Two Artificial Diet Formulations For Troides Helena Linne Larvae (Lepidoptera : Papilionidae) In Bantimurung-Bulusaraung National Park, South Sulawesi

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Abstract: The Common Birdwing *Troides helena* Linne (Lepidoptera : Papilionidae) is the one of butterflies species protected under Indonesian laws i.e. PP 7 and 8 year 1999 and UU no.5 year 1990. *T. helena* including on the list of International Trade in Endangered Species of Wild Flora and Fauna (CITES) Appendix II. The artificial diet for *T. helena* never done and we were used mung bean sprout and red bean as the substitute of wheat germ that more cheap and easy to find in our areas in South Sulawesi. The result was showed the high percentage of *T. helena* larvae survival on red bean 57,05% more higher than mung bean sprout 51,66%. *A. tagala* leaves as a control given a highest is 61,36%. The larvae weight before pre-pupae stage was highest on *A. tagala* leaves 4,86 mg. The lowest larvae weight on the mung bean sprout 2,13 mg. The red bean was given the higher larvae weight on artificial diet is 3,22 mg. This results different to pupae weight in every diet. The pupae weight on mung bean sprout is 2,55 mg compare to red bean 2,45 mg. The male emergence from red bean was higher 71,20% and lower on mung bean sprout 55%. The female from red bean was higher 48,08 % and 33% on mung bean sprout. *T. helena* male was emerged from mung bean sprout dan red bean almost be abnormal individual. Most of *T. helena* males has a crippling wings and the abdoment coherent in their exuviae. They has a short living periode about 4-6 h on their sites. On several *T. helena* female, we found few eggs reduce on her ovary (based on the dissection after her death). The formulation of artificial diet that suitable for *T. helena* larvae is red bean based the results on larvae survival and adult emergences. The composition of red bean almost similar to *A. tagala* leaves (control).

Keywords: *Troides helena*, artificial diet, mung bean sprout, red bean

1. INTRODUCTION

The Common Birdwing *Troides helena* Linne (Lepidoptera: Papilionidae) is one of butterflies listed in the International Trade in Endangered Species of Wild Flora and Fauna (CITES) Appendix II (Salmah *et al.*, 2000)[18]. In Indonesia, there are regulations about the trade and utilization of wild animals and plants, *i.e.* PP 7 and 8 year 1999 and UU no.5 year 1990 (accessible at http://www.dephut.go.id/index). Butterflies protected under Indonesian laws includes 19 species in the genera of *Ornithoptera*, *Troides*, *Trogonoptera* and one species from Nymphalidae, the Sulawesi lacewing butterfly, *Cethosia myrina* (Peggie, 2011)[17]. The difference between *T. helena* with the other butterflies is that several males have structural scales that beneath also have yellow pigment (Endo and Ueda, 2004; Peggie, 2011)[7,17]. In South Sulawesi, *T. helena* commonly found in Bantimurung-Bulusaraung National Park, Maros District. *T. helena* larvae is known to feed on the *Aristolochia tagala* leaves (Aristolochiae). Nishida *et al.*, (1993)[13] and Tsukada and Nishiyama (1982)[21] found that major secondary metabolites from these plants is Aristolochic acid with various concentrations found in the tribe of Troidini butterflies ranging from zero to more than 150 µg per insect. This component was higher approximately 718,5 µg in the body of female *T. magellanus* and 3,3 µg in the wing pair (Mebs and Schneider, 2002)[14]. The *T. helena* larvae use aristolochic acid as the feeding deterrence to protect from predators especially sparrows (Nishida and Fukami, 1989)[12]. Currently, the greatest thread of deforestation made the *T. helena* lose their host plant and move to the other areas. Lepidopteran larvae need the nutrition and fatty acid such as linoleat and linolenat in their development (Astuti, 1992)[2]. In fact, the larvae stage as the main factor of insect to be an adult (Braby, 2000; Chapman, 1998)[4,5]. Commonly insects reared on plant tissue might not develop as well as when they are reared on artificial diet because the plant secondary chemistry and lower nutritional value might arrest their optimal development (Blanco *et al.*, 2008)[3]. Insect artificial diet are essential tools for insect mass rearing research (Glass and Pan, 1982)[10]. One of the artificial diets used as protein source for insect is wheat germ (Cohen, 2003; Moore, *1985*)[6,15]. Wheat germ is a tiny part of a wheat kernel that contain a high protein and source of vitamin E (Anonymous, 2011)[1]. Unfortunately, wheat germ as the main component for artificial diet is very difficult to find in our the Southern part of Sulawesi. In butterflies artificial diet, we could modified the nutrition inside and add other component that suitable for insect target (Fordyce and Nice, 2008; Mebs and Schneider, 2002)[8,14]. Because the great deforestation in our butterflies habitat, so that *T. helena* larva need a specific artificial diet to improve their presence. Currently, the

