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Artificial Diet for Rearing of *Conogethes punctiferalis* Guenee (Lepidoptera : Crambidae)

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Abstract

This experiment was conducted to develop an artificial diet for rearing of castor shoot and capsule borer, *Conogethes punctiferalis* Guenee under laboratory conditions. The results revealed that artificial diet-1 yielded a larval survival rate of 95.46 per cent, mean pupal weights of 61.15 mg for females and 50.24 mg for males, fecundity rate of 39.02 eggs per females and took shortest period to complete the life cycle (31.48d). These outcomes indicated that *C. punctiferalis* adapted well to the artificial diet and successive rearing conditions. The diet could serve as a viable alternative to the natural host plants for consecutive rearing of the insects.

Keywords : artificial diet, *Conogethes punctiferalis*

The castor shoot and capsule borer, commonly called as yellow peach moth *Conogethes punctiferalis* Guenee (Lepidoptera : Crambidae) is major insect pest of castor, *Ricinus communis* L. and is widely distributed in south and East Asia, Australia, New Zealand and Papua New Guinea (CAB International, 2011). The larva of *C. punctiferalis* is highly destructive and typically polyphagous attacking more than 120 wild and cultivated plants *viz*., peach, apple, pine trees, chestnut, durian, citrus, papaya, cardamom, ginger, egg plant, sunflower, maize and forestry crops (Lu, *et al*., 2010; Li *et al.,* 2015) and cause a huge yield loss of more than 55 per cent in castor (Ganesha, 2012). Effective management of *C. punctiferalis,* often relies on sound integrated pest management (IPM) strategy. To develop and improve IPM strategies, studies were carried out to understand its bio-ecology, physiology and toxicology. One of the pre-requisites for conducting these studies is availability of a large number of healthy eggs, uniformly developed larvae, pupae and adults for testing. Hence, a successful artificial diet for rearing *C. punctiferalis* in laboratory is highly desirable to facilitate studies for developing sound IPM programmes.

The development of artificial diets, pioneered by Vanderzant *et al.* (1962), facilitated the continuous production of insects. Since then, many species of lepidopterans, coleopterans and dipterans have been successfully reared under controlled laboratory conditions (Gupta *et al.,* 2005). The rearing of *C. punctiferalis* on meridic diets proposed by Honda *et al.* (1979) suggested that colonies fed on these diets had a larger variation in the development duration than the colonies fed on natural host plant materials. However, they still could not produced large number of uniform larvae due to low larval survival and adult emergence, insufficient nutrition of diets *etc*. Considering the sparse information on artificial diet for mass-rearing *C. punctiferalis,* the present study on artificial diet was planned.

Material and Methods

Insects: The initial *C. punctiferalis* population was established with a collection of larvae from castor fields at the Dryland Research Station, University of Agricultural Sciences, GKVK, Bengaluru (13^0 05" N, 77^0 34" E with 924m MSL), Karnataka, India during 2015-16 and the borer population was maintained on fresh castor for one generation. The stock borer culture was held in laboratory under the 26 ± 1^0C, 70-80 per cent relative humidity and a photo period of 16 : 8 h light : dark.

Artificial diets: The semi-synthetic diet formulated for *Conogethes* sp. (Ambanna, 2014) was used as a starting medium for preparation of meridic diet for castor shoot and capsule borer and the diet was modified by the addition of young capsule powder as a token stimuli and variation of casein amount as mentioned below :

1. Artificial diet 1 (Castor young capsule based meridic diet) - Castor leaf powder as a token stimuli.

2. Artificial diet 2 (Plain meridic diet) - without the addition of any capsule powder and reduced amount of casein.
3. Natural diet- castor capsule as a control.

The ingredients for the diets were divided into three parts (A, B&C) as shown in Table I. Part A: The ingredients were weighed and kept separately before mixing. Castor young capsule powder was homogenized with 400 ml of water in a blender. The homogeneous mixture was mixed with soybean powder, casein, yeast extract powder and sucrose in a stainless steel pot. The mixture was autoclaved for 30 min at 125^0C. Part B: Agar with 300 ml of distilled water was heated to boiling to dissolve the agar completely. At this point, this part was poured into part A, blended for 3min, and allowed to cool for future use. Part C: The weighed ascorbic acid, Wesson's salt, sorbic acid, multivitamin multimineral capsules, vitamin E, methyl parahydroxy benzoate and streptomycin sulphate were dissolved in 75 ml of distilled water. The solution was added to the mixture of part A and B and blended for 3min. Before the mixture became cold, the diet was poured plastic vials (5 x 4 cm) filling $3/4^{th}$ the volulme and sealed with plastic cling cap, and the diet was allowed to solidify to room temperature.

