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Bindu K. Jose
Research scholar
Forest Entomology
Kerala Forest Research Institute, Peechi- 680653
Thrissur, Kerala, India
Email: bindukjose@gmail.com

Sajeev TV
Scientist E1, Head,
Forest Entomology,
Kerala Forest Research Institute, Peechi- 680653
Thrissur, Kerala, India
Email: tvsajeev@gmail.com

Sudheendrakumar VV
Rtd. Scientist,
Forest Protection Division,
Kerala Forest Research Institute, Peechi- 680653
Thrissur, Kerala, India
Email: vvsudheendrakumar@gmail.com

Correspondence:
Bindu K. Jose
Research scholar
Forest Entomology
Kerala Forest Research Institute, Peechi- 680653
Thrissur, Kerala, India
Email: bindukjose@gmail.com

Role of protein and lipids in artificial diets of teak defoliator moth: *Hyblaea puera*

Bindu K. Jose, Sajeev TV, Sudheendrakumar VV

ABSTRACT
This work assessed the importance of proteins and lipids in artificial diets used for growing the teak defoliator *Hyblaea puera* Cramer. Development of a completely artificial diet for rearing *H. puera* became an urgent need as they are used for the production of a biocontrol agent, the *Hyblaea puera* nucleopolyhedrosis virus. Different forms and quantities of proteins and lipids were examined, and bioassays were carried out both with *H. puera* larvae and adults. Addition of proper type and quantity of protein in the artificial diet gave good larval, pupal and adult survival of *H. puera*, together with an adequate life span and good fecundity. This study showed that the type of protein is a major factor in the food acceptance and development of 1st and 2nd instar larvae of *H. puera*. Even if lipid is an essential factor in lepidopteran growth, the addition of lipid in the form of egg yolk and coconut oil caused early mortality.

Keywords: Proteins, lipids, *Hyblaea puera*, insect diet, insect-growth

1. Introduction

In the preparation of artificial diets for insects, proteins and lipids are very important as they are necessary for proper growth. Proteins have an important position in insect biochemical processes and have major functional roles in their diet. Protein functions as a source of energy and as structural material in insects. Proteins are good emulsifiers having buffering and stabilizing capacity, and they bind water to form gels, bring additional elasticity and make films at interfaces between diet components. These properties of proteins can alter the consistency of the diet and hence these factors need to be taken care of in the preparation of artificial diets for insects [2, 8]. They are also known to be essential in the binding of fats and flavours, and also help in storage [3, 7].

Insects need lipids for sclerotisation and metamorphosis, and they also function as nutrient transporters [1]. Many insect artificial diets become successful after the addition of lipids such as cholesterol [4, 5, 6]. However, being insoluble in water, incorporation of lipid into an insect diet is a complicated issue [10].

The current study tested various proteins, lipid sources and their role in an artificial diet for *Hyblaea puera* (teak defoliator) to ensure proper growth. *H. puera* is a serious pest of teak and it can cause 44% loss in the wood-growth increment. Presently the production of a biological control agent, the *Hyblaea puera* nucleopolyhedrosis virus (*Hp*NPV), against this pest depends on the availability of larval stages of *H. puera*. The virus mass multiplies inside the body of live *H. puera*. An artificial diet is essential for the mass rearing of the insects which are to be used for virus production. A diet developed by Mathew *et al.* [9] supports only the final instars of the insect. As a part of the development of a complete artificial diet for *H. puera,* the importance of protein and lipids has now been recognized and tested. Both quantitative and qualitative studies have been conducted to customize the functions of proteins and lipids in the artificial diet.

2. Methods

In order to work out the efficiency of proteins and lipids in the growth of the insect, different foodstuffs containing varying amounts of proteins and lipids were tested.

2.1 Insects for the study
Pupae of *H. puera* collected from Nilambur teak plantations were surface sterilized by using 0.5% sodium hypochlorite solution for 5 minutes followed by thorough washing under tap water. The pupae were then air dried and placed in glass bottles (20 cm high and 10 cm wide) for emergence. After emergence, the moths were sexed by means of morphological features [10] and then transferred to a wooden cage (15 x 15 x 15 cm) for mating. They were provided with 10 % (v/v) honey solution on small pieces of sponge (5 x 3 cm). On the second day, moths were sexed, and pairs were set and transferred to wide-mouth bottles (20 x 10 cm) for oviposition. The eggs were transferred to glass bottles provided with fresh tender teak leaves. Every alternate day, the larvae were transferred to a new sterile glass bottle with new teak leaves to avoid contamination. First-instar larvae were selected for the experiment.