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artificial diet for *T. helena* in South Sulawesi has never been done. Formulation artificial diet for *T. helena* can increase the number of butterflies in mass rearing and keep from endangered species status. The purpose of the present study was to modify the Morton's artificial diet to make it more suitable for *T. helena* larvae. Mung bean sprout and red bean were used as the substitute component for wheat germ. The materials are very cheap and easy to find in butterflies habitat. In addition, dried leaves of *A. tagala* were added into the artificial diet as the feeding attractant for *T. helena* larvae.

2. MATERIALS AND METHODS

2.1 The Insects

T. helena pupae were collected on August 2012 from their habitat in forest on Bantimurung-Bulusaraung National Park, Maros District South Sulawesi Province Indonesia. The collected pupae were reared in the laboratory screen cage (2 x 1 x 2 m; width x length x height) at 21 - 27°C and 73 - 90,67% relative humidity. The emerged butterflies were transferred into the field screen cage (5 x 12 x 6 m; width x length x height) for mass rearing. The host plant *A. tagala* were growth to facilitate mating and oviposition of female butterflies. Flowers plant of *Ixora paludosa* and *Clerodendron japonicum* inside the screen cage were supplied as the nectar sources for butterflies. The honey-water 10% was given as the supplementary food for *T. helena* adults (Soewarno, 2010)[20]

2.2 Component and preparation of artificial diets

The recipe of the artificial diets used in the current study is shown in Table 1. Our artificial diet differed from that devised by Morton (1979)[16] in that wheat germ, Wesson salts, formaldehyde, potassium hydroxide, acetic acid and choline chloride were omitted. Mung bean sprout and red bean were added as the subtitute of wheat germ. Soybean and red bean were crushed separated, filtered with an 80 mesh sieve and placed in the plastic container (Gan *et al.*, 2011)[9]. The *A. tagala* leaves selected be carefully, washed and dried at the room temperature about 3 to 5 days. The dried leaves were crushed and keep into the plastic container. The mung bean sprout and 10 mL distilled water were grinding. All of the dry component thoroughly mixed together on the erlenmeyer. The green agar and distilled water were boiled for 3-10 min, with stirring. When the solution was about 75-85°C, the mung bean sprout and all of the dry component were added. The same procedure we used for red bean artificial diet. Chloramfenikol and sorbic acid were used as the anti-microbial. Two types of artificial diet without wheat germ were prepared : one containing the mung bean sprout and the other containing red bean. Finally, the warm artificial diet was dispensed into plastic containers (5 x 4 cm; diameter x height) up until about 0,5 cm from container based. and leave to dry for 24 h. The artificial diets were stored in freezer at 4°C. The diets then were brought to room temperature before used it.