Rearing procedure: To compare two formulated artificial diets with the natural diet, 30 neonate larvae obtained from stock culture were transferred into each screw capped plastic vial (5 x 4 cm) using a fine hair brush. The vials were covered with a transparent plastic lid with small holes for ventilation. From the 2^{nd} to 5^{th} generations, the population was solely reared on the artificial diets. Larval survival and development were checked daily. When the larvae reached penultimate stage, the cloth (100 x 75mm) was placed in the diet blocks to provide pupation sites. After pupation, the newly formed pupae were collected from rearing vials, sexed, numbered, weighed and placed in plastic boxes (15 x 6 cm) for adult emergence. Pupal survival, duration and adult emergence were observed daily. Each treatment was replicated five times with a total of 150 larvae per diet treatment per generation.

For each diet treatment, newly emerged adults (1:1; female: male) were paired and released into the ventilated glass cages (60 x 60 x 60 cm) containing castor inflorescence (raceme). The panicle and young capsules were placed in 500 ml conical flask with water to mimic natural ambience and fed with 10 per cent honey solution soaked in cotton swabs/wads, black cloth for mating and oviposition. Eggs deposited on plant parts, cotton swabs and black cloth were collected and counted. Collection of eggs was continued until female in the cage. Adult longevity also was recorded. Eggs were counted under a microscope (Nikon SMZ25, 1x, WD: 60) and placed into the petri dishes (8cm). The number of hatched F_1 larvae was counted daily.

Castor panicle with flowers and young capsules collected from castor field in Dryland Research Station, University of Agricultural Sciences, GKVK, Bengaluru was used as a control diet treatment. Rearing conditions and experimental procedures were the same as that of the artificial diet treatments. The total life cycle mean of four generations were cumulated for statistical analysis.

Statistical analysis: The biological parameters including incubation period, larval development, larval survival (ratio of larvae to pupae), pupal duration, weight and survival rate (ratio of pupae to adult), pre-oviposition duration and egg hatchability over mean four generations were recorded, and compared among the diet treatments using analysis of variance (ANOVA). Significant differences of mean of four generations in different treatments (diets) were tested. All analysis was performed with SPSS 16.0 statistical software. The life table parameters were calculated for each diet using Jack knife analysis; each parameter was compared at 95 per cent confidence interval.

RESULTS AND DISCUSSION

The data on the number of days required to complete each insect stage is presented in Fig. 1. Duration of the larval (16.37 d) and pupal stages (7.12 d) on the artificial diet-1 was shorter than the artificial diet-2 and the natural diet. There was no

TABLE I

Composition of ingredients in the artificial diet used to rear C. punctiferalis

Parts	Ingredient	Artificial diet 1 (Quantity)	Artificial diet 2 Quantity	Functions
A	Soybean powder (Commercially available)	130gm	100 gm	As a main ingredient/carrier
	Castor leaf powder (Locally prepared)	80 gm	-	As a token stimuli to initiate and maintain continuous feeding
	Yeast powder (RM027-500G, HiMedia Lab. Pvt Ltd.)	25gm	25gm	Source of protein and vitamin
	Casein (GRM497-500G, HiMedia Lab. Pvt Ltd.)	20gm	10gm	A protein source which provides amino acids and Carbohydrates for tissues
	Sucrose (GRM134-500G, HiMedia Lab. Pvt Ltd.)	15 gm	15 gm	As a source of sugar
	Distilled water	400ml	400ml	As a solvent for diet ingredients
B	Agar-agar (GRM666-500G, HiMedia Lab. Pvt Ltd.)	15gm	15gm	Solidifying agent
	Distilled water	300ml	300ml	As solvent
C	Ascorbic acid (PCT0207-100G, HiMedia Lab. Pvt Ltd.)	3gm	3gm	Normal growth and development, egg hatching and pupal survives
	Wesson's salt mixture (TS1100, HiMedia Lab. Pvt Ltd.)	1.5gm	1.5gm	As source of salt required to maintain membrane structure and function
	Sorbic acid (FD236-0.200g, HiMedia Lab. Pvt Ltd.)	1gm	1gm	As preservative so that the diet does not deteriorate
	Multivitamin multimineral capsules (BECADEXAMIN, GlaxoSmithKiline. Pvt Ltd.)	2 nos.	2 nos.	SuppliesVitamins
	Methyle parahydroxy benzoate (GRM1291-500G,	2 gm	2 gm	As food and flovor ingredient, stimulant
	Strepomycine sulphate (CMS220-5G, HiMedia Lab. Pvt Ltd.)	0.5gm	0.5gm	As an antibiotic for reducing microbial contamination
	Vit. E capsule USP 400mg (Evion 400, MERCK Ltd.)	2 nos.	2 nos.	As a source of vitamins for reproduction