2.2. Template diet ingredients
An artificial diet developed for the last instars of teak defoliators by Mathew *et al* (9) was modified (unpublished) and used as the template for the present study. The template diet (starter diet for experiments) contains macro-ingredients like Bengal gram (7.5 g), agar (2.5 g), casein (2.5 g), yeast (2.5 g), teak leaf powder (2.5 g) and micro-ingredients like Wesson's salt mixture (2 g), multivitamin (1 tab), ascorbic acid (1.5 g), vitamin E (1 tab), sorbic acid (0.1 g), streptomycin (0.025 g), ampicillin (0.025 g), methyl *para*-hydroxybenzoate (0.1 g) and water (300 ml).

2.3 Diet preparation
Agar was added to half the required quantity of distilled water in a beaker, stirred with a glass rod and brought to boil. Macro- and micro-ingredients were mixed together using a blender and added to the other half of the water, into which the agar solution cooled to 60 °C as then slowly poured. After mixing thoroughly, this hot diet was poured into the container used for testing.

2.4 Tested ingredients
2.4.1 Protein
Experiments with four diets using soybean powder (5, 15 and 25 g), casein (10, 20 and 30 g), Bengal gram (30, 35 and 40 g), and wheat germ (30, 35 and 40 g) were prepared for bioassay against the 1st-instar larvae of *H. puera*. Each ingredient was added along with the macronutrients in the 250 ml template diet. The prepared diets were cooled and allowed to solidify in Petri plates and were presented to 1st-instar teak-defoliator larvae. Observations on larval survival were taken at 24 h intervals.

2.4.2 Lipids
Three different diets with smashed egg yolk (2, 3 and 5 ml), coconut oil (2, 3 and 5 ml) and cholesterol powder (95%) (2, 5 and 8 g) were prepared in order to test the role of lipids in the development of *H. puera*. Three different experiments were conducted and observations on larval survival taken at 24 h intervals.
Control batches of insects were reared separately on tender teak leaves for each set of experiments and data analysis was done using Statistical Product and Service Solution (SPSS).

3. Results
3.1 Protein
Table 1 shows the mean survival and life span of *H. puera* larva reared on diets with different types of protein.

Table 1: Mean survival and life span of *H. puera* larva on various protein sources

Type of protein	Mean survival (%)	Mean life span (days)
Control (teak leaf)	73.3 ± 1.93^a	12.6 ± 0.19^a
Bengal gram	16.7 ± 1.92^b	20.4 ± 0.26^b
Soybean flour	15.5 ± 1.11^b	18.3 ± .04^c
Casein	17.7 ± 1.01^b	20.1 ± 0.08^b
Wheat germ	69. 2 ± 1.24^a	13. 8 ± 0.28^a

In a column, differences between values followed by the same letters are not statistically significant (0.05).

Among the diets, wheat germ performed satisfactorily (69.3%) when evaluated against the control (teak leaf). A significant difference was observed in the survival of insects reared on teak leaf compared to the larvae reared on the other three diets. The mean larval-life span was 12.7 days in the control group and 13.8 in the wheat-germ diet but it was extended by 6-8 days in the treatments. On teak leaf and wheat germ, the larvae took about 2 days of developmental period for each instar, but this period was extended by 1-4 days in the case of 1st- and 2nd-instar larvae in the treatments.

3.2 Lipids
The mean survival and life span of *H. puera* larvae reared on diets with different types of lipids is presented in Table 2.

Table 2: Mean survival and lifespan of *H. puera* larvae on diets with various lipid sources

Type of lipids	Mean survival (%)	Mean lifespan (days)
Control (teak leaf)	72.2 ± 1.11^a	12.1 ± 0.09^a
Egg yolk	0	0
Coconut oil	0	0
Cholesterol	29.9 ± 1.36^b	19.7 ± 0.5^b

In a column, differences between values followed by the same letters are not statistically significant (0.05)

The best survival rate was observed in insects reared on teak leaf. Complete mortality of the larvae occurred in insects reared on the diets with egg yolk and coconut oil. In the cholesterol-added diet, larval survival was 30.0%.

3.3. Bioassays with a selected protein source

The performance of the artificial diet containing added wheat germ was evaluated on the basis of the life parameters such as larval period, survival percentage, pupation period and rate, adult emergence percentage and the longevity and oviposition rate of *H. puera* (Table 3). The observations were taken over three generations of the defoliator.

