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A MODIFIED ARTIFICIAL DIET FOR REARING POTATO TUBER MOTH, *PHTHORIMAEA OPERCULELLA* (ZELLER)

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ABSTRACT: An artificial diet and rearing system was standardized and validated for potato tuber moth larvae [*Phthorimaea operculella (Zeller)*] under laboratory conditions. Potato tuber moth (PTM) completed its life cycle on artificial diet and produced a new generation. Bionomics of potato tuber moth, reared on this artificial diet, were compared to those reared on potato leaves. The percent survival of the larvae on exclusive artificial diet (treatment-1) was less (51.2%) than on potato leaves (89.6%). Survival could be increased significantly on artificial diet (89.2%) when the neonates were initially reared on potato leaves up to first instar and then released on artificial diet for completion of rest of the larval stages (treatment-2). The mean durations of different life cycle stages were longer on artificial diet (treatment-1 and treatment-2) as compared to control reared on potato leaves. Numbers of the hatchlings per adult pair per day were greater in control than in artificial diet. The diet and the rearing system described here (treatment-2) will be useful for calculating toxicological data of many insecticides.

INTRODUCTION

Potato tuber moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), is one of the major economic pests of potato in tropical and sub-tropical regions. Damage to the stored potatoes has been estimated to be about 20% in Bihar, 30-40% in plateau region, 18-85% in the north-eastern hills and 25-50% in Himachal Pradesh (3). The biology and ecology of potato tuber moth have been extensively studied in India (6, 7), but no attempts have been made to develop artificial diet for rearing this important pest under laboratory conditions. Development of artificial diet would be exceptionally useful for maintaining potato tuber moth cultures round the year and also it can be used for determining the effectiveness of insecticidal agents by incorporating them into such diets. Possibility of homogenous mixing of test compounds in artificial diet ensures better and accurate information on lethal concentrations (LC) as compared to the conventional methods, in which the test substances are applied to leaves and tubers.

Although there are few reports on rearing of PTM on artificial or semi-artificial diet (1, 2, 4, 5), when used in our laboratory there were negligible to very low rates of larval survival on these diets. Singh and Charles (5) diet supporting low larval survival was better than others. Therefore, the original Singh and Charles diet was modified for improving survival and fecundity of PTM. Here, we report the bionomics of PTM reared on this modified artificial diet under laboratory conditions with data on fecundity, duration of different life-cycle stages and survival.

MATERIALS AND METHODS

Insect culture

P. operculella adults were collected from potato fields of Central Potato Research Institute, Shimla. (31°N 77°E; 2200 m asl) and mated in 21x8 cm plastic containers at 26 °C under total darkness. The containers were

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covered with double layered muslin cloth and had filter paper in between. Cotton soaked with sucrose solution (10%) was kept on a small Petri dish in these containers as a food source for adult moths. Filter papers, on which eggs were laid, were collected every 2 days and kept in separate container for hatching. The neonates were then transferred on whole potato tubers by fine camel hairbrush immediately after hatching. Pupae were collected from these infested tubers and allowed to grow into adults. The adults were reared again in the same manner and a homogenous culture was established.

Diet preparation

The diet reported by Singh and Charles (5) was modified as shown in **Table 1**. For preparation of artificial diet, cholesterol (group-II) was dissolved in excess dichloromethane, stirred with group-I components and the mixture was left for 24 h in the laminar flow air bench after which the mixture was dissolved in 75 ml of water and the pH was adjusted to 6.5 by adding 4N potassium hydroxide solution. To this 5 ml emulsion of group-III components was added and the mixture was autoclaved at 120 ^{o}C (105 KPa) for 20 minutes and then cooled to 60 ^{o}C. Vitamins and sugars of group-IV were dissolved in 15 ml of distilled water and added to the autoclaved diet after filter sterilization. Elements of group V (sorbic acid dissolved in 95% ethanol and Penicillin G and streptomycin sulfate dissolved in distilled water) were then added after filter sterilization. Volume of the diet was made to 120 ml with sterile water. After proper mixing, 1 cm thick layer of the diet was poured in the 12-well insect rearing plates (M/S Tarsons Products Pvt. Ltd., 33/1, Netaji Subhash Road, Kolkata 700 001, India, Cat. No. 980020). The diet was dried overnight in a laminar airflow bench on which larvae were kept on the next day for feeding.