2.3. The effect of artificial diets to *T. helena* larvae

The solid artificial diets of mung bean sprout were cut into 1 cm x 1 cm x 1 cm thick pieces and placed in plastic containers (1 pieces; 5 x 4 cm, diameter x height). The one of first instar larvae was placed on the food. The same procedure was done for red bean artificial diet. There were ten replication used for each artificial diet. *A. tagala* leaves were used as the control food. Everyday artificial diet and control were replaced and recorded. Number of survival, larva and pupae development and sex ratio of adult butterflies were recorded. Before pre-pupae stage, the larvae were transferred in to plastic cup (5 x 15 cm; diameter x height) and given a woody stick as the site for pupae stage. The data were analyzed using microcomputer analysis (SPSS, Excel) and significant differences were tested using Duncan's multiple range method.

3. RESULT AND DISCUSSION

In this study, the two types of artificial diets formulation for *T. helena* larvae is shown on Table 1 with mung bean sprout and red bean used as the substitue for wheat germ.

Table 1. Composition of two artificial diet for mass rearing of *Troides helena larvae*

Component of Morton's artificial diet	Amount	Modified artificial diet recipes	
		Mung bean sprout	Red bean
Soy flour (g)	7.0	7.0	7.0
Wheat germ (g)	6.0	-	-
Yeast (g)	6.0	5.5	5.5
Sucrose (g)	3.6	3.6	3.6
Aristolochia tagala dried leaves (g)	1.5	1.5	1.5
Wesson salts (g)	1.0	-	-
Ascorbic acid (g)	0.4	0.4	0.4
Vitamin B (g)	-	0.2	0.2
Potassium sorbate (g)	0.2	-	-
Methyl-para hydrobenzoate (g)	0.15	-	-
Aureomycine (g)	0.023	-	-
Chloramfenikol (g)	-	0.1	0.1
Sorbic acid (g)	-	0.1	0.1
Mung bean sprout (g)	-	6.0	-
Red bean flour (g)	-	-	6.0
Agar (g)	1.9	1.9	1.9
Formaldehyde solution (10%)	0.43	-	-
Potassium hydroxide (4M)	0.8	-	-
Acetic acid (25%)	1.14	-	-
Choline chloride solution (50%)	0.23	-	-
Distilled water (mL)	to mass	250	250

The high percentage of *T. helena* larvae survival on artificial diet is shown on Table 2. *T. helena* larvae survival shown on *A. tagala* leaves as a control gave a highest survival of 61.36%. Use of red bean as artificial diet showed survival level of 57.05% which is higher than use of mung bean sprout (51.66%).

Table 2. The effect of artificial diets on *T. helena* development

Diet	Larvae survival (%)	Weight (mg)		Adult emergence (%)	
		Larvae	Pupae	Male	Female
Mung bean sprout	$51{,}66^{\pm 3{,}09a}$	$2{,}13^{\pm 0{,}33a}$	$2{,}55^{\pm 0{,}35a}$	$55{,}00^{\pm 2{,}16a}$	$33{,}19^{\pm 0{,}01a}$
Red bean	$57{,}05^{\pm 6{,}50b}$	$3{,}22^{\pm 1{,}12a}$	$2{,}45^{\pm 0{,}33a}$	$71{,}20^{\pm 0{,}11c}$	$48{,}08^{\pm 1{,}01ab}$
A.tagala leaves (control)	$61{,}36^{\pm 5{,}79b}$	$4{,}86^{\pm 3{,}62b}$	$4{,}65^{\pm 1{,}21a}$	$90{,}11^{\pm 2{,}75b}$	$70{,}34^{\pm 4{,}01c}$

The larvae weight before pre-pupae stage was highest on *A. tagala* leaves 4.86 mg compared to the artificial diets used. The mung bean sprout diet treatment showed the lowest larva weight of 2.13 mg and red bean diet shows a bit higher larvae weight (3,22 mg) compared to mung bean. On the contrary, pupae weight in every diet shows similar figures of 2.55 mg and 2.45 mg for pupae weight fed on artificial diet containing mung bean sprout and red bean, respectively.

