REARING OF CROCIDOLOMIA BINOTALIS (ZELLER) (LEPIDOPTERA: PYRALIDAE) ON THE ARTIFICIAL DIETS

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Abstract- Studies on rearing of *Crocidolomia binotalis* (Zeller) on different artificial diets by using insect diet recipe based on insect diet recipe from Kurihara, Jack bean diet and egg yolk *Couturnix japonica* (Squil) diet, and cabagge fresh leaves diet as a its natural diet. The insect rearing was conducted under controlled laboratory conditions of 27±2°C, 65 ± 5% RH, during 2015; in the Laboratory of Entomology, Faculty of Biology University of Gadjah Mada, Yogyakarta, Indonesia. The results showed that the larval stage of *C. binotalis* on fresh cabagge leaves diets resulted in highest percentage survival rates among all the parameters of larval stages of *C. binotalis* (90%). However, the use of Jack bean and quail egg yolk as artificial diets showed percentage survival rates respectively were 70% and 66 %. The pupal weighst of *C. binotalis* feed on jack bean and egg yolk diets were lower than feed on cabagge fresh leaves and Kurihara recipe diet. The incubation period for eggs was 4 days (range 3-6 days). The percentage of egg hatched of the insect reared on fresh cabagge leaved and Kurihara diet was 44.33- 51.67% , however eggs from imago that the larvae feed on Jack bean and eggs yolk are not hatched. The squil eggs yolk diet with improvinng some more protein engridients.is recommended to be an alternative diet for rearing larva *C. binotalis* coloni under laboratory conditon.

Key words- *Crocidolomia binotalis*, cabbage, jack bean, quail egg yolks

I. INTRODUCTION

Crocidolomia binotalis Zeller (Lepidoptera: Pyralidae), is harmful and economically important caterpillars on cabbage crop in Indonesia.this insect is the secondary pest of cabbage, may become a serious problem in the dry season. [1] The larvae of *C. binotalis* is a common pest of cruciferous crops with a worldwide distribution in tropical and temperate regions. The distribution area of the insect is reported in South and Southeast Asia, Australia, South Africa, Tanzania and the Pacific Islands [2]. Insect pests were reared on artificial diets for mass production of the insect for insect pest control strategies. The use of artificial diet for rearing insect has many benefit such as it does not need lot of numbers area for growing host plant for natural insect diet and the insect diet can be supplied continously. The insect artificial diet is defined than any diet that not the natural insect diet [3],[4]. The insects artificial diets usually contain numerous ingredients that are expensive and time consuming to prepare. Costs and preparation times can be reduced by careful selection of diet ingredients for rearing healthy insects [3.], [4], [5.].[6]. Balanced nutrient intake plays a very important role in the normal growth and reproductive cycle of insects and allows continuous rearing over a many generations. Considerable effort has been invested in the development of cost-effective diets based on the use of cheaper ingredients, or by reducing and eliminating nonessential ingredient [7][8].[9].[10]. The artificial diet developed by Kurihara [11] has been use for rearing *C. binotalis* for 45 generations in the laboratory of Insect Toxicology, the Institute of Physical and Chemical Research (IPCR, Wako, Saitama, 351-01, Japan.

The objective of this study was to examine the effect of different diets on the length of development, percent survival, pupa weight, and female fecundity of an established laboratory colony of *C. binotalis.*

II. DETAILS EXPERIMENTAL

2.1 The Rearing Insects

Crocidolomia binotalis larvae were collected from the cabagge crops in Kopeng, Magelang District, Central Java Province, Indonesia. The collected larvae were reared in the plastic boxes in the laboratory. The emerged moths were transferred into the screen cage (40 x 40 x 40 cm; width x length x height) for mass rearing. The host plant, cabbage was grown in the plastic bags (25 x 30 cm.) and put inside the sceen cage to supplies the nectar for facilitate mating and oviposition of female moths. The honey was diluted with water to 10% (v/v) was given as the supplementary food for the moths. The sex ratio and weight of the resulting pupae were recorded. At adult emergence, 30 females and an equal number of males from the same diet treatment were selected for mating in single pairs, transferred to screen cage (20x20x20 cm). The number of eggs that hatched and the number of larvae that survived from eggs to pupation, the pupal sex ratio, larval development time, pupal weight, and duration of the pupal stage were recorded.

