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Research Article

A Successful Laboratory Mass Rearing System Of Spotted Pod Borer, Maruca Vitrata (Crambidae: Lepidoptera) On A Black-gram Based Semi-Synthetic Diet

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Abstract

Background: The spotted pod borer Maruca vitrata Fabricius (Lepidoptera: Crambidae) is a serious pantropical pest of legume crops. A suitable semisynthetic diet is desirable for producing uniform insects for commercial purposes or research.

Results: To mass rear M. vitrata under laboratory conditions, a study was carried out and aimed to develop a blackgram-based semisynthetic diet (D-B). Comparison of several biological, reproductive and digestive parameters for three successive generations of M. vitrata reared on four different semisynthetic diets, viz., D-J, D-JR, D-NEW, black gram-based semisynthetic diet (D-BAS) and black gram natural diet, was successfully attempted. Larvae fed a blackgram-based semisynthetic diet (D-BAS) recorded the shortest developmental time (29.37 days) and the highest food consumption (64.21 mg) and fecal production (20.83 mg), resulting in the maximum larval and pupal weights (48.46 and 47.11 mg, respectively). The insects fed the D-NEW and blackleg natural diets recorded the longest developmental period (32.90 and 34.48 days) with minimum larval weights (44.03 and 48.64 mg) and pupal weights (40.39 and 49.43 mg), respectively. The highest percent pupation (82.53), adult emergence (78.22), larval (6.50) and pupal growth index (12.86) were recorded for larvae fed a blackgram-based semisynthetic diet (D-BAS). The highest sex ratio (1.81) and fecundity (43.94 eggs) were recorded on the blackgram semisynthetic diet (D-BAS). The highest relative consumption rate (0.23 gg-1day-1), relative growth rate (0.084 gg-1day1), relative metabolic rate (0.073 gg-1day-1), approximate digestibility (67.49%) and metabolic costs (46.51%) and lowest efficiency of conversion of ingested food (36.09%) and efficiency of conversion of digested food (53.49%) were recorded for the blacklegy semisynthetic diet (D-BAS).

Conclusions: A black gram-based semisynthetic diet was found to be superior, exhibiting a high rate of egg production, growth, survival and reproduction compared to other diets, but was not significantly better than the natural diet with black gram flowers. The blackgram-based semisynthetic diet was suitable for the continuous rearing of M. vitrata to produce uniformly sized insects of predictable performance without a loss of vigor or a decline in reproductive potential. A blackgram-based semisynthetic diet can be used as an efficient alternative to a natural diet for rearing in laboratory conditions.

Keywords: Blackgram-based Semisynthetic Diet, M. Vitrata, Growth, Reproductive, Nutritional Indices.

1. Introduction

The aim of this study was to develop and standardize a semi-synthetic diet suitable for the continuous mass rearing of *M. vitrata* without loss of vigor or reproductive potential in the laboratory for various purposes. In general, host insects may be reared either on their natural food or semisynthetic diets. The rearing of the pest insect in the laboratory requires the availability of good quality food. In general, semi-synthetic diets used for insect rearing in laboratories are produced and marketed by firms for rearing a specific pest. The maintenance of a continuous host insect population in the laboratory on its natural diet involves various problems, viz., the availability of a suitable host plant or its preferred parts throughout the year. Hence, the development of a suitable semisynthetic diet is highly essential. Although it may be difficult and time consuming initially to establish or standardize different components of the diet for continuous maintenance of host insect culture in any laboratory, once optimized, they may prove to be simple to prepare and easy to use for further studies. With proper quality control procedures, it is possible to produce insects on semisynthetic diets that are comparable and sometimes superior to those raised on their natural food.

Many studies have focused on developing an economical diet formula and cost-effective rearing techniques for *M. vitrata.* However, there were many issues related to the availability of ingredients for the development of semisynthetic diets for the continuous rearing of this species, such as cowpea flower powder, Taiwan sesbania leaves, and Taiwan sesbania seed powder [1, 2]. In addition, the rearing density of the larvae in most previous studies was one larva per container or less than five larvae per container, except for Liu and Hwang, and an adequate number of mating pairs was maintained for the experimental population by using a semisynthetic diet [1-5]. Therefore, semisynthetic diets for this species need to be improved for continuous rearing in the laboratory to produce a large number of uniformly sized insects without compromising the loss of vigor or reproductive potential. Good knowledge of insect rearing procedures in the laboratory is important to study their life cycle, behavior, feeding habits, and susceptibility or resistance to chemical pesticides.

