SOME INITIAL RESULTS OF REARING HONEY BEE (*APIS CERANA)* IN LABORATORY

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Abstract

In this study we conducted to rear worker honey bee (*Apis cerana*) from larvae to adult stage in the laboratory by using plastic well plates. Our study results were showed that honey bee larvae *Apis cerana* could be reared in the laboratory. The adult worker bee started to emerge on day 17 from grafting. The emergence of worker bee peak on day 18 and declined thereafter. The average survival rate from larvae to pre-pupae stage was 74.6%. The average survival rates from pre-pupae to adult stage and from larvae to adult stage were 40.7 % and 30.4 % respectively.

Keywords: ***Apis cerana, Honey bee, emergence, survival rates, Laboratory***

INTRODUCTION

Honey bees *Apis cerana* Farbricius 1793 is one of nine species of stingbees in the world (Sheppard *et al.*, 2000). This honey bee species has been domesticated, kept and honey-harvested for a long time in Asian countries (Chinh, 1996). However, honey bee in general as well as honey bee species *A. cerana* is declining. Therefore, the development of honey bee research tool to enhance our understanding of honey bee biology and prevent honey bee population from declining is an urgent need. An important tool for this research is the rearing of honey bee larvae *in vitro* (i.e. in the laboratory and in the absence of nurse bees) (Crailsheime *et al*., 2013).

For a long time, rearing *A. mellifera* worker larvae in the laboratory have been conducted by researchers. Rhein (1933) collected royal jelly from queen cells and fed it to larvae in the laboratory. Only workers were produced. Many attempts have been made to rear honey bee *in vitro* (Weaver, 1955; 1974; Jay, 1963; Rembold *et al*., 1974, 1981; Shuel *et al*., 1978).

However, most studies were conducted on *A. mellifera*. In this study, rearing *A. cerana* larvae were conducted by using basic diets 6% gluco, 6% fructose, 1% yeast and 50% royal jelly.

MATERIALS AND METHODS

The studies were carried out in the Honey bee Lab, Department of Agricultural Biology, NAAS. The standard methods for artificial rearing *A. mellifera* larvae (Huang, 2009; Crailsheim *et al*., 2013) were applied to rear *A. cerana* larvae. The larval honey bee diets include 6 grams of D-glucose (6%), 6 grams of D-fructose (6%), 1 gram of yeast (1%) and 37 ml of water. Before grafting larvae diets was pre-warmed (34 ºC, 15 minutes). Each well of 48-well cell culture plate was added 100 µL of diet. First instar larvae (hatched within 24 hrs) are transferred into cells by grafting tool. The plates are placed in a desiccator (10% sulfuric acid solution), kept in an incubator at 34 ºC, 95% RH. Each day, excessive diet was removed by a vacuum. Next, pre-warmed fresh diet is added to the larval (Table 1). Larval mortality is recorded each day.

Table 1. Amount of diet to add to the larval per day.

Days	1	3	4	5	6	Total
Amount (µl)	100	120	120	130	130	600

When a larva defecates, it is transferred to a pupation plate. Pupating plates are 24-well plates matted with 2 layers of dust-free Kimwipes. The mature larvae were dried on 2 layers of pre-cleaned Kimwipes and transferred to the pupation plates by a modified grafting tool. The pupation plates are kept in an incubator at 34 ºC and 75% RH until adult bees emerge. After 3 days the bees were inspected for survival to pupation plates.

RESULTS AND DISCUSSION

1. The growth and developmental time from larvae to adults

When larvae developed their body gradually increased in size and often moved (Fig 1).