2.2 Component and preparation of artificial diets

The recipe of the artificial diets used in the current study is shown in Table 1. The standard diet, widely used for rearing *C. binotalis* was modified from insect diet recipe by Kurihara et. al. (1986). The diets were prepared as follows: the fresh *organic cabagge* leaves washed and dried in the oven at the

temperature 50 ^{o}C for 3 days. The dried leaves were crushed and keep into the plastic containers. The bean and 10 mL distilled water were grinding. The ingredients were added in a beaker glass thoroughly mixed together on the baker glass. The agar and distilled water were boiled for 3-10 min, with stirring. When the agar solution was about 50°C, the bean and all of the dry component were added. The completed diets were blended with a kitchen mixer. Finally, the diet was poured into sterile plastic tray of 400 ml (25 ×10×2.5 cm) and allowed to solidify at room temperature. The same procedure was used for Jack bean and eggs yolk Squil artificial diet. Two types of artificial diet without casein and cholesterol were prepared. Finally, the artificial diets then were brought to room temperature (30 ^{0}C) before used it.

Table 1. The composition of artificial diet used for rearing *C. binotalis* (Kurihara,1987).

No.	ingredients	weight
1.	*Cabbage leaf powder*	12,0 g
2.	Agar	10,5 g
3.	***Casein***	15 g
4.	*Dried yeast(Ebios)*	12 g
5.	*Cellulose*	6 g
6.	***Cholesterol***	0,3 g
7.	*Choline chloride*	0,6 g
8.	NaCl	2,4 g
9.	*Ascorbic acid*	0,9 g
10.	*Folic acid*	0,6 g
11.	Chloramphenicol	0,1 g
12.	*Acetic acid*	0,5 ml
13.	Twen 20	0,3 ml
14.	*Rapessed oil*	2,4 ml
15.	Benzoat	1 gr
16.	Distilled water	500 ml

Table 2. The The composition of modified artificial diet used for rearing *C. Binotalis* (Kurihara,1987).

No.	ingredients	weight
1.	*Cabbage leaf powder*	12,0 g
2.	Agar	10,5 g
3.	***Jack bean***	15 g
4.	*Dried yeast(Ebios)*	12 g
5.	*Cellulose*	6 g
6.	*Squil egg yolk*	0.3 g
7.	*Choline chloride*	0,6 g
8.	NaCl	2,4 g
9.	*Ascorbic acid*	0,9 g
10.	*Folic acid*	0,6 g
11.	Chloramphenicol	0,1 g
12.	*Acetic acid*	0,5 ml
13.	Twen 20	0,3 ml
14.	*Rapessed oil*	2,4 ml
15.	Benzoat	1 gr
16.	Distilled water	500 ml

2.3. The effect of artificial diets to *C. binotulis* larvae.

The solid artificial diets of bean based diets 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 ten of first instar larvae was placed on the food. The same procedure was done for the modified artificial diets. There were 3 replication used for each artificial diet. The fresh cabagge leaves were used as the control food diet. Everyday artificial diet and control were replaced and recorded. Number of survival, larvae and pupae development and sex ratio of adult moth were recorded. Before pre-pupae stage, the larvae were transferred in to plastic container full with sand as the site for pupae stage. The data were statisticly analyzed using Anava method and significant differences were tested using Duncan's Multiple Range Method.

