Carbohydrates in insect artificial diet: an evaluation study with *Hyblaea puera* Cramer

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ABSTRACT

Production of a variety of biocontrol agents and bioassay experiments using insects require laboratory reared insects. The success of such year round rearing programs requires an artificial diet on which all life stages of the insect can be reared. Precise understanding of the various components of the diet is needed to design them in such a way that it adequately mimics the natural diet. The teak defoliator *Hyblaea puera* was taken as the test organism to study the role of carbohydrates in lepidopteran artificial diet. Modification of the existing diet (template diet) of *Hyblaea puera* for last instars was done by adding different quantities of wheat, rice, starch, oats, sucrose and Isabgol husk powder individually. Bioassays against the early larval instars of teak defoliator with these modified diets had deleterious effect on larval growth parameters. All the diet combinations tried except wheat germ gave survival rate of less than 25 per cent. But the addition of wheat germ to the template diet brought the experiment a step forward and the survival rates of insects were 69 per cent. Present study enlightened the role and importance of correct form and quantity of carbohydrate in insect artificial diets.

Keywords: Carbohydrates, *Hyblaea puera*, Wheat germ, Insect growth, Insect artificial diet

INTRODUCTION

The success of insect rearing program relies on artificial diets since natural host could invite contamination under laboratory conditions. When continuous rearing of the insect for many generations is attempted, it is the diet that determines the body size and vitality of the insects. Among the diet components insects use carbohydrates as building materials and fuels [2].

Carbohydrate play a major role in insect development like metabolism, metamorphosis, development of flight muscles, reproduction and embryonic development, insect behavior and as reserve food during diapauses [1,7]. Some carbohydrates cannot be utilized by most of the insects, but they may be useful as fillers that help intestinal mobility. Some insects especially phytophagous insects are unsuccessful to survive on artificial diet that are low in carbohydrates [5]. The type of carbohydrate must be suited to the specific insect.

To date, nutritional studies have focused primarily on determining which carbohydrates are necessary to support optimum growth, development, reproductive activity and survival of individual species. For most species, glucose, fructose and sucrose are nutritionally adequate sugars. The quantitative requirements of carbohydrates vary according to age, sex and metamorphic stages.

The present study focused on the type and quantity of carbohydrate source required for the development of artificial diet for rearing a serious pest of teak (*Tectona grandis*) *Hyblaea puera* (teak defoliator). An artificial diet used to rear *H. puera* [6] supported only the last (3^{rd} to 5^{th}) larval

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instars. The initial larval stages (1st and 2nd) require tender teak foliage to feed. As carbohydrates are principal component in an insect diet, different food varieties as sources for carbohydrates were tested during this work. Better forms of carbohydrates in terms of utilization efficiency in *H. puera* were evaluated.

MATERIALS AND METHODS

For the selection of a suitable carbohydrate source which can support all larval instars of teak defoliator pests, a template diet which was partially modified form of diet developed by Mathew *et. al*., in 1990 was used for this study. Bioassays were carried out using 1st instar larvae of *H. puera* to understand the effect of tested ingredients on growth parameters.

Template diet

Agar(2.5g), Casein (2.5g), yeast (2.5g), teak leaf powder (2.5g), Wesson's salt mixture (2g), multivitamin (1 tab), ascorbic acid (1.5 g),vitamin E (1tab), sorbic acid (0.1g), streptomycin (0.025g), ampicillin (0.025g), methyl parahydroxyl benzoate (0.1g) and water (350 ml) were used for the preparation of template diet. The prepared semisolid diet was poured in to the center part of the Petriplate and allowed for solidification.

Test insect

Pupae of *H. puera* collected from Nilambur teak plantations were surface sterilized, washed and placed in glass bottles of 20 cm height and 10 cm wide for emergence. After emergence the moths were sexed by means of morphological features (Sudheendrakumar, 2004) and were transferred to a wooden cage (15 cm x 15 cm x 15 cm) for mating. They were provided with 10 % (v/v) honey solution on small sponge pieces (5 cm x 3 cm). On the second day a pair of moths was transferred to wide mouth glass bottles (20 cm x 10 cm) for oviposition. The eggs were transferred to glass bottles provided with fresh tender teak leaves. Newly hatched first instar larvae were selected for the experiment.

Carbohydrate Sources for Experiment

With a view to develop a diet which could support all larval stages of the insect right from the first instar seven carbohydrate sources were analysed. These included commercially available rice powder (10g, 20g and 30g), whole wheat flour (10g, 20g and 30g), starch flour (10g, 20g and 30g), fried powdered oats (10g, 20g and 30g), table sugar (5g, 10g and 15g), powdered wheat germ (10g, 20g and 30g) and fiber obtained from Isabgol (*Psyllium*) husk (5g, 10g, 15g). Each of these carbohydrate sources were mixed with the macro and micro ingredients of the template diet, added with heated agar and presented to first instar larvae on Petri plates.

Bioassays

The performance of the diet was evaluated with reference to the life parameters of *H. puera.* For experiments several addition studies with different carbohydrate sources in the template diet were conducted and did bioassays against the 1st instar larvae of *H. puera*. Survival rate and life span of the larvae were recorded in all carbohydrate sources throughout the experimentation period. In the final stage, three generations of the *H. puera* were maintained on the selected carbohydrate added diet. Control batch of insects were reared on teak leaves. Observations on larval survival were made at 24 h intervals.

RESULTS AND DISCUSSION

Results of the present study are furnished in the following table 1. Mean survival and life span were calculated.

Survival and life span of *H. puera* on different carbohydrate sources

Results of the effect of different carbohydrate sources on life span and survival of *H. puera* are listed in Table 1.