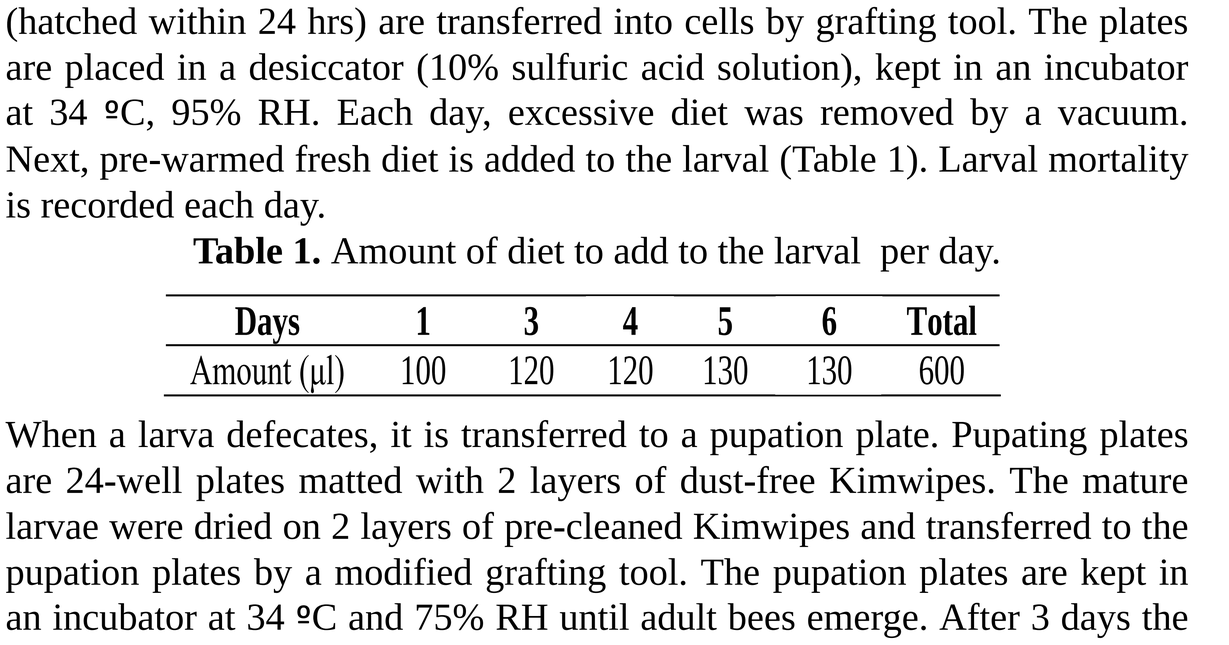

Fig1. The development of *A. cerana* larvae rearing *in vitro*. A: day 1; B: day 3; C: day 5; D: day 7; E: day 11; F: day 13; G day 15; H: day 17.

It could be observed the surviving young larvae had respiratory movement and a light reflecting surface under a stereomicroscope for young larvae on day 3, and day 4 from grafting. From day 5, larvae movement could be seen with the naked eye. On the contrary dead larvae could be recognized by absence of movement, lack of turgidity, flattened body. Larvae defecated by day 6 or day 7 after grafting. In our experiment defecation were indicated by the presence of uric acid crystals and the appearance of light yellow feces (Fig 2) When a larva defecates it is important to clean excessive diets and feces off the larvae and transferring to pupating plate.