III. RESULTS AND DISCUSSION

The three types of artificial diets formulation and fresh cabagge leaves diet for *C. binotalis* larvae is shown on Figure 1 A,B,C, and D. The highest larvae weight was showed on fresh cabagge leaves because it is the suitable host plant for *C. binotalis* (Figure 4.) The artificial Diets used Jack bean and egg yolk found to be suitable for *C. binotalis* although the artificial diets (Figure 1-3) have a complete nutrition, the absence of casein and cholesterol will affect the development of the insect, especially, the total duration from egg to adult stage was shown on Table 3. The total duration of the insect developments were reared on jack bean was 30.05 days, squil egg yolk diet was 32.60 days, Kurihara diet was 48.87 days and 48.17 days for fresh cabagge leaves diets . Significan difference was observed in the larva development stage when reared on three type of artificial diets to fresh cabagge leaves. In the development of imago stage, significant difference was observed in the imago reared from jack bean and squil egg yolk were difference to the larvae reared on the other diets. However the larvae reared on Kurihara diet was not significant difference to larvae reared on fresh cabagge leaves. The duration both the larvae and pupal periods when reared on jack bean and squil egg yolk were shorthern than reared on Kurihara and fresh cabbage leaves diets (Kurihara, 1987).

Table 3. Life cycle of *Crocidolomia binotalis* reared on three artificial diets and fresh cabagge leaves..

Type of diets	Duration of development (day)				
	Eggs	Larva	Prepupa	Pupa	Imago
Fresh Cabagge leaves	4,16±0,057^{a}	11,34 ± 0,216^{a}	1,39±0,055^{a}	8,14±0,064^{a}	23,14 ± 1,16^{a}
Kurihara	4,20±0,00^{a}	11,33 ± 0,086^{ab}	1,40±0,015^{a}	8,23±0,014^{a}	22,51 ± 2,17^{a}
Jack Bean	4,16±0,057^{a}	11,98 ± 0,348^{b}	1,41±0,031^{a}	8,30±0,068^{a}	4,20 ± 0,67^{c}
Squil egg yolk	4,13±0,057^{a}	11,57 ± 0,032^{b}	1,43±0,086^{a}	8,39 ± 0,054^{a}	7,08 ± 1,09^{b}

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Kurihara	4,20±0,00^{a}	11,33 ± 0,086^{ab}	1,40±0,015^{a}	8,23±0,014^{a}	22,51 ± 2,17^{a}
Jack Bean	4,16±0,057^{a}	11,98 ± 0,348^{b}	1,41±0,031^{a}	8,30±0,068^{a}	4,20 ± 0,67^{c}
Squil egg yolk	4,13±0,057^{a}	11,57 ± 0,032^{b}	1,43±0,086^{a}	8,39 ± 0,054^{a}	7,08 ± 1,09^{b}

Notes : Number follow with a same charactersand colum showed non significant at 5 %.

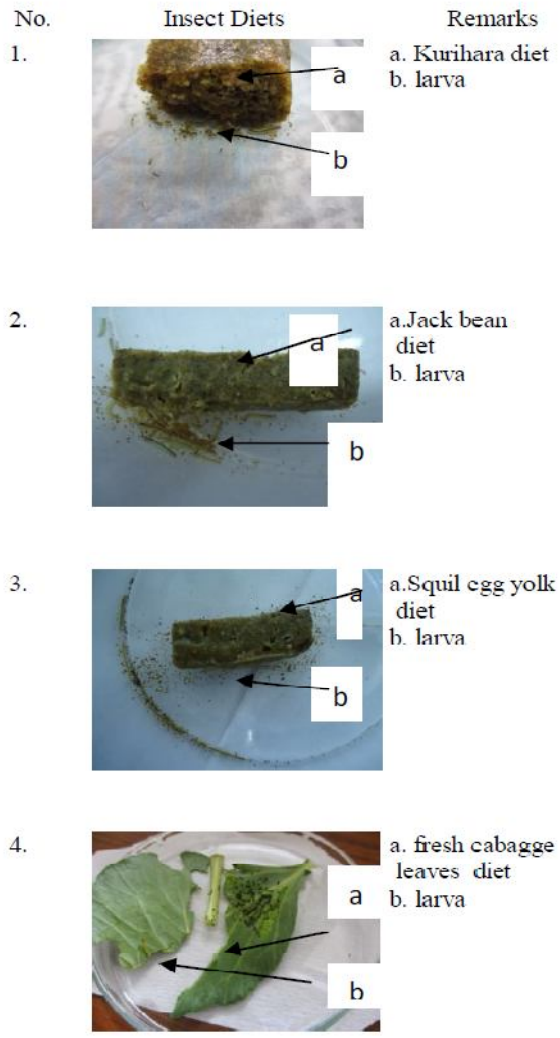


Figure 1-4. Larvae of *C. binotalis* reared on three types of artificial diets and fresh cabagge leaves.