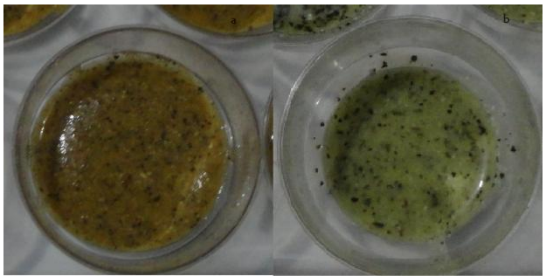

Fig. 1 Two types of *T. helena* artificial diet.
a) red bean; b) mung bean sprout

Insect mass rearing technology has used the red bean as the important protein source in artificial diet (Fig.1). It was applied to *Spodoptera exigua* (Hùbner) and *Crocidolomia binotalis* in laboratory (Sari and Prijono, 2004)[19]. The highest larvae weight was showed on *A. tagala* leaves because it is the suitable host plant for *T. helena*. Mung bean and red bean as the artificial diet with modified ingredients found to be suitable for *T. helena* larvae. Mebs and Schneider (2002)[14] state that although the artificial diet has a complete nutrition, the absence of volatile oils and secondary metabolytes will affect the development of the target insect. The aristolochic acid on *A. tagala* leaves act as the clue for *T. helena* to identify their host. Emergence of male butterflies when using red bean as artificial diet was higher for about 71.2% compared to mung bean sprout (55%). Similarly, female from red bean diet treatment was slightly higher than female emergence in mung bean sprout treatment shown by the female emergence percentage of 48.08% and 33% for red bean and mung bean sprout diet treatments, respectively. However, the male *T. helena* emerged from the artificial diets made from mung bean sprout and red bean almost be abnormal individual. Fig. 2 shows that most of *T. helena* males had a crippling wings (c) and the abdoment coherent in their exuviae (b). In addition, the individuals had a short living periode about 4-6 h on their sites. On several *T. helena* females, few eggs reduction in the ovary was observed (a) (based on the dissection after the death of the butterflies). In addition, the female failed to conduct the copulation process. The crippling wings phenomenon on *T. helena* males was assumed to be related to the absence of linseed oil in formulation of the artificial diet used in the recent study. Astuti (1992)[2] state that the presence linseed oil on the artificial diet can decrease the crippling wings on the *Papilio demoleus* adults. The cytosterol on the citrus leaves has many contribution on *P. demoleus* development. The similar result was showed on Holloway *et al.*, (1991)[11] on *Bicyclus spp.* which needs a cytosterol compound in their wings pattern and development. Other assumption is that the artificial diet was unbalance to support the growth and development of the butterfly. This resulted in abnormality in wings development and reproduction in the organ of the female. Moore (1985)[15] state that the precise amount of nutrition in the diet in the larvae stage will affect on the development of the larvae to be an adult. Natural composition of *A. tagala* leaves contains amino acid, fatty acid and vitamins different to the present artificial diet in Fig. 1. Blanco *et al.*, (2009)[3] state that handling and transfer process of the larvae to the new blocks of artificial diet can affect their organ development. During the process, larvae must learn to accept the artificial diet new blocks and unlikely to cope with the materials. Other limiting factor for *T. helena* adults development is presence of predatory ant (*Oecophylla smaragdina*) observed in the screen cages of the recent study (Fig. 2 d). Nishida and Fukami (1989)[12] state that among the common predators for *T. helena* adults are gecko, ants and spiders.

Fig. 2 *Troides helena* adults.

a) female with her ovary and eggs inside; b) male abdoment coherent in his exuviae;
c) crippling male wings; d) male was attacked by predatory ants

4. CONCLUSION

The formulation of artificial diet that suitable for *T. helena* larvae is red bean based the results on larvae survival and adult emergences. The composition of red bean almost similar to *A. tagala* leaves (control). The absence of linseed oil on the artificial diet has contributed to crippling adult in their wings development.

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