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Micronutrients - Significance and function in growth and survival of insects – A case study

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ABSTRACT

Eight different micronutrients (Wesson's salt mixture, ascorbic acid, potassium, calcium, sodium, ferrous, vitamin E and a mixture of vitamins) in different combinations were given to Hyblaea puera in order to understand their role in insect growth. Larval growth period, survival rate, pupation rate, pupal size, pupal weight, moth emergence rate, moth behavior and fecundity rates were analyzed. Ascorbic acid increased feeding rate , Wesson's salt shows marginal increase in the pupal weight and vitamin E caused statistically significant increase in fecundity rate of H. puera. The possibility of addition of selected micronutrients in a continuous H. puera rearing program was also evaluated.

Key words: micronutrients, *Hyblaea puera*, artificial diet, insect development

INTRODUCTION

Micronutrients are metals or metal containing inorganic compounds. They function as intracellular messangers or enzymatic co factors. Micronutrients as the name implicates are required in small quantities for the proper execution of vital activities in living things [1, 2, 6]. As in vertebrate the essentiality and intake of micronutrients [7, 10] is well established in insect nutrition also [8]. Micronutrients have its own importance in insect diet particularly in the preparation of insect artificial diet. They require micronutrients for proper growth and survival [1]. In the case of lepidopteron insects the absence or presence of micronutrients in the diet can influence on its behaviour and immunocompetance [5]. The knowledge about the role of vitamin in insect nutrition is limited.

Role of micronutrients in the growth and survival of an ecconomically important forest insect *Hyblaea puera* was analysed in the present study. *H. puera* is a serious pest of teak and it causes 44 per cent loss in the wood growth increment. Presently the production of a biological control agent against this pest, the *Hyblaea puera* nuclear polyherdosis virus (*Hp*NPV) is depended on the availability of larval stages of *H. puera*. Since teak is a deciduous tree, a complete artificial diet for *H. puera* is essential to ensure the availability of this insects in all seasons for different studies and experimental purpose. Wesson's salt mixture, ascorbic acid, potassium, calcium, sodium, ferrous phosphate, vitamin E and a mixture of commercialy available vitamin mixture were tested. Efficiancy of these micronutrients on development, reproduction and survival of the insect *Hyblaea puera* were tested by doing bioassays with all instars.

MATERIALS AND METHODS

Diet experiments were carried out by bringing modifications in the template diet. Different micronutrients were added in the template diet to know their functions in the survival and growth of larval stages.

Template diet for experiments

A partially modified form of diet [4] was used as the template diet. Wheat germ (30g), agar (7.5g), casein (7.5g), yeast (2.5g), teak leaf powder (2.5g), sorbic acid (0.1g), streptomycin (0.025g), methyl parahydroxyl benzoate (0.1g) and water (350ml) were used for the preparation of experimental diet. Control batch of insects were maintained on teak leaves.

Test insects *Hyblaea puera*

Pupae of *H. puera* collected from Nilambur teak plantations were surface sterilized by using 0.5 per cent sodium hypochloride 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 [11] and were transferred to a cage (15 cm x 15 cm x 15 cm) for mating. They were provided with 10 per cent (v/v) honey solution on small sponge pieces (5 cm x 3 cm). On the second day moths were sexed, pairs were set and transferred to a wide mouth bottles (20 cm x 10 cm) for oviposition. The eggs were transferred to glass bottles provided with fresh tender teak leaves. After hatching the first instar larvae taken out for experiments.

Micronutrients

The following eight substances were used to supply micronutrients in *H. puera* artificial diet. Each preparation were incorporated in to the diet at the time of preparation and their effects on insect development were evaluated .