Table 1. Composition of modified diet.

Ingredients	Singh and Charles (1977) diet (Quantity$^{-120\ ml}$)	Modified diet (Quantity$^{-120\ ml}$)
Group-I		
Agar	2.5 g	2.5g
Cellulose powder	10 g	13.0 g
Lactic casein	3.5 g	3.5 g
Wheat germ	3.0 g	3.0 g
Wesson's salt mixture	1.0 g	1.0 g
Group-II		
Cholesterol	0.1 g	0.1 g
Group-III		
Linoleic acid	0.25 g	0.25 g
Tween 80	0.05 g	0.05 g
Group-IV		
Sucrose	3.0 g	3.0 g
Glucose	0.5 g	0.5 g
Inositol	0.04 g	0.04 g
Ascorbic acid	0.40 g	0.40 g
Choline chloride	0.20 g	0.20 g
Niacinamide	2.0 mg	2.0 mg
Calcium pantothenate	2.0 mg	2.0 mg
Thiamine hydrochloride	0.5 mg	0.5 mg
Riboflavin	1.0 mg	1.0 mg
Pyridoxine hydrochloride	0.5 mg	0.5 mg
Folic acid	0.5 mg	0.5 mg
Biotin	0.04 mg	0.04 mg
B_{12}	0.004 mg	0.004 mg
Group-V		
Sorbic acid	176 mg	176 mg
Nipagin M	132 mg	-
Streptomycin	-	0.003 mg
Penicillin G	-	0.003 mg

Rearing of larvae

Feeding on potato leaves: For experimental control, all larval stages of potato tuber moth were reared on potato leaves fixed in 20 ml vials containing water. Five neonate larvae were transferred on each potato leaf with camel hair brush, immediately after hatching. Observations were taken from ten potato leaves infested with 5 larvae each. Fresh leaves were changed after every third day and the larvae from old leaves were transferred on the new leaves for feeding. Pupae were collected from these leaves and after determination of their sex, they were allowed to develop into the adults in 1:1 ratio (female: male) in plastic containers (21 × 8 cm) and allowed to mate. Eggs were collected every second day. The rearing was carried out in the BOD incubator at 26° C.

Feeding on artificial diet: The diet was prepared as described earlier. While rearing the larvae on artificial diet, two different treatments were used. In treatment-1, the larvae were transferred to the wells of the insect rearing plates (2 larvae/well) immediately after hatching. In treatment-2, the larvae were initially fed on potato leaves (@ 5 larvae per leaf) till the first instar stage and then the surviving second instars were transferred to the artificial diet (2 larvae/well). Each treatment was replicated thrice. In both the treatments, larvae were reared at 26 °C in the BOD incubator. The pupae from each treatment were allowed to develop into adults in the plastic containers (21 x 8 cm) and eggs were collected every second day.

Parameters for comparing efficiency of artificial diet

Duration of developmental stages, percent survival, fecundity and percent hatch were used to determine efficiency of artificial diet. Duration of egg stage was time period between laying of egg masses till hatching of neonates. Instars were identified by head capsule width. The larvae turned greenish and reddish on potato leaves and artificial diet, respectively prior to pupation. Pupae were identified by their relative inactivity, barrel shape and silken cocoon around them. For calculating the number of eggs per pair per day and percent hatching, observations were taken from ten pairs of the adults.

Data analysis

For duration of life cycle stages and per-cent survival, experiments were laid out in two factorial randomized complete block design with treatments and larval stages. To comply with the assumption of normal distribution, per-cent survival data were arc sine transformed before performing analysis of variance (ANOVA). For statistical analyses of fecundity (number of eggs/pair/day) and percent hatch, single factor randomized complete block design was employed. The percent hatching data were also arc sine transformed prior to statistical analysis. Where the F-statistics were significant, the means were separated using least significant difference (LSD) test at 5% level of significance and presented in respective tables. All the statistical analyses were carried out using MSTAT-C software package.

