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Artificial diet for mass-rearing of melon borer, *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae)

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ABSTRACT: The melon borer, *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae), is a serious pest of tropical and subtropical cucurbitaceous vegetables. A suitable artificial diet is desirable for producing uniform insects for commercial purposes or research. Four new artificial diets (D-1, D-2, D-3 and D-4) and bitter gourd, the natural host plant of *D. indica*, were used for rearing *D. indica*, and the life parameters were compared. The results indicated that insects could complete a full life cycle after 3 generations, only when the larvae were fed bitter gourd or the diet D-1.The new artificial diet, D-1 was formulated based on bitter gourd leaves, *Momordica charantia* (L.) and chick pea, *Cicer arietinum* L. Developmental parameters like egg hatching, larval duration and longevity of the adult reared on the D-1 artificial diet were found to be significantly improved relative to the other three diets (D-2, D-3 and D-4), but were not significantly better than those reared on the host-plant bitter gourd. However, the rearing efficiency (i.e., larval - pupal survival, developmental duration of pupa and fecundity of adults,) on the D-1 diet was on par with the rearing efficiency on bitter gourd. There were no significant changes in reproductive potential after five successive generations of rearing on the new diet. These results indicated that the newly developed diet could serve as a viable alternative to bitter gourd plant for continuous rearing of *D. indica.*

Keywords: *Diaphania indica,* artificial diet, reproductive potential, mass production

INTROUCTION

Diaphania indica (Saunders) (Lepidoptera: Pyralidae), known as melon borer, is one of the key pests of cucurbitaceous vegetables like cucumber, muskmelon, gherkin, bottle gourd, bitter gourd, snake gourd and so on (Pandy, 1977; Ravi et al., 1998; Tripathi & Pandy, 1973, Segeren 1983, Viraktamath *et al.,* 2003). *D. indica* has been reported from South America, the Indian subcontinent, Far East, South East Asia, the Pacific islands, Australia, and Africa, as causing damage to one or the other cucurbit round the year (Ke, Li, Xu & Zheng, 1988; Peter & David, 1990; Ravi et al., 1997, 1998; Radhakrishnan & Natarajan, 2009, Capinera, 2001; Peter & David, 1991). The larvae of *D. indica* feed on flowers, leaves and fruits of cucurbits and cause 14% - 30% yield loss in different cucurbit crops (Jhala *et al.,* 2005; Singh and Naik, 2006). In order to make and streamline pest control strategies, studies must be focused on the biology, bionomics, behaviors, and ecology of the pest. One has to coordinate these studies for the availability of a nonstop and satisfactory supply of high quality experimental insects. Development of artificial diet has a distinct advantage in that the insect can be reared throughout the year.There were not many serious attempts to mass multiply *D. indica* in the laboratory. However Ranganath *et al.* (2006) concentrated on developing a cost-effective mass rearing techniques for *D. indica*. Nevertheless, there are various issues related to the artificial diet for the continuous rearing of this species. The disadvantages include difficulty in the accessibility of some of the components such as tender gherk in fruit powder throughout the year and incapability of the diet tosupport the egg and first instar development. Therefore, artificial diet for this species should be enhanced for nonstop rising in the laboratory to deliver a large amount of uniform insects. Hence the point of this study was to build up an artificial diet suitable for the constant rearing of *D. Indica* without a loss of vigor or reproductive potential.

MATERIALS AND METHODS

Experimental insects

A laboratory culture of *D. indica* was established in the Bio control laboratory of Indian Institute of Horticultural Research (ICAR-IIHR), Bengaluru, India (12° 08' N, 77° 35' E). The initial culture of the insect was collected from the cucurbit vegetable field of IIHR. The population was maintained on one of the host-plant, Bitter gourd at 26 ± 1°C temperature and a photoperiod of 14:10 L:D and 60 ± 10% RH. The eggs were collected daily, and the newly hatched larvae were fed on bitter gourd leaves until pupation. Upon emergence, five pairs were confined to small plastic boxes (500 ml capacity) covered with black muslin cloth. The adults were fed with 10% honey solution.