Table 3: *H. puera* on new wheat germ added diet.

generation	age interval (x)	No of living at the beginning (lx)		no dying during age interval 'x' (dx)		'dx' as % of lx (100qx)		log (lx)		Difference between successive log 'lx' values (kx).	
		test	con	test	con	test	con	test	con	test	con
1	Instar 1	90	90	17	10	18.88	11.11	1.95	1.95	0	0
	Instar 2	73	80	7	7	9.58	8.75	1.86	1.9	0.09	0.05
	Instar 3	66	73	6	7	9.09	9.58	1.81	1.86	0.05	0.04
	Instar 4	60	66	3	1	5	1.51	1.77	1.81	0.04	0.05
	Instar 5	57	65	6	7	10.52	10.75	1.75	1.81	0.02	0
	Pupa	51	58	1	4	1.96	6.89	1.7	1.76	0.05	0.05
	Adult	50	54					1.69	1.73	0.01	0.03
	Egg (3 pairs)	129.3	146.25								
90 each 1st instar larvae from test and control were opted for 2nd generation experiments											
2	Instar 1	90	90	13	10	14.44	11.11	1.95	1.95	0	0
	Instar 2	77	80	6	8	7.79	10	1.88	1.9	0.07	0.05
	Instar 3	71	72	5	5	7.04	6.94	1.85	1.86	0.03	0.04
	Instar 4	66	67	3	3	4.54	4.47	1.81	1.82	0.04	0.04
	Instar 5	63	64	3	5	4.76	7.69	1.79	1.8	0.02	0.02
	Pupa	61	59	2	5	3.27	8.47	1.78	1.77	0.01	0.03
	Adult	57	56	4	3			1.75	1.74	0.03	0.03
	Egg (3 pairs)	298.49	276.44								
90 each 1st instar larvae from test and control were opted for 3rd generation experiments											
3	Instar 1	90	90	9	9	10	10	1.95	1.95	0	0
	Instar 2	81	81	6	4	7.4	4.93	1.9	1.9	0.05	0.05
	Instar 3	75	77	4	5	5.33	6.49	1.87	1.88	0.03	0.02
	Instar 4	71	72	2	4	2.81	5.55	1.85	1.85	0.02	0.03
	Instar 5	69	68	0	2	0	2.94	1.83	1.83	0.02	0.02
	Pupa	68	66	4	3	5.88	4.54	1.83	1.81	0	0.02
	Adult	64	63								
	Egg (3 pairs)	332.44	315.53								

Even though the first generation on an artificial diet recorded a slight reduction in larval survival (63.3%) to that on teak leaf (72.2%), from the second generation onwards the survival rate (70.0%) was satisfactory and comparable with that in the control group (71.1%). Mortality of early instars was comparable with the control batch from the third generation onwards. The pupation rate in all generations of the experimental batch was equal to or more than that of the control group. The fecundity of experimental batch (129) in first generation was also less in than the control (146), and the difference was only 17 numbers of eggs. In second and third generations, the fecundity rates of the experimental batches of insects were more than those of the control group of insects.

4. Conclusion

The study indicated that the type of protein is a major factor in the food acceptance and development of 1st- and 2nd-instar larvae of *H. puera* grown on an artificial diet. The survival and lifespan of larvae reared on the diet with added wheat germ (Fig 1) was similar to the control group of insects on teak leaves.

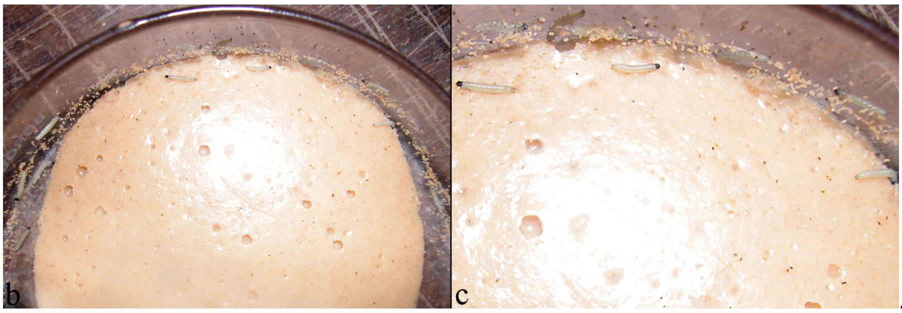

Fig 1: 1st- and 2nd-instar larvae of *H. puera* grown on the diet with added wheat germ.

Three generations of insects could grow successfully on the diet with added wheat germ. But the addition of extra amounts of protein did not increase larval growth and may reduce the palatability of the diet.

Even though lipid is an essential factor in lepidopteran growth, the addition of lipid in the form of egg yolk and coconut oil caused all the insects to die at the 1st- instar stage. The addition of cholesteol also reduced the survival rate while increasing the life span by one and half times. Hence the addition of extra lipid was not a crucial factor for the survival of larvae on the experimental artificial diet.

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