1. Wesson's Salt Mixture: Commercial preparation of this salt was not available in our market. Hence it was prepared by mixing calcium carbonate (21g), copper sulphate (0.039g), ferric phosphate (1.470g), manganese sulphate (0.020g), magnesium sulphate (9g), pottassium aluminium sulphate (0.009g), pottasium chloride (12g), pottassium dihydrogen phosphate (31g), pottassium iodide (0.005g), sodium chloride (10.5g), sodium fluoride (0.057g) and tricalcium phosphate (14.9g). Different quantities like 1g, 1.5g and 2g of Wesson's salt mixture dissolved in alcohol were added to the template diet and bioassayed against 1st instar larvae of *H. puera*. Observations recorded at 24 h intervals.
2. Ascorbic acid: Ascorbid acid (1g, 1.5g, 2g) was dissolved in alcohol and added to the template diet. Bioassay with 1st instar larvae of *H. puera* was conducted and observations recorded at 24 h intervals.
3. Vitamin mixture: contains 75 mg calcium, 0.1 g carbohydrate, 0.5 mg copper, 0.38 g fat, 30 mg ferrous fumarate, 0.15 mg folic acid, 42.5 mg ginseng extract, 0.1 mg iodine, 3 mg magnesium sulphate, 0.5 mg manganese, 10 mg nicotinamide, 58 mg phosphate, 2 mg potassium sulphate, 0.2 g protein, A 2500 IU vitamin, 11 mg vitamin B, 1 mg vitamin B12, 1.5 mg vitamin B2, 1 mg vitamin B6, 50 mg vitamin C, 200 IU vitamin D, 5 mg vitamin E, and 10 mg zinc oxide. The contents dissolved in alcohol and 1ml,1.5ml and 2ml of the solution were added to the template diet. The diet was presented to first instar larvae and observations on larval survival were made daily.
4. Potassium**:** potassium was added to the diet in liquid form. 1ml, 1.5ml and 2 ml of potassium added to the 250ml of template diet and given to the 1st instar larvae of *H. puera*. Observations done daily.
5. Calcium: powdered calcium tablets were used for these experiments. 1g, 1.5g and 2g of calcium powder was added to the diet and experimented against 1st instar larvae of *H. puera*.
6. Sodium: 1g, 1.5g and 2g of sodium salts were incorporated to the tested diet and bioassay against 1st instar larvae of test insect. Daily observations were conducted.
7. Vitamin E: Semi solid form of Vitamin E obtained from capsules was used for the study. 1st instar larvae of *H.puera* were used for the experiments. All insect life parameters were observed daily.
8. Ferrous phosphate: Addition of ferrous phosphate (1g, 1.5g and 2g powdered) in artificial diet had done and experimented with 1st instar larva of *H. puera*. Observations were conducted daily.

Bioassays

Bioassays were carried out with the various diet developed in the course of this study. Observations were made on the response of the test insect on specific time interval. Initially first instar larvae of *H. puera* were used for the study. The experiments with 1st and 2nd instar larvae were carried out in petriplates of radius 4.5cm. The diet designated for testing was poured in to the center of the petriplate and after solidification the larvae were released. On 3rd instar onwards larvae were reared on the plastid rearing tubes (5.5cmX2.3cm) till pupation. After emergence moths are allowed to mate and eggs were collected. Thirty larvae each in three replicates were maintained for every study. In all cases teak leaves were used as the control.

RESULTS

Effects of micronutrients on *H. puera* larval growth

The following tables show the influence and efficiencies of micronutrients on larval survival, pupal survival, pupal weight, adult emergence and fecundity. Mean life span was also calculated to analyse the role of micronutrients in development process of insects.

Table 1: Micronutrients effects on *H.puera* growth parameters and survival

Type of micronutrients	Larval Survival (%)	Pupal Survival(%)	Pupal weight (g)	Adult Emergence(%)	Fecundity (number)	Mean life span
Control	70.66 ± 1.92^a	90.45 ± 2.05^a	0.23 ± 0.003^a	93.02 ± 0.7^a	284.97 ± 3.58^a	17.32 ±0.31^a
Vitamin mixture	71.11 ± 1.11^a	90.54 ± 2.81^a	0.22 ± 0.01^a	93.95 ± 0.45^a	282.84 ± 6.33^a	19.7 ± 0.4^b
Wesson's salt mixture	71.54 ± 1.11^a	89.05 ± 1.67^a	0.29 ± 0.003^b	93.68 ± 0.54^a	289.53 ± 9.28^a	17.29 ± 0.28^a
Ascorbic acid in alcohol	70.3 ± 2.93^a	90.73 ± 2.41^a	0.2 ± 0.1^c	90.59 ± 0.66^b	273.48 ± 5.1^a	21.03 ± 0.24^d
pottassium	67.77 ± 1.2^b	91.91 ± 4.22^a	0.19 ± 0.008^c	89.83 ± 0.21^b	271.17 ± 7.8^a	19.71 ± 0.39^b
sodium	65.22 ± 2.4^b	89.4 ± 2.3^a	0.19 ± 0.02^c	90.2 ± 1.11^b	275.54 ±3.21^a	21.7 ± 0.24^d
calcium	70.62 ± 1.32^a	90.55 ± 1.2^a	0.22 ± 0.31^a	90.6 ± 0.03^b	286 ± 4.3^a	20.35 ± 1.4^c
ferrous	66.47±1.7^b	91.44 ± 42^a	0.23 ± 0.011^a	91 ± 1.1^b	291 ± 5.1^a	21.06 ± 2.1^d
Vitamin E	71.2 ±1.21^a	90.1 ± 14^a	0.2 ± 0.54^c	90.42 ± 0.65^b	346 ± 1.12^b	20.2 ± 0.21^c