The percentage of larval survival rate of *C. binotulis* reared on squile egg diets was significantly different compare to the fresh cabagge leaves and Kurihara diets but was not significance difference to jack bean diet. The percentage of larval survival reared on fresh cabagge leaves and Kurihara and Jack bean were not significant difference. The fresh cabagge leaves diet gave a highest survival rate (90%), As the use of Kurihara and Jack bean were respectively 76.6%, and 66.6 %. (see Table 4.)

Tabel 4. Percentage of survival rate of *C. binotalis* reared on three artificial diets and fresh cabagge leaves.

Types of insect diet	Larva survival rate (%)
Fresh Cabagge leaves	9,00 ± 1,00[a]
Kurihara	7,66 ± 1,15 [ab]
Jack Bean	7,00 ± 1,00[b]
Squil egg yolk	6,66 ± 0,57

Notes : Number follow with a same characters and colum showed non significant at 5 %.

The Pupal weight can be seen on Table 5. There is no significant difference observed in the development of pupal stage when the larvae reared on fresh cabagge, Kurihara, Jack bean, and Squil eggs yolk diets. In addition, the weight of the pupa was not significant difference. Those were 0.039 g, 0.038 g, 0.036 g, and 0.36 g.

Table 5. The Pupal weight of *C. binotalis*

Type of insect diet	The pupal weight/g
Fresh Cabagge leaves	0,039 ± 0,0016 a
Kurihara	0,038 ± 0,019 a
Jack Bean	0,036 ± 0,024 a
Squil egg yolk	0,036 ± 0,011 a

Notes : Number follow with a same characters and colum showed non significant at 5 %.

The mean fecundity of the insect was 44.5 % on fresh cabagge leaves diet, 39.1 on Kurihara diet, 0 % on Jack bean and squil egg yolk. (see Table 6.) Significant difference was observed in the fecundity of the insect when reared on fresh cabagge leaves diet, however the other three type of diets were not significant difference.

Table 6. The percentage of fecundity of of *C. binotalis.*

Type of diet	Fecundity of the insect /%
Fresh Cabagge leaves	44.5000 ± 10.13520 [a]
Kurihara	39.1667 ± 7.30677 [b]
Jack Bean	0.0000 ± 0.00000[0]
Squil egg yolk	0.0000 ± 0.00000 [0]

Notes : Number follow with a same characters andcolum showed non significant at 5 %.

IV. DISCUSSION

The use of fresh cabagge leaves and Kurihara standard diets for rearing *C. binotalis* this study as those diets has been used successfully for many years in the continuous rearing of *C. binotalis* for 35 generations. Fresh cabagge leaves which has natural composition of cabagge leaves contains amino acid, fatty acid and vitamins different to the present artificial diet in Table 1 [11]. Kurihara state that handling and transfer process of the larvae to the new portion of artificial diet can affect their organ development, during the process, larvae must learn to accept the artificial. This artificial diets includes a variety of ingridient such as Casein and cholesterol that are expensive. Therefore the simplified diet described used for *C. binotalis* was less expensive. The total price of Kurihara diet was IDR. 91,056; Jack bean IDR. 31,216; squil egg yolk IDR. 74,056. Insects reared on each type of media showed shorther duration of larva l survival, developmental

rates, indicating that the simplified diet provides non optimal balance of nutrients. For this reason the artificial diets described here was prepared without cholesterol and casein, all of which were present in the standard diet. Protein is important in the insect diet, it will influences in the survival and development of inseect. [12]. Casein is used as a primary protein supply was substituted in this diet by squil egg yolk which a cheap source of protein that suppies all the amino acid without physiological system effect to the insect. [13]. I was reported that squil egg yolk contained 844 mg cholesterol. The diet is considered suitable for insect rearing should be evaluated for several generations to determine the capablelity of the diet for maintaining biological parameters, such as insect survival rate, reproduction and normal behavior [14]. The jack bean and squil egg yolk diets presented here has now been used for over three generations of *C. binotalis* rearing in the Laboratory of Entomology, Faculty of Biology, UGM. Therefore the diets offers considerable promise for mass production.