Component and Preparation of artificial diet:

Diet for mass multiplication of *D. indica* was based on artificial diet used for multiplication of *D. indica* (Ranganath *et al.,* 2006). Compositions of the diets are detailed in Table 1. Four nutritional media were tested for the artificial diet studies of *D. indica.*

All the ingredients except agar, ascorbic acid,

Table 1. Composition of different artificial diets (for 100 ml) prepared for mass rearing of *Diaphania indica*

Ingredient (g/ml)	Diet 1	Diet 2	Diet 3	Diet 4
Distilled water	72	72	72	72
Agar	2.5	2.5	2.5	2.5
Sucrose	2.6	2.6	2.6	2.6
Dextrose	0.5	0.5	0.5	0.5
Ascorbic acid	2	2	2	2
Methyl-Para hydroxyl benzoate	0.112	0.112	0.112	0.112
Sorbic acid	0.3	0.3	0.3	0.3
Vitamin tablet	3	3	3	3
Streptomycin sulphate	0.015	0.015	0.015	0.015
Casein	-	6.5	-	-
Chickpea	-	-	6.5	-
Peptone	6.5	-	-	6.5
KOH (4M)	0.5	0.5	0.5	0.5
Bittergourd leaf	10	-	-	-
Cucumber leaf	-	10	-	-
Ridge gourd leaf	-	-	10	-
Bottle gourd leaf	-	-	-	10

vitamin tablet and casein or peptone were mixed well in a blender until smooth. Agar was dissolved in 72 ml boiling water, blended with the pasted ingredients and stirred for 5 minutes.Then autoclaved at 121ºC temperature and 15 LBS pressure for 30 minutes.After autoclaving the mixture was allowed to cool to 60º c,and then the remaining ingredients like ascorbic acid, vitamin tablet and casein or peptone were added and stirred for 2 minutes.After mixing all the ingredients and cooling to room temperature,the diets were dispensed into separate containers and refrigerated at 4° Celsius.

Observations on biological parameters (comparisons of different diets)

The suitability of four diets for continuous lab rearing was estimated by comparing its effectiveness with those reared on one of the most preferred host plant, bitter gourd. Ten first instar larvae transferred to a diet cup (size) constituted a single replication. Each diet was replicated ten times. Larvae were reared to pupal stage in these cups. Diet cups containing larvae were maintained at room temperature in the laboratory temperature (mean maximum 28.6° Celsius and mean minimum 20.4º Celsius). The artificial diet was replaced weekly once, and the bitter gourd leaves were replaced daily.

Efficiency of different diets was compared in terms of growth parameters like survival rates, egg hatching, developmental durations of egg, larvae and pupae and longevity. For fecundity, unit of two males and three females represented a single replication. 10 pairs of adults were used to measure the adult longevity.

Rearing successive generations on the diet

To evaluate the effect of different diet on the reproductive potential of *D. indica*, all of the life parameters were compared for 5 successive generations on diet 1.The methods of estimation was same as described above and the larval density per diet cup was 15 larvae .Ten replications were maintained for each generation.

Statistical analysis

Statistical analyses were performed utilizing SPSS 21. one-way analysis of variance (ANOVA)was utilized to analyze survival rates, egg hatching, developmental durations of egg, larvae and pupae and longevity, and Duncan's multiple range test ($p < 0.05$) was used to

Table 2. Survival and developmental duration of *D. indica* reared on different artificial diets in the first generation.

Diet type	Survival (%)			Developmental Duration (Days)			Body weight (mg)		Longevity (Days)		Fecundity (Eggs/Female)
	Larva	Pupa	Egg	Larva	Pupa	Egg	Female pupa	Male Pupa	Female	Male	Mean ±SD
Natural host	87.87±2.84[a]	90.36±15.98[a]	94.24±2.9[a]	20.79±0.77[a]	8.60±0.67[a]	3.52±0.49[a]	0.072±0.003[a]	0.06±0.006[a]	18.01±0.52[a]	9.00±0.40[a]	50.40±1.38[a]
Diet 1	80.4±3.02[b]	89.99±2.06[a]	88.86±8.03[b]	24.93±1.16[b]	8.50±0.71[a]	3.48±0.67[a]	0.073±0.003[a]	0.06±0.003[a]	17.98±0.69[a]	8.88±0.48[a]	48.92±0.97[a]
Diet 2	69.35±2.20[c]	80.85±3.05[b]	70.33±11.52[c]	27.40±0.77[c]	10.13±0.33[c]	4.01±0.29[b]	0.062±0.003[ab]	0.05±0.003[b]	16.02±0.95[b]	7.99±0.47[b]	40.02±2.36[b]
Diet 3	67.64±10.96[c]	80.67±2.48[b]	72.45±2.73[c]	27.37±.79[c]	9.43±0.68[b]	4.02±0.45[b]	0.062±0.03[ab]	0.05±0.002[b]	16.14±0.75[b]	7.92±0.06[b]	38.99±9.08[b]
Diet 4	51.94±5.30[d]	68.14±2.01[c]	59.60±8.65[d]	31.58±0.77[d]	9.91±0.54[c]	4.05±0.24[b]	0.05±0.056[b]	0.05±0.001[b]	14.52±2.22[c]	7.09±0.25[c]	25.10±0.59[c]