Table 1. Mean survival and lifespan of *H. puera* larvae in diets with different carbohydrate sources.

Carbohydrates	No of *H. puera* tested		Mean survival (%)	Mean life span (days)
	replicates	Total number		
Control (teak leaf)	3	90	77.77 ± 1.11[a]	13.03 ± 0.23[a]
Whole wheat flour	3	90	19.99 ± 1.92[be]	18.55 ± 0.29[bc]
Rice	3	90	7.77 ± 4.00[c]	19.34 ± 0.4[bc]
Starch	3	90	15.55 ± 1.11[b]	19.75 ± 0.46[c]
Oats	3	90	24.93 ± 1.73[be]	20.91 ± 0.16[d]
Sucrose	3	90	21.61 ± 1.46[be]	19.94± 0.43[c]
Wheat germ	3	90	69. 26 ± 1.24[a]	13. 84 ± 0.28[a]
Isabgol husk fiber	3	90	23.33 ± 3.85[be]	18.74 ± 0.49[bc]

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

It can be seen that the highest survival in the treatment with wheat germ (69%) was comparable with that of the control (77%). The performances of the diets containing wheat, oats, sucrose and isabgol husk fiber were similar. The lowest survival was recorded in the diet with rice powder. The larval life span was normal in the insects reared on teak leaf and wheat germ, but it got extended by 5 – 7 days in all other treatments. In all other treatments larval life span gets extended by 1-2 days and very rarely did the larvae cross from second to third instar.

Bioassay with the selected diet against *H. puera*

The life table of *H. puera* reared on the wheat germ based diet is given in table 2. The performance of the diet was evaluated on the basis of the life parameters like larval period, survival percentage, pupation period and rate, adult emergence percentage, longevity and oviposition rate. The observations were taken up to three generations of defoliator.

In all generations the mortality rate in the early stage of larvae was more than that of control. Even though the generations of *H. puera* on artificial diet recorded slight reduction on larval survival than the generation on teak leaf, the survival rate was satisfactory and comparable with that in the control group. The pupation rate in all generations of the experimental batch was more or less equal to the control group. The fecundity rate of experimental batch was also reduced than the control, but it was negligible.

Table 2. Life table of *H. puera* on new wheat germ added diet.

g	x	lx		dx		100qx		log(lx)		kx	
		T	C	T	C	T	C	T	C	T	C
1	Instar 1	90	90	11	5	12.22	5.55	1.95	1.95	0	0
	Instar 2	79	85	13	9	16.45	10.58	1.89	1.92	0.06	0.03
	Instar 3	66	76	7	6	10.6	7.81	1.82	1.88	0.07	0.04
	Instar 4	59	70	6	2	10.16	2.85	1.77	1.84	0.05	0.04
	Instar 5	53	68	2	3	3.38	4.41	1.72	1.83	0.05	0.01
	Pupa	51	65	17	8	33.33	12.30	1.70	1.81	0.02	0.02
	Adult	34	57								
	Egg(3pairs)	734	813								
90 each 1st instar larvae from test and control were used for 2nd generation											
2	Instar 1	90	90	14	9	15.55	10	1.95	1.95	0	0
	Instar 2	76	81	8	8	10.52	9.87	1.88	1.9	0.07	0.05
	Instar 3	68	73	11	7	16.17	9.58	1.83	1.86	0.05	0.04
	Instar 4	57	66	9	6	15.78	9.09	1.75	1.81	0.08	0.05
	Instar 5	48	60	7	2	14.58	3.33	1.68	1.77	0.07	0.04
	Pupa	41	58	11	7	26.82	12.06	1.61	1.76	0.07	0.01
	Adult	30	51								
	Eggs (3pairs)	756	881.33								
90 each 1st instar larvae from test and control were used for 3rd generation											
3	Instar 1	90	90	12	8	13.33	8.88	1.95	1.95	0	0
	Instar 2	78	82	11	4	14.10	4.87	1.89	1.91	0.06	0.04
	Instar 3	67	68	12	6	17.9	8.82	1.82	1.83	0.07	0.08
	Instar 4	55	61	9	7	16.36	11.47	1.74	1.78	0.08	0.05
	Instar 5	46	54	12	7	26.08	12.96	1.66	1.73	0.08	0.05
	Pupa	34	47	7	8	20.58	17.02	1.53	1.67	0.13	0.06
	Adult	27	40								
	Egg (3 pairs)	808.66	793								

The life Table contains; generation (g), number living at the beginning (lx), number dying during age interval x (dx), dx as % of lx (100qx), log value of initial no (log(lx)) and difference between successive log(lx)values (kx).

CONCLUSION

The result showed that the newly selected carbohydrate source- the wheat germ-was capable of rearing larval stages of *H. puera*. All the parameters like survival rate, pupation rate, larval period, pupal period, adult emergence and fecundity were comparable to the control group on teak leaf. Addition of wheat, rice, starch, oats and sucrose as additional carbohydrate source in the template diet had deleterios effect on the larval survival. Isabgoal husk powder was added to the template diet to enhace both the starch and fibre content. But there was no difference in either larval surval or life span of the larvae.

Wheat germ - the reproductive element of wheat is a part of wheat kennel [4]. Wheat germ is a rich source of carbohydrate and it also contain an array of aminoacids and essential lipids including monosaturated and polysaturated fatty acids [8]. It is a rich source of minerals and vitamins [3] which are required for the growth of lepidopteran insects. The form of carbohydrate present in wheat germ supports the growth of *H. puera* larva from 1st instar onwards.

Apart from the nutritive profile, use in many successful insect diets and cost effectiveness have made wheat germ a major conent of the insect artificial diet. Inclusion of wheat germ gave significant break through in the diet development process of *H.puera* for all the larval instars.

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