Values in the table show the quantities in 1000g of artificial diets

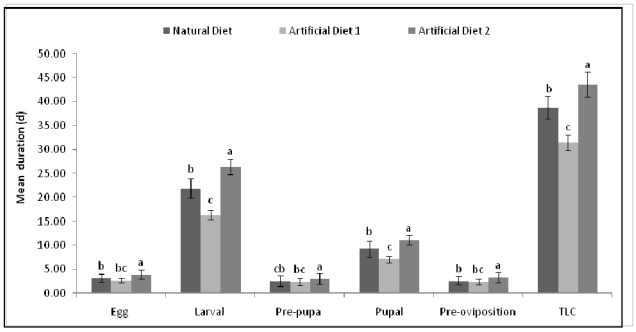

Fig.1: Duration of developmental stages (egg to adult, N=30) of *Conogethes punctiferalis* reared onarticifial diets (1 and 2) and natural host. TLC-total life cycle. Bars represent Means±SE; significant differences among three host plants are indicated by letters over each bar (*P>0.05*, LSD test).

significant difference in pre-oviposition period among the diets. On artificial diet-2, the insect took longest period to complete life cycle (43.56±2.60 d) and it was statistically significant from the duration required to complete the total life cycle on artificial diet-1 and natural diet. Artificial diet-1, wherein castor capsule powder was incorporated as on ingredient, the insect took minimum number of days to complete the life cycle (31.48±1.50 d). In natural diet, the duration required was in between the two artificial diets (38.78±2.34 d). The total life cycle of *C. punctiferalis* reared on artificial diet-1 was shorter than the artificial diet-2 and the natural diet over four generations (Fig. 2). Therefore, for a balanced diet and optimum yield of quality insects. The artificial diet should be inclusive of both essential and non-essential ingredients. Further, the texture and structure of the artificial diet should be attractive, so that the insect feeding on artificial diet has stimulating effect. Li *et al.* (2015) reported that a generation developmental time for *C. punctiferalis* on artificial diet ranged from 42.4 d to 63.3 d.

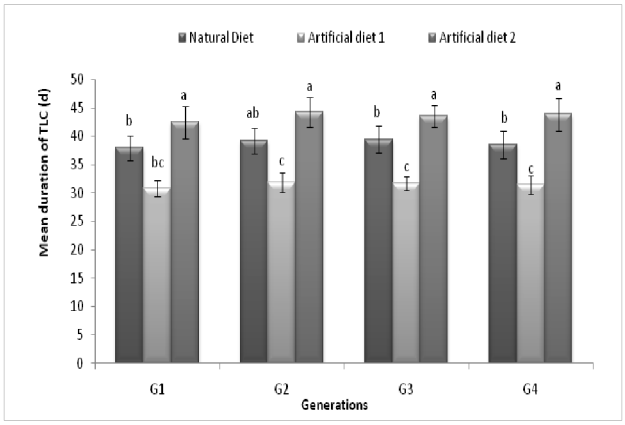

Fig.2: Total life cycle of *C. punctiferalis* reared on three diets *viz.*, artificial diet-1, artificial diet-2 and natural diet over four successive generations. Generations (G1, G2, G3 and 4); Bars represent Means ± SE; significant differences among three diets over four generations were indicated by letters over each bar (P>0.05, LSD test).

Data on quantitative and qualitative parameters of *C. punctiferalis* reared on artificial diets is presented in Table II. Insect fed with artificial diet-1 laid maximum number of eggs (39.02±1.67). This was statistically significant and higher than the number of egg laid by females reared on natural and artificial diet-2. A similar trend in values with respect to pupal weight and adult longevity was observed (Table II).

TABLE II
Pupal weight, adult longevity and fecundity of Conogethes punctiferalis *reared on articifial and natural diets*

Diets	Pupal Weight (mg)		Adult Longevity (d)		Mean** no. eggs per
	Female	Male	Female	Male	Female
Natural Diet	53.27±0.47b	40.72±0.83b	8.23±0.30b	7.03±0.30ab	24.41±0.31b
Artificial Diet 1	61.15±1.05a	49.24±1.03a	8.05±0.42c	6.15±0.29c	39.02±1.67a
Artificial Diet 2	49.42±0.57ab	39.67±0.93b	9.45±0.35a	7.67±0.14a	19.64±0.84ab

Means ± SE (N=15) within a column and followed by the same letter are not significantly different at (*P>0.05*; LSD test); ** mean of 50 females (N-=50).