To date, a large number of semisynthetic diets have been developed, and many insect species are being reared successfully on these diets. Understanding the biology and standardization of different diets for mass multiplication of *M. vitrata* on semisynthetic diets will yield valuable information for strategizing the management options of the pest. Therefore, a semisynthetic diet for *M. vitrata* needs to be improved for mass rearing in the laboratory to produce a large number of uniformly sized insects that can be used further for a variety of experimental purposes.

2. Methods

2.1. Rearing of m. Vitrata

Mass rearing of spotted pod borers was carried out in the Department of Entomology, Agricultural College, Bapatla. The second instar larvae of M. vitrata were collected from a blackgram field on an agricultural college farm. Similarly, a baseline population of M. vitrata was established and maintained under laboratory conditions and reared on blackgram flowers and pods as a natural diet for three generations at 26 ± 1 °C, with a photoperiod of 14:10 L:D and 60 ± 10% RH, except for adult mating and oviposition, when they were kept at 85–90% RH. The eggs were collected daily, and the newly hatched larvae were used for further experimentation. Twenty newly hatched larvae were transferred to transparent plastic jars (10 cm diameter) and reared with either semisynthetic diets or on blackgram flowers and pods. The semisynthetic diet was replaced every three days, and the black grams of flowers and pods were replaced every two days. A corrugated paper was put into the box as a pupation site when the larvae entered the prepupal stage, which was determined by a change in the body color from light pink to light green.

2.2. Preparation Of Blackgram-Based Semi-Synthetic Diet

The following diet mixture treatments were evaluated or assessed for successful rearing of *M. vitrata* in the laboratory.

1) D-JR, Jackai and Raulston. ; 2) (D-BAS) A semisynthetic diet based on blackgram leaves, flowers and flour; 3) D-J diet, as reported by Jin et al. ; 4) D-NEW diet, as reported by Liu and Hwang; 5) Blackgram flowers and pods as a natural diet for comparison [2, 3, 5].

The composition of the standard diet is given in Table 1. A blacklegy semisynthetic diet was prepared in five steps as detailed below.

Blackgram seeds, leaves and flowers were baked in an oven at 100 °C for 6 h and ground to a fine powder in a 250 ml stainless steel blender. (2) Agar was mixed with 1/4th of the final volume of total water content. (3) The major nutritional and antimicrobial ingredients, blackgram flour, leaf factor, blackgram flower powder, brewer's yeast, sorbic acid, and methyl-parahydroxybenzoate, were combined and added to the remaining volume of the water. (4) The first two parts (2 and 3) of the diet were blended and stirred for 3 min separately and then autoclaved at 121 °C and 15 psi for 15 min together. After autoclaving, the two components were blended for 5–6 min in the blender until cooled to approximately 60 °C; then, the third part of the diet, including ascorbic acid, a vitamin mixture, and approximately 50 ml of distilled water, was added to the mixture. (5) Formaldehyde was added to the mixture and blended for 2 min. After mixing all the ingredients and cooling to room temperature, the paste-like diet was dispensed into appropriate containers and stored at 4 °C.

2.3. Rearing of m. Vitrata on semi-synthetic diet

The neonate larvae of M. vitrata were transferred to Petri plates containing diet cubes and were allowed to feed. The second instar larvae were isolated into multicavity cell trays and were fed with preweighed diet cubes. The diet was changed every three days and was replaced with a new diet cube of a known weight. (Plate 1)

Daily morphometric observations, i.e., larval period, larval weights, food consumed (mg), fecal weight (mg), duration of the prepupal period (days), pupal period (days), pupal weight (mg measured after 24 h of pupation) and adult longevity (days), were recorded. In addition, overall generation indices, such as survival percent, sex ratio, fecundity, egg incubation period (days), larval growth index, pupal growth index, and nutritional indices, such as RCR, RGR, RMR, ECD, ECI, AD and CM, were also calculated.