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

The diets containing vitamin mixture,Wesson's salt mixture, ascorbic acid, calcium, vitamin E and control gave comparable larval survival. While pupal survival was uniform across the treatments. However, there was significant variation in pupal weight among the treatments. Diet with Wesson's salt gave better pupal weight than the control. Except for the diet containing Vitamin E, there was no significant difference in fecundity of moths between treatments. Notable increase was observed in fecundity for the larvae reared in diet containing Vitamin E. The result indicated that except for the increase in fecunduty influenced by the addition of vitamin E and pupal weight increase by the addition of Wesson's salt there was no variation between tretments with respect insect growth.

Feeding response of *H. puera* on different micronutrients presence

The feeding responses of *H. puera* larva towards the addition of specific micronutrients are examined to know their feeding behaviour, role in larval growth, pupal weight and size, adult weight and size, influence on egg laying capacity and egg hatching. Results are furnished in table 2.

Table 2: Behavioural response (on feeding and growth) of defoliator larvae and diet quality upon deletion of specific ingredients from diet

Micronutrient	Behvioural response	
	Feeding	**Gowth and reproduction**
Vitamin mixture	Normal	Normal
Wesson's salt	Normal	Pupal weight increased, moth activities were normal
Ascorbic acid	Slightly phagostimulant	normal
pottassium	Normal	Normal
sodium	Normal	Normal growth, reduced fecundity
calcium	Normal	Normal
Ferrous	Normal	Normal
Vitamin E	Normal	Normal growth, high fecundity

It can be seen that vitamin mixture, Wesson's salt mixture, ascorbic acid, potassium, sodium, calcium, ferrous and vitamin E bring no change in larval feeding. However ascorbic acid caused increase in feeding but growth and reproduction parameters remain as normal. Wesson's salt incorporation increases pupal weight still it doesn't affect the moth emergence or activities. Vitamin E caused significant increase in fecundity.

DISCUSSION

Nutritional deficiencies in insects have been linked with such vague symptoms like poor growth rate, lowered fecundity or reduced body weight [1]. Present study ascribed the functions of eight different micronutrients

(Wesson's salt mixture, ascorbic acid, potassium, calcium, sodium, ferrous, vitamin E and a mixture of vitamins) on growth, survival and reproduction of *H. puera*.

Ascorbic acid is essential in insect diet [9] and serve as a phagostimulat in phytophagous insect [3]. In the current study the addition of ascorbic acid increased feeding rate of the insect. The production of ascorbic acid within *H. puera* is not known and most of the phytophagous insects donot produce this vitamin by themselves (Lehinger *et al*,1993). Hence addition of ascorbic acid in the artificial diet of teak defoliator *H.puera* is advocated. Addition of Wesson's salt shows marginal increase in the pupal weight but all other parameters like moth emergence, survival, larval growth and survival, fecundity rate and egg hatching were normal compared to the control. Considering the high cost and intricacy of ingredients or the non availabity in our market Wesson's salt is not recommended to include in the artificial diet for *H. puera*. Larvae fed in vitamin E added diet caused statistically significant increase in fecundity rate than the diets with out. Therefore addition of vitamin E in arrtificial diet for *H. puera* is practical and suggestable. The study showed that vitamins and mineral incorporated with the other ingredients of experimented control artificial diet is sufficient to rear *H. puera*. Extra addition of other micro nutrients will not improve growth performance in *H. puera*. Exception is for ascorbic acid, Wesson's salt mixture and vitamin E and their addition to a continuous rearing system of *H.puera* is advisable.

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