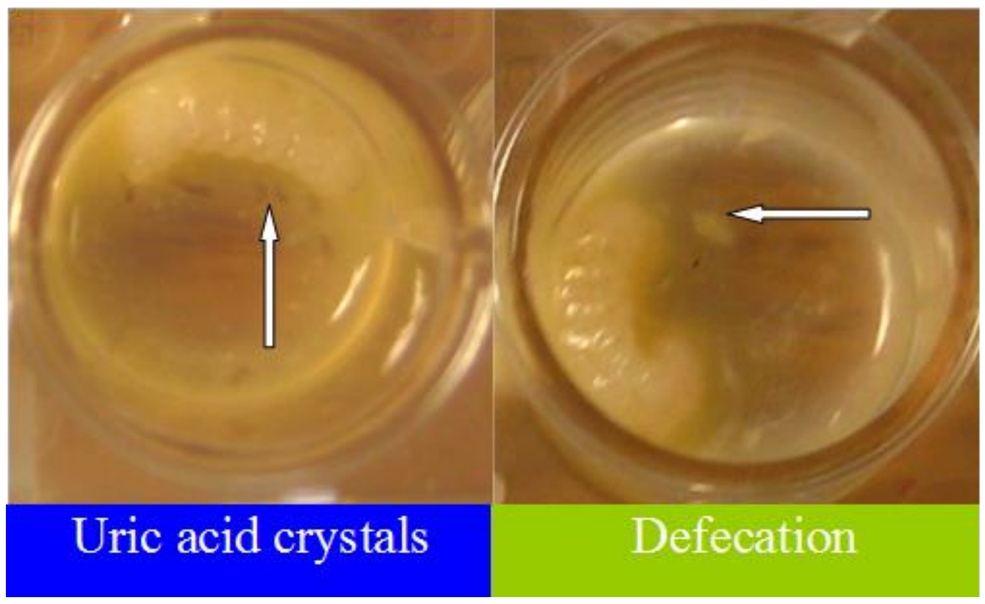

Fig 2. Defecated larvae with transparent uric acid and feces

2. The developmental time of larvae from grafting to emerging

Fig. 3 demonstrates the emerge time of *A. cerana* worker bee reared *in vitro*. The observation on the emerge of 68 worker bees showed that 17.6% worker bee emerged on day 17 after grafting, worker bees emerged peak on day 18 (57.4%) and then declined

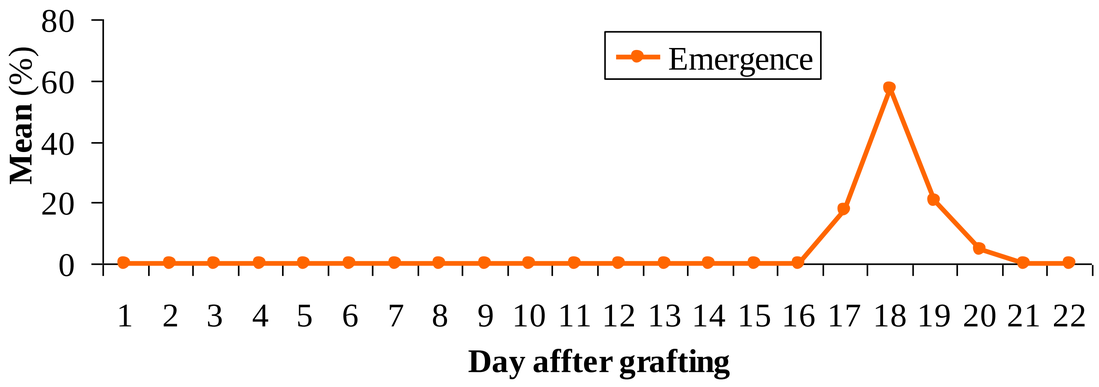

Fig. 3. The on set of adult emerge rearing in vitro

3. Survival rates

Table 2 showed the survival rates of *A. cerana* honey bee larvae reared *in vitro* for two trials with a total of 224 larvae was grafted.

Table 2. The average survival rates of *A. cerana* larvae rearing in vitro

Trials	No. of grafted larvae	No. of pre-pupae	No. of emerged adults	Survival rates (%)		
				Larvae to pre-pupae	Pre-pupae to adults	Larvae to adults
1	135	86	26	63.7	30.2	19.3
2	89	81	42	91.1	51.9	47.2
Total	**224**	**167**	**68**	**74.6**	**40.7**	**30.4**

The average survival rate from larvae to pre-pupe was 74.6%. While of those from pre-pupae to adults and from larvae to adults were only 40.7% and 30.4% respectively. This is considerably much lower than these of larvae to adults on *A. mellifera* reported 50% up to 80% (Peng *et al.*, 1992; Aupinel *et al*., 2005; Huang, 2009, Kaftanoglu *et al.*, 2011; Chan, 2012). Our data demonstrated that most of them died in pre-pupae to adult stage. Young honey bee larvae is fed several times daily with royal jelly, a high postdefecation mortality were reported by (Weaver, 1955; Jay, 1963).