Values (mean ± SD) followed by different letters in the same row were significantly different according to Tukey's HSD test ($p < 0.05$).

Table 3. Male and female sex ratio of pupae produced per generation.

Generations	Observed			Expected 1:1 ratio	
	Male	Female	Total	Male	Female
1	275	329	604	302	302
2	154	150	304	152	152
3	160	136	296	148	148
4	134	122	256	128	128
5	16	24	40	20	20
Total	739NS	761NS	1500	750	750

Ns=not significant at 0.05 level of significance; p=0.07.

Table 5. Efficiency comparison of Diet 1 with natural host plant in the fifth generation.

Life parameter		Natural	D-1	T test
Survival or Hatching	Larvae	80.14±5.11	78.34±8.01	NS
	Pupae	88.39±3.62	86.50±6.94	NS
Percentage (%)	Eggs	92.63±0.92	86.23±1.87	*
Developmental duration (days)	Larval stage	19.85±1.18	24.28±1.64	*
	Pupal stage	8.91±0.72	9.08±0.91	NS
	Egg stage	3.30±.52	3.29±.51	NS
Body weight (mg)	Female pupae	0.071±.007	0.070±.008	NS
	Male pupae	0.059±0.006	0.058±0.004	NS

* Significant at p=0.05
NS Non Significant

Table 4. Comparison of life parameters of *D. indica* reared on D-1 in succession for five generations

Generation	Survival or Hatching percentage (%)			Pupal wt (mg)		Longevity (days)		
	Larva	Pupa	Egg	Female pupae	Male pupae	Female	Male	Fecundity
1st	80.48±3.02NS	89.99±2.06NS	88.86±8.03a	0.073±0.003NS	0.06±0.003a	17.98±0.69a	8.88±0.48a	48.92±0.97
2nd	80.28±1.79NS	87.63±6.23NS	87.57±0.77ab	0.072±.01NS	0.059±.003b	16.78±1.19b	8.07±0.82ab	48.86±.93
3rd	79.96±2.42NS	87.45±6.98NS	86.91±1.32b	0.070±.01NS	0.058±.004b	16.58±1.81b	7.71±1.25b	48.66±1.31
4th	78.11±6.14NS	87.02±7.21NS	86.70±1.32b	0.071±.008NS	0.059±.003b	15.94±2.57b	7.45±1.77b	48.31±2.18
5th	78.34±8.01NS	86.50±6.94NS	86.23±1.87b	0.071±.008NS	0.058±0.004b	15.86±2.28b	7.36±2.16b	48.46±1.47

Values (mean ± SD) followed by different letters in the same row were significantly different according to Tukey's HSD test ($p < 0.05$).

compare the means. Independent-samples *t* test was used to compare the data for all the life parameters measured on the artificial diet and the bitter gourd and chi^2 test was used to compare the male female ratio.