CONCLUSIONS

The suitability of three artificial diets especially diet which contained jack bean dan squil egg yolk for the larvae *C. binotalis* were evaluated. The parameters of biology larvae *C. binotalis* were including life cycle duration, pupal weight, female fecundity, and survival rates were studied. The life cycle of insects were reared on Jack bean and squil eggs yolk were shorter than the fresh cabagges and Kurihara diets. The morphological of larval and pupal stages were not impaired by the artificial diets. Therefore, based on the results, the squil eggs yolk is recommended to be an alternative diet for rearing larva *C. binotalis* coloni under laboratory conditon

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REFERENCES

[1] Sastrosiswojo, S., and Setiawati, W. 1990. *Biology and Control of Crocidolomia binotalis in Indonesia*. Lembang Horticultural Research (LEHRI). *Bandung 40391: Indonesia. Hal 81-87*.

[2] Kalshoven, L.G.E. 1981. Pests of crops in Indonesia. Revised and translated by P.A. van der Laan, Univ. of Amsterdam with the assistance of G.H.L. Rothschild, Jakarta: P. T. Ichtiar Baru - van Hoeve, 701 p.

[3] Singh, P. E., and R. F. Moore [eds]. 1985. Handbook of insect rearing. Elsevier, Amsterdam.

[4] Moore RF (1985). Artificial Diets : Development and Improvement In: Singh P and Moore RF (Eds). Handbook of Insect Rearing Vol. 1. Elsevier, New York: p. 47-70.

[5] Shapiro, M., R. A. Bell, and C. D. Owens. 1981. *In vivo* mass production of gypsy moth nucleopolyhedrosis virus, pp. 633-655. *In* C. C. and M. M.L [eds.], The Gypsy Moth: Research Toward Integrated Pest Management. USDA, Washington D.C.

[6] Shapiro, M. 1986. *In vivo* production of baculoviruses, pp. 31-62. *In* R. R. Granados and B. A. Federici [eds.], The biology of baculoviruses, vol. II. CRC Press, Boca Raton, FL.

[7] Bell, R. A., C. D. Owens, M. Shapiro, and J. R. Tardif. 1981. Mass rearing and virus production, pp. 599-655. *In* Doane, C.C. and McManus, M.L. [eds], The Gypsy moth: Research toward integrated pest management. USDA Forest Service, Technical Bulletin 1584, Washington DC.

[8] Hunter-Fujita, F. R., P. F. Entwistle, H. F. Evans, and N. E. Crook [eds]. 1998. Insect viruses and pest management. Wiley, Chichester, United Kingdom.

[9] Morimoto, M., K. Matsuda, and Y. Ohta. 2004. Evaluation of calcium-alginate gel as an artificial diet medium for bioassays on common cutworms. J. Agric. Food Chem. 52: 4737-4739.

[10] Cappellozza, L., S. Cappellozza, and A. Saviane. 2005. Artificial diet rearing system for the silkworm *Bombyx mori* (Lepidoptera: Bombycidae): effect of vitamin C deprivation on larval growth and cocoon production. Appl. Entomol. Zool. 40: 405-412.

[11] Kurihara, M., Tatsuki, S.; Sutrisno, S.; and J. I.Fukami. 1987. Artificial Diet for the Large Cabbage Heart Caterpillar, *Crocidolomia binotalis (Zell)* (Lepidoptera : Pyralidae). *Applied Entomology and Zoology*, 22. 232-234.

[12] Lee, K., S. Simpson, and K. Wilson. 2008. Dietary protein-quality influences melanization and immune function in an insect. Func. Ecol. 22: 1052-1061.

[13] Anonymous, 2007., *Human Nutritition*. Research Center of Agricultural Reasearch and Service. (USDA) of United State Development of Agriculture. *The USDA Food Search of Windows*.

[14] Cohen, A. C. 2001. Formalizing insect rearing and artificial diet technology. Am. Entomol. 47: 198-206.

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