Larval growth index = $\frac{\text{\% Pupation}}{\text{Larval period(days)}}$

Pupal growth index = $\frac{\text{\% Adult emergence}}{\text{Pupal period (days)}}$

Relative metabolic rate - RMR (g/g/day) = $\frac{M}{(B \times T)}$

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Relative consumption rate - RCR ($gg^{-1}day^{-1}$) =

$$\frac{\text{Weight of food ingested}}{\text{Average larval body weight per day}}$$

Relative growth rate - RGR ($gg^{-1}day^{-1}$) =

$$\frac{\text{Increase in larval body weight}}{\text{Average insect body weight per day}} \times 100$$

Approximate digestibility -AD (%) =

$$\frac{\text{Weight of food ingested} - \text{Weight of faeces}}{\text{Weight of food ingested}} \times 100$$

$$\text{ECD (\%)} = \frac{\text{Weight gained by larvae during feeding period}}{\text{Weight of food ingested}} \times 100$$

$$\text{ECD (\%)} = \frac{\text{Weight gained by larvae during feeding period}}{\text{Weight of food ingested} - \text{Weight of faeces}} \times 100$$

Metabolic Cost (CM) (%) = 100 – ECD

where T: duration of feeding period (days); F: weight of fecal matter produced (g) during T; B: weight gain by larvae (g) during T; : mean weight of larvae (g) during T; I: weight of food consumed (g) during T; I – F: food assimilated (g) during T; and M: (I – F) – B: food metabolized during T (g).

3. Results

Biological Parameters: *M. vitrata* development on a black gram-based semisynthetic diet was as good as that on other semisynthetic diets but not comparable to that on a natural black gram diet. The results suggested that there was a significant influence of different diets on the growth parameters of *M. vitrata.* There was a significant difference between the egg incubation periods when the larvae were fed different diets, ranging between 2.75 and 3.17 days. The shortest larval durations were recorded when the larvae were fed D-B (14.24 days), D-JR (15.12 days), D-J (16.34 days) and blackleg natural diet (17.08 days) diets compared to D-NEW (17.47 days) diets. Similarly, the lowest pupal durations were recorded for the larvae that were fed the D-B, D-J, and D-NEW diets (6.05, 6.08 and 6.17 days, respectively) compared to the D-JR (6.73 days) and blackleg natural diets (7.04 days), whereas the adult life span was reduced on the D-NEW (6.28 days) and D-B (6.33 days) diets compared to the D-J, D-JR, and blackleg natural diets (6.50 and 6.50 and 7.20 days, respectively). (Table 2 and Figure 1)

Larvae fed the natural blackgram diet and the D-B and D-JR semisynthetic diets were heavier (ranging from 45.55 to 55.73 mg) than the larvae fed the D-J (45.17 mg) and D-NEW diets (44.03 mg) (Table 3 and Figure 2). Similarly, heavier pupae resulted on blackgram natural diets, D-B and D-JR (ranging between 49.43 and 42.30 mg), compared to D-J (40.74 mg) and D-NEW (40.39 mg) (Table 4 and Figure 3). The fecundity was higher on the blackgram natural diet (52.03 eggs) and blackgram semisynthetic diet (43.94 eggs), whereas it was the lowest on the D-J diet (23.57 eggs), indicating the preference of blackgram as its natural host (Table 5 and Figure 4).

The highest percent pupation was recorded on the blackgram natural diet, which was 85.39%, and the blackgram-based semisynthetic diet, which was 82.53%, followed by the D-JR, D-NEW and D-J diets, which recorded 77.67, 67.78 and 61.46%, respectively. The lowest pupal mortality was recorded on the blackgram natural diet (14.61%). Among all semisynthetic diets, the lowest mortality was 18.64 percent on the blackleg semisynthetic diet, followed by the D-JR, D-NEW and D-J diets, which recorded 22.33, 32.22 and 38.54 percent mortality, respectively. The highest percent adult emergence was observed in larvae fed the blackleg natural diet (83.94%) and the blackleg semisynthetic diet (78.22%), followed by the D-JR, D-NEW and D-J diets, which recorded 72.92, 63.21 and 57.21 percent emergence, respectively (Table 6 and Figure 5). The maximum larval growth index was recorded on the D-B diet at 6.50. The pupal growth index of *M. vitrata* in three generations was high on the D-B diet at 12.86 (PGI), and this was closely followed by the blackgram natural diet (11.63 PGI) (Table 7 and Figure 6).