RESULTS AND DISCUSSION

Observations on biological parameters of *D. indica* reared on different artificial diets in the first generation. The comparisons of all the life parameters of *D. indica* reared on four new artificial diets and host plant bitter gourd are shown in Table 2. Pupal survival for *D. indica* reared on D-1 and natural host was on par and significantly different from the other diets (pupal survival: $F = 36.92$; df = 4,145; $p = 0.000$). Larval survival and egg hatching for insects reared on D-1 diet were significantly higher than for those on other diets, but were lower than those on host-plant bitter gourd (Larval survival: $F = 219.92$; df = 4,195; $p = 0.000$; egg hatching: $F = 174.768$; df = 4, 254; $p = 0.000$). The developmental durations of eggs and pupa on D-1 were significantly shorter than those reared on D2, D3 and D4 and was on par with that reared on host plant (eggs: $F = 20.44$; df = 2, 245; $p = 0.000$; pupa: $F = 45.49$; df = 4, 145; $p = 0.000$).). Male and Female adult longevity on D-1 was longer than that on other 4 and on par with that on host-plant bitter gourd. (Female adult longevity $F = 30.46$; df = 4, 95; $p = 0.000$; Male adult longevity $F = 56.72$; df = 4, 95; $p = 0.000$). The fecundity of females obtained on D-1 was significantly greater than those on the remaining four diets. Regardless of the way that the females reared on D-1produced fewer eggs than those reared on host-plant bitter gourd, the distinction was not statistically significant (Table 3) (F = 111.89; df = 4, 95; p = 0.000). Hence considering different developmental parameters, D1 was on par with the natural host and was superior than other diets (D1, D2 and D3).

The developmental duration of larvae on D-1 was significantly lesser than for those on the other three diets (F = 830.87; df = 4, 195; p = 0.000). Pupal body weight on host-plant bitter gourd was on par with D-1 and significantly greater than that on D2, D3 and D4 (female pupae: F = 3.14; df = 4, 119; p = 0.017; male pupae: F = 50.47; df= 4, 180; p = 0.000.

Comparison of life parameters of *D. indica* reared on D-1 in succession for five generations

The adults produced after second generation from the diets D-2, D-3 and D-4 were either failed to lay fertilized eggs or failed to hatch in a significant numbers, hence couldn't considerer for further generation studies. The biological performances of the 5 successive generations fed D-1were compared by measuring developmental duration, survival and hatching, body weight, longevity, and fecundity.

Survival or Hatching percentage

There was no significant difference in the egg and larval survival rate after across generations, except for pupa (Table 4). The pupal survival in the first generation was superior to the pupal survival in all other generations (F = 3.45; df = 4, 245; p= 0.009). But it was on par among second, third, fourth and fifth generations.

Developmental duration

Across generations the development period from larva to egg ranged from 31.00 ± 0.75 to 44 ± 0.87 days. Regardless of generations, the larval development period ranged from 20.50 ± 0.17 to 32.00 ± 0.71 days. In breeding depression effects on the quality of the next generation population appeared from fourth generation. The variations from the normal pupae produced were observed such as irregularly shaped pupae or incomplete pupal development from fourth generation, which failed to emerge. This added to production of non-vigorous adults and influenced the reproductivecapacity of the population. The delayed development at generation 5 is attributed to longer larval period. But the duration of pupal period did not drastically change with generation (Figure 3).

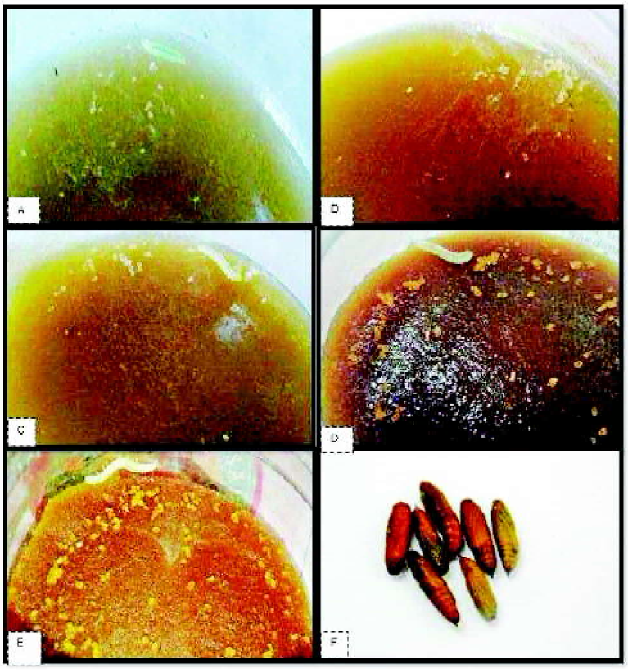

Fig. 1. Different instars of *Diaphania indica* feeding on artificial diet D-1 A) First instar, B) Second instar C) third instar, D) fourth instar E) fifth instar and F) pupa