TABLE III

Viability (%) of eggs, larval and pupal stages of C. punctiferalis *on artificial and natural diets*

Diets	Mean (%) survival		
	Eggs	Larvae	Pupae
Natural diet	79.20±3.85ab (N=35)	84.70±0.50b (N=45)	82.35±1.80a (N=30)
Artificial diet 1	92.45±1.30a (N=35)	95.46±1.85a (N=45)	91.50±2.55a (N=30)
Artificial diet 2	63.75±2.85b (N=35)	72.9±1.87ab (N=45)	69.84±1.30b (N=30)

Means ± SE within a column and followed by the same letter are not significantly different at ($P>0.05$; LSD test).

The pupal weight was higher on the artificial diet-1 compared to all the diets (61.02 mg per female, 49.24 mg per male). Therefore, artificial diet-1 proved superior over natural and artificial diet-2. Accordingly, the artificial diet formulated by Ambanna (2014) for *C. punctiferalis* rearing resulted that minimum pupal weight (0.37 mg) on plain semi-synthetic diet was significantly lower than natural and castor diet. Li *et al.* (2015) reported that mean pupal weights of 73.6 mg for males and 77.3 mg for females and a fecundity rate of 97.9 eggs / female.

Survival rates of eggs, larvae and pupae also were significantly affected by diets. Li *et al.* (2014) observed that the survival and reproduction of *C. punctiferalis*were impacted by the choice of the host plant material incorporated in meridic diets. The viability of the egg stage from females reared on artificial diet-1 (92.45%) was higher than artificial diet-2 (63.75%) and natural diet (79.20%) (Table III). Percentage survival of larvae from egg to pupation and pupae from larvae to adult emergence on Artificial diet-1 proved superior over natural and artificial diet-2 and the data showed statistical significant difference among the three diets offered aid lebitum to *C. punctiferalis* (Table III). Possibly, the high viability values obtained on artificial diet-1 are related to the fact that artificial diet have more quantity and equilibrium of nutrients required for the insect development. Cohen (2004) suggested that relative amounts of components of artificial insect diet impact performance and fitness of insects. Li *et al.* (2015) found that highest concentration of chestnut meal contained diets resulted in enhanced survival rate, shortened developmental duration, increased pupal weight, and increased number of eggs produced by females.

TABLE IV

Fertility life table of C. punctiferalis *from the parameters of moths reared on artificial diets (1&2) and natural diet. Mean generation time (T), net reproductive rate (R_0) and intrinsic rate of increase (r_m)*

Diets	Parameters		
	T^1(d)	R_o^1	r_m^1
Natural diet	38.75 8b	145.2b	0. 067
Artificial diet 1	30.90 c	210.8c	0.071
Artificial diet 2	44.65 a	95.45a	0.034

[1] parameters (N=4)followed by the same letter do not differ by the Jaccknife test

Generation time (T) for artificial diet-1 was shorter than that with natural diet and artificial diet-2 (Table IV). These biological parameters produced a net reproductive rate (*Ro*) (The rate of population increased in each generations) of 145.2, 210.8 and 95.45 on the natural, artificial diet-1and artificial diet-2, respectively. The r_m value is an indicator of fitness, with a higher value indicating a higher level of fitness. The r_m value for *C. punctiferlis* larvae fed on the artificial diet-1 (0. 071) was higher than natural diet (0.067) and artificial diet-2 (0.034). Li *et al.* (2015) reported that the r_m value of 0.074 for the cohort fed on artificial diet indicating a higher level of fitness than the cohort fed on fresh corn and other diets.

In summary, the development of a successive rearing method using an artificial diet for *C. punctiferalis* under laboratory or other artificial conditions was successful. We believe that castor capsule powder and less aomunt of casein were key components for the succes of this diet in enhancing *C. punctiferalis* performance. This artificial diet also has the following favorable characters. The diet is economical for rearing of *C. punctiferalis*; most materials used are common foodstuff and chemicals that are easily accessible; It making procedure is simple and easy to follow. The cost of Rs. 70-80/- for 1,000g of diet is enough to rear 150 larvae to pupae. This diet possesses favourable properties for the successive rearing. Larvae feeding on it exhibited superior performance in multiple life-history traits during four continuous generations of rearing. The diet is efficient to use. The whole process is convenient, time-efficient, and requires less labour than the conventional rearing.

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