Nutritional Indices: Food consumption by spotted pod borer larvae was found to be directly related to weight gain. The highest amount of food was consumed by the heaviest larva, i.e., on the blackgram natural diet (68.80 mg) with high fecal matter production (21.94 mg), followed by the D-B diet (64.21 mg with fecal matter 20.83 mg). The lowest amount of food consumption (53.83, 53.85 and 53.95 mg) and fecal weights (17.82, 17.93 and 24.06 mg) were recorded by the insects that were fed the D-J, D-JR and D-NEW diets, respectively (Table 8 and Figure 7). The RCR, RGR and RMR values were highest for the D-B diet (0.23, 0.084 and 0.073 g g-1 day-1, respectively) and lowest for the D-NEW diet (RCR, RGR and RMR were 0.16, 0.061 and 0.030 g g^{-1} day^{-1}, respectively) (Table 9 and Figure 8). The highest AD and CM values of the D-B semisynthetic diet (67.49 and 46.51%, respectively) were high, followed by the black gram natural diet (68.10 and 42.81%, respectively). The highest ECI and ECD values were recorded with the D-J (43.73%) and NEW diets (67.30%) (Table 10 and Figure 9).

4. Discussion

The shortest egg incubation period of 2.75 observed on the blackgram-based semisynthetic diet (D-B) is in close agreement with Wang et al., who reported an average incubation period of 2.2 ± 0.4 days on a soybean-based artificial diet [6]. These variations might be due to changes in the nutritional quality of diets, protein content, temperature and humidity conditions in the laboratory. Larvae grown on a blackgram-based semis-synthetic diet (D-B) recorded the lowest larval developmental time of 14.24 days, which was in close conformity with reports of Ochieng and Owuor, who reported a larval period that lasted for 8 to 14 days on cowpea [7]. On a semisynthetic diet, the larval period was reported to range from 13.5 to 14.3 days [8]. Wesson salt and choline chloride are indispensable in insect diets and may determine the growth and survival of insects. Prolongation in the developmental time indicates a compensatory action, where larvae subjected to a poorer nutritional source tend to compensate for food stress by lengthening the immature stage. The larvae that fed on both D-NEW and D-J diets were both adversely affected, as they had a prolonged larva-adult period and reduced larval and pupal weight. The quality of the food consumed in the first few instars plays an important role in the development of insects.

The developmental time was found to be the lowest with the D-B semisynthetic diet (29.37 days), which was significantly superior to the rest of the diets. The results indicated that including wheat germ, sucrose, brewer's yeast and ascorbic acid can provide sufficient nutrition for normal insect growth, survival, and fecundity and sustain the completion of multiple life cycles. The results of the present investigation were in concurrence with those reported by Booker, in which the total life cycle lasted for approximately 30 to 35 days [9]. The highest larval weight was recorded on the natural blackgram diet (55.73 mg), which might be due to the protein content in blackgram flour and brewer's yeast. In addition to blackgram flour and brewer's yeast, wheat germ and sucrose are also important protein components of insect semisynthetic diets [10]. The pupal weights of the present results are in line with the reports of Arulmozhi, who reported female pupal weights of 44.40 mg on a cowpea diet and 37.8 mg on a soybean semisynthetic diet. Pupal weights were higher on the natural diet and are contrary to the finding of Chi et al. [4, 11]. They reported a higher pupal weight on the artificial diet than on the natural diet. This weight gain on the artificial diet in their study may be related to the amount of blackgram flour added to the diet.

The highest sex ratio values recorded for the larvae fed the blacklegic natural diet and D-B semisynthetic diet were 2.36 and 1.81, respectively. The diet developed by Ochieng and Bungu showed a decline in larval performance after a number of successive laboratory generations, while Jackai and Raulston were unable to obtain adequate mating pairs for studies on oviposition. However, the present studies indicated that there was no problem in obtaining adequate mating pairs, and a sex ratio of 1:1 was maintained throughout where mated females produced the expected number of viable eggs [3, 8, 12]. This might be due to the feeding habit of larvae, climatic conditions and the addition of choline chloride, which affected egg and sperm production. The observations are in accordance with Karmarkar, who reported the fecundity of *M. vitrata* females as 37 to 81 eggs; similarly, Vaidya also reported 35 to 81 eggs [13, 14] . Variation in the number of eggs laid might be due to the feeding habits of larval stages, and climatic conditions and choline chloride added to the diet might have affected egg and sperm production. In addition, the present research revealed that relative humidity in the culture chamber was very important during mating when 85–90% was needed or adults failed to mate.