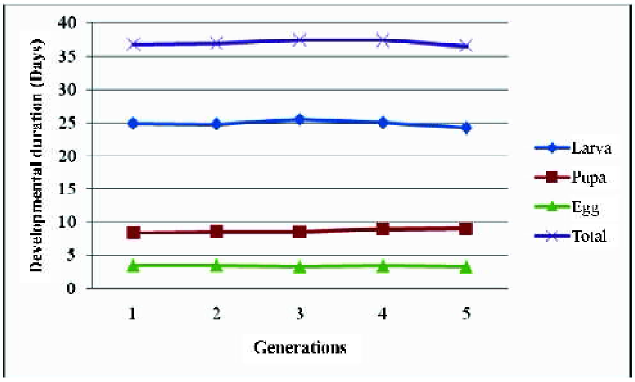

Figure 2. Duration of insect developmental stages of *D. indica* reared for five generations in D1 artificial diet

Comparison of life parameters of *D. indica* reared on D-1 and the natural host (bitter gourd) in succession for five generations.

Longevity and fecundity

Except for the developmental duration required for egg and larva, other biological parameters evaluated such as fecundity, larval mortality and pupal weight of the insect fed on artificial diet and its natural host, bitter gourd leaves were not significantly different (Figure 2). Sex

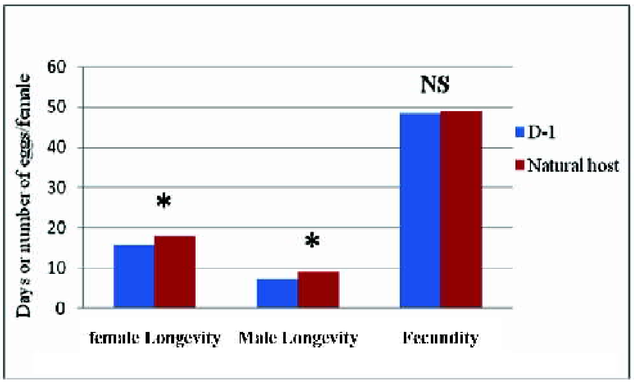

Fig. 3. Comparison of longevity and fecundity of *Diaphania indica* reared on D-1 and on the natural host bitter gourd
* Significant at p=0.05
NS - not significant (*t*-test)

ratio (Female: Male) did not differ between generations when reared either on artificial or natural host (0.85: 1). This indicates that the artificial diet 1(D-1) is the best diet which is as good as the natural food for *D. indica*, except for the number of days required to complete egg and larval stages life cycle.

Other life parameters

Table 5 gives a comparative analysis of developmental period, fecundity, mortality, pupal weight, adult longevity and survival (%) of *D. indica* on its natural host and the artificial diet in the fifth generation.

This study demonstrated that *D. indica* per formed excellently on D-1 compared to other artificial diets, but not compared to host plant bitter gourd. Important life parameters of insects reared on D-1, including larval pupal survival, body weight and fecundity, were similar to those reared on host plant bitter gourd. Whereas the other parameters like egg hatchability and larval developmental period were significantly different. These results imply that the new artificial diet D-1 can be used as an alternative to natural hosts for rearing purposes. Factors like sex ratio, ages of the virgin female and male at mating, delayed female mating and the number times the male has previously mated will seriously affect the reproductive potential of an insect (Stewart and Philogène 1983; Foster and Ayers 1996; Proshold 1996; Michereff *et al.,* 2004). However, in our study, the fecundity of *D. indica* adult female after 5 successive generations of laboratory rearing was comparable and was on par with that reared on host plant.

The unsprayed cucurbitaceous vegetables and weeds are storehouse of natural enemies contributing to regulation of pests of cucurbits. Development of Insecticide Resistance (IR) in *D.indica* (Ravi, 1998) and outbreak of minor pests such as sucking pests can bring about pest management problems in cucurbitaceous crops. Availability of artificial diet to rear *D. indica* facilitates mass production of natural enemies in the laboratory, which could be utilized in pest management programmes. All things considered, this study showed that, the new artificial diet supported a high rate of survival comparable to those reared on the host-plant (bitter gourd). The new artificial diet was suitable for the continuous rearing of *D. indica* to get uniform insects of excellent performance without a decline in vigor or reproductive potential.

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