RESULTS AND DISCUSSION

Establishment of larvae in the diet

Three different published diets (1, 2, 5) were tried to rear PTM in the present study. Although the neonates tried to feed and penetrate in two diets (1, 2) in initial stages, they were unable to establish and eventually died in the first and second instar stages. The larvae fed successfully on the Singh and Charles diet but very few (4-5%) of them were able to complete the life cycle. We, therefore, modified this diet to achieve better survival of the potato tuber moth. The PTM larvae penetrated modified medium **(Fig. 1A)**, made tunnels inside the diet and produced piles of the excreta on the surface of the diet **(Fig. 1B)**. Similar excreta piles were produced by larvae fed on leaves or tubers. The larvae pupated inside the diet **(Fig. 1C, D)**. The collected pupae were able to grow into adults, mate and lay eggs completing whole life cycle on this diet.

Durations of the developmental stages

Duration of the potato tuber moth life cycle was significantly shorter on potato leaves than in treatments 1 and 2 with artificial diet **(Table 2)**. The period between egg laying and

Fig. 1. Feeding behaviour of PTM larvae on artificial diet. (A) The larvae released on top of the diet surface quickly burrowed in the diet and moved to the bottom of the diet inside the well; (B) piles of excreta accumulated at the bottom of the well due to feeding of larvae on diet; (C) larvae started making cocoons at the bottom of the well and (D) pupated.

hatching in treatment-1 was significantly higher than potato leaves and treatment-2. For other larval stages, the lengths of the instars were significantly shorter when they were reared on the potato leaves than on artificial diet-based treatment-1 and treatment-2. Similarly, the duration of the pupae was also significantly longer in artificial diet than on potato leaves.

Effect of artificial diet on survival of larvae, pupae and adults

Survival of first instars was significantly better when they were reared on potato leaves and treatment-2 with artificial diet **(Table 3)**. Similarly, the survival of second instars to fourth instar larval stages was significantly higher on potato leaves and treatment-2. No significant difference in survival of larvae in treatment-2 and in potato leaves was observed. However, survival of the pupae was best in treatment-2. There was no significant difference for survival of adults fed on potato leaves and artificial diet.

Number of egg masses and percent hatching

Potato tuber moths reared on the potato leaves produced significantly more egg masses/pair/day than those reared on the artificial diet **(Table 4)**. The percent hatching was also higher in leaf-fed potato tuber moths compared to artificial diet treatments.

In the present study, three earlier reported artificial diets (1, 2, 5) were tried. In first 2 diets, the PTM larvae were unable to establish and eventually died, while in third diet the larvae fed successfully but very few (4-5%) of them were able to complete their life cycle. Therefore, none of these diets qualified for use as control in the experiments. The diets described by Gleave *et al.* (2) and Badegana and Ngameni (1) were more compact and mortality of PTM larvae on these diets was due to their inability to penetrate into the diet. On the contrary, diet described by Singh and

Table 2. Durations of life cycle stages of potato tuber moth on different diets.

Growth stage	Duration (days) on different diets		
	Potato leaves (control)	Treatment -1	Treatment -2
Egg incubation	3	3	3
1st instar	3.2	4.8	3.7
2nd-4th instar	13.4	16.8	17.0
Pupa	6.6	8.4	8.0
Adult	4.3	5.1	5.3
Total duration of life cycle(1st instar to adult)	27.5	35.1	34.0
	Treatment	Growth stage	Treatment x Growth stage
F	29.71	617.81	3.88
df for F-value	2, 28	3, 28	6, 28
Standard error of mean	0.152	0.196	0.339
CD (P $\leq$ 0.05)	0.439	0.567	0.982

Table 3. Survival of growth stages of potato tuber moth on different diets.

