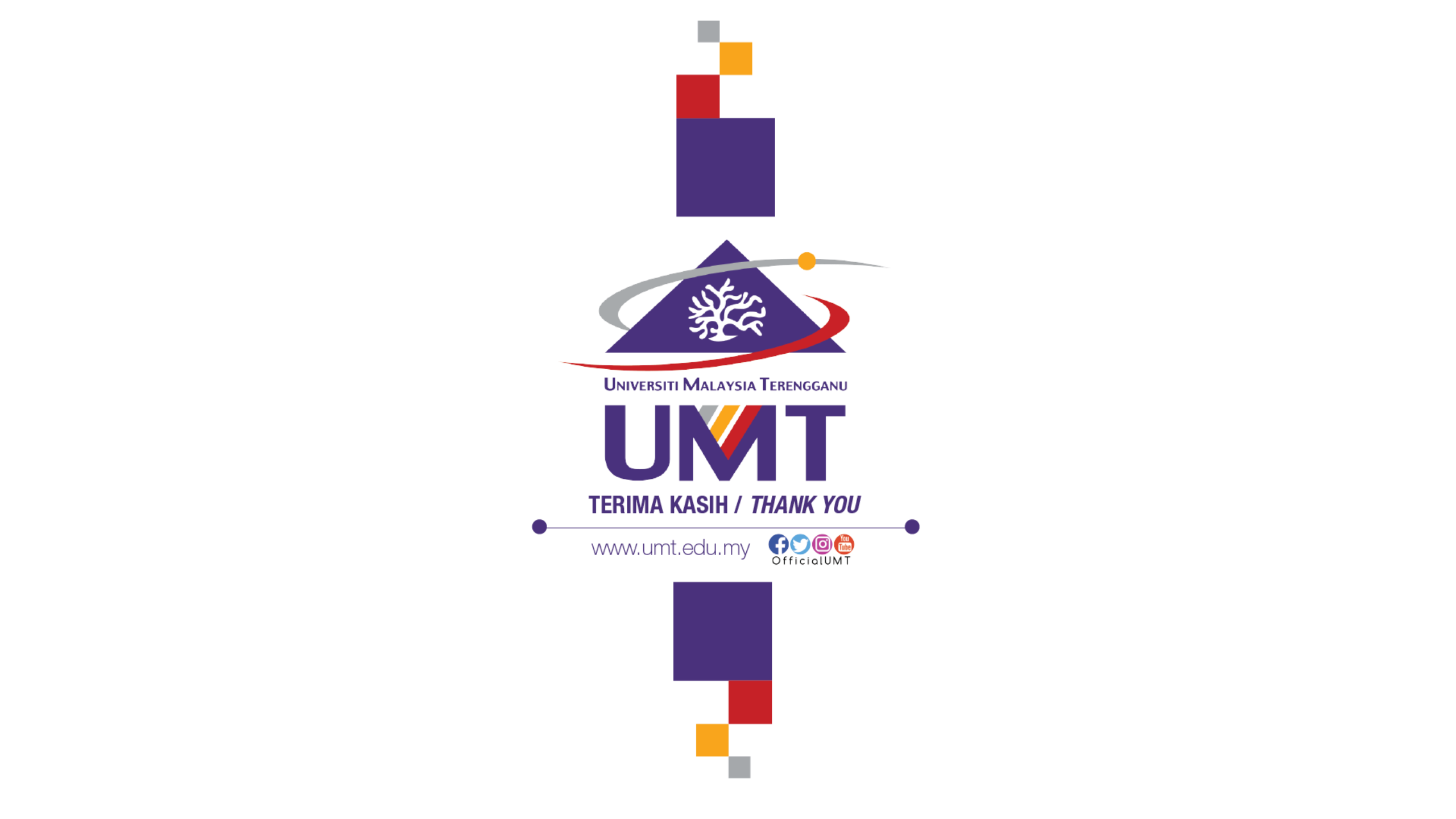
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