Higher percent pupation among all semisynthetic diets was on the blackgram semisynthetic diet (81.36%). The present results are in close agreement with the results of Wang et al., who reported 65% on soybean and 72% on a cowpea artificial diet. Slight variations could be due to changes in protein level and moisture content in artificial diets [6]. High water content in a diet was reported to cause drowning of larvae and asphyxiation of adults [15, 16]. Lingappa found that low agar content in the diet increased larval mortality [17]. In our observation, a high mortality rate appeared in the larval stage, especially in the neonate larval stage, which could at least partially be attributed to the inappropriate water and agar content in the D-J and D-JR. The results obtained were in close agreement with Jackai and Raulston, who reported 65 percent adult emergence on a semisynthetic diet [3]. The variations might be due to changes in diet, mainly the protein level, which is an important growth supplement for insects.

The present results were in close conformity with the results obtained by Ramasubramanian and Sundara babu, who reported a 4.41 larval growth index on pigeonpea, and Ganapathy, who reported 6.50 on pigeonpea [18, 19, 20]. The variations might be due to diet composition and dependence on the feeding capacity of the larval stage. Those insects bred on unsuitable diets would have a longer larval developmental time and probably a low level of pupation. The present results on the pupal growth index were similar to those of Jackai and Raulston, who recorded an 11.59 growth index on a semisynthetic diet [3]. Growth index (GI) values, obtained by expressing the percentage of pupation over the larval growth developmental time, provided a good measure of the suitability of a given diet [21].

According to Pinto et al., the physical attributes of the diet, i.e., the texture and moisture content, greatly influenced the insect's capacity to consume and digest food [22]. Thus, among the evaluated diets, the water content in the D-J diet was higher than those of the remaining treatments, which promoted greater surface stiffness compared to other diets and thus might have resulted in less food ingestion and consumption of the D-J diet.

The present results revealed that the highest AD value was recorded in the D-B diet (67.49%), which was in accordance with Shwetha et al., who reported the highest approximate digestibility when larvae were fed a chickpea diet (47.99%), which was found on par with the pigeonpea host (48.81%) [23]. The lowest approximate digestibility was reported for the cowpea diet (42.15%). The present results partially correlated with the results of Shwetha et al., who evaluated the efficiency of the conversion of ingested food into body matter and found the significantly highest ECI with pigeonpea hosts (69.92%), whereas the lowest ECI was recorded for cowpea diets (60.07%) [23]. The degree of food utilization depends on the digestibility of food and the efficiency with which digested food is converted into biomass. The ECI is an overall measure of an insect's ability to utilize ingested food for growth and development.

The blacklegy natural diet and D-B semisynthetic diet recorded ECD values of 57.19 and 53.49 percent, respectively. Although there was an increase in AD, it did not compensate for the decrease in ECD, which accordingly led to a reduced growth rate. According to Lazarevic and Peric-Mataruga,

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a reduction in growth is a general response for phytophagous insects due to changes in the general host and a shift to a new host [24]. The proportion of digested food that is actually transformed into net insect biomass is denoted by ECD. The results of the digestive parameters studied are in agreement with Talaee et al., who evaluated the nutritional indices of whole instars of *S. exigua* on different sugar beet genotypes [25]. The ECI was reported to range from 11.78 to 24.65, ECD values were in the range of 13.74 to 28.46 percent, RGR ranged from 1.78 to 5.71 mg mg^{-1} day^{-1} and RGR ranged from 0.29 to 0.87 for different genotypes.

5. Conclusions

Among all the semisynthetic diets tested, the black gram-based semisynthetic diet was found to be superior, exhibiting a high rate of growth, survival and reproduction. The blackgram-based semisynthetic diet was suitable for the continuous rearing of M. vitrata to produce uniformly sized insects of predictable performance without a loss of vigor or a decline in reproductive potential. A blackgram-based semi-synthetic diet can be used as an efficient alternative to a natural diet for rearing in laboratory conditions.

Abbreviations

D-BAS, diet reported by Anusha and Sairam Kumar ; D-J, diet reported by Jin et al.; D-JR, diet reported by Jackai and Raulston ; D-New, new artificial diet [3, 5].

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