Growth stage	Percent survival on different diets		
	Potato leaves (control)	Treatment -1	Treatment -2
1st instar	98.6(84.6)*	60.0(50.7)	96.6(79.6)
2nd-4th instar	93.3(75.7)	54.0(47.3)	91.3(72.9)
Pupa	84.0(66.6)	46.6(43.1)	86.6(68.6)
Adult	82.6(65.7)	44.0(41.5)	82.6(65.5)
Mean survival of all stages	89.6(73.2)	51.2(45.7)	89.2(71.7)
	Treatment	Growth stage	Treatment × Growth stage
F	451.24	57.23	2.82
df for F-value	2, 22	3, 22	6, 22
Standard error of mean**	2.257	2.606	4.514
CD ($P \leq 0.05$)***	2.13	2.46	3.01

* Figures in parenthesis are means of arc sine transformed data.
** and *** Standard error of mean and CD values are for arc sine transformed means.

Table 4. Effect of diets on fecundity and percent hatch in potato tuber moth.

Treatments	Eggs/pair/day	Hatch (%)
Potato leaves (control)	37.8	77.4 (61.6)*
Treatment –1	23.3	35.9 (36.8)
Treatment –2	33.7	70.3 (57.0)
F value (calculated)	1004.09	6318.41
df	2, 28	2, 28
Standard error of mean	0.291	0.182**
CD ($P \leq 0.05$)	0.86	0.54***

* Figures in parenthesis are means of arc sine transformed data.
** and *** Standard error of mean and CD value is for transformed means.

Charles (5) was relatively coarser and penetration of PTM larvae on this diet was possibly due to the fibers of the cellulose, which modified the physical texture of the diet. On this diet we observed that majority of larvae died and there was bacterial/fungal growth around the dead larvae. These observations prompted us to modify Singh and Charles (5) diet by increasing cellulose content from 10 g to 13 g and replacing nipagin M with streptomycin and penicillin G **(Table 1)**. Increased cellulose content and wide spectrum antibiotics helped in the rapid establishment of the larvae in the medium and thus a homogenous population could be obtained. The larvae of potato tuber moth are very delicate and often get drowned in water film on the surface of this diet. In order to avoid this, artificial diet was dried overnight in the laminar airflow bench.

In general the durations of various stages of potato tuber moth were much longer on artificial diet (in both treatment 1 and treatment 2) than on potato leaves (control). This is obvious because potato leaves are natural diet of the pest and contain all essential nutrients required for its growth.

Singh and Charles (5) reported egg incubation period of 3 days on potato tubers as well as on artificial diet and same was observed in our studies. The larval and pupal durations reported by Sharaby and Saleh (4) on potato tubers were similar to our observations on potato leaves. However, larval and pupal periods observed in our experiments were longer than those reported by Singh and Charles (5). These variations in the duration of various stages of potato tuber moth can be due to the differences in the culture conditions. Singh and Charles (5) maintained potato tuber moth cultures at 30 °C with 16 h photoperiod while in our experiments they were maintained at 26 °C in dark.

Under treatment 2, in which the first instars were initially fed on potato leaves, the duration of first instar was almost same as observed in those fed on potato leaves. However, the durations of other stages were longer when larvae were transferred to artificial diet. This indicates presence of enhancing nutritional factors in potato leaves, which probably are not present in artificial diet. The survival of the different stages of potato tuber moth on artificial diet in treatment 2 was almost the same as in control. Better survival in treatment 2 may possibly be due to the better establishment of pre-fed older larvae on the diet. It also appears that there is some critical nutritional requirement during first instar stage, which is met by feeding the larvae on potato leaves. The PTM reared on potato leaves also exhibited better egg laying, fecundity and percent hatch. Thus, there appears a possibility of improving bionomics of potato tuber moth by supplementing artificial diet by filter sterilized leaf/tuber extract.

The cost of 120 ml diet comes to Rs. 18 from which 100 pupae can be obtained.

CONCLUSION

Reproductive bionomics of PTM was better on potato leaves as compared to artificial diet. However, for round the year availability of PTM cultures, one has to resort to rearing PTM on modified diet using treatment-2. Moreover, artificial diet will be more useful for accurate and precise evaluation of potential insect control agents including insecticidal crystal proteins (cry proteins) by their homogenous incorporation in the diet. Continuous availability of PTM larvae would also help in the mass rearing of the natural enemies like granulosis viruses and parasites for integrated pest management strategies.

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