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Development of Tapioca Based Oligidic Diet and its Effects of Mass Rearing Performance (Biology) on Lepidopteran Pest of *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae)

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ABSTRACT

This research mainly focused the objective is to study the effect of tapioca based oligidic diet and its effect on growth and reproductive parameters in tobacco caterpillar larvae of *Spodoptera litura* (Fabricius). These results indicated an artificial diet for mass rearing of the tobacco caterpillar *Spodoptera litura* (Fabricius) from the neonate to adult stage was developed at 27±1°C, 65±5% RH and 16:8 h scoto/photo-phase regime. The impact of a tapioca-based artificial diet on the developmental rate, life history parameters and fertility was examined over five consecutive generations for the *Spodoptera litura* armyworm Hubner (Lepidoptera: Noctuidae), a highly polyphagous pest of many agricultural crops. The study showed that when fed the tapioca-based artificial diet during larval stage, larval and pupal developmental period, percentage of pupation, pupal weight, emergence rate of male and female, longevity, fecundity and hatching were non-significantly different than that of the control agar-based artificial diet. Moreover, the cost to rear on tapioca-based diet approached 2.13 times less than the cost of rearing on the agar-based artificial diet. The mean biological parameters based on rearing of ten continuous generations showed higher pupation (67.0±3.2), emergence (77.0±5.6) and fecundity (234.2±50.1 eggs) as compared to the most preferred natural food, castor leaf whose respective recorded values are 80, 75, 60% and 480 eggs. The artificial diet developed is suitable for mass rearing *S. litura* successfully throughout the year for laboratory and field experimentation as well as for commercial production of nuclear polyhedrosis viruses.

Key words: *Spodoptera litura*, oligidic diet, tapioca, caterpillar larva

INTRODUCTION

The cotton boll, *Spodoptera litura* (Fabricius) Hubner is one of the major series pests in an agricultural field. The cotton armyworm *Spodoptera litura* Hubner (Lepidoptera: Noctuidae) is a major threat to intensive agriculture (Dhir *et al.*, 1992; Malarvannan *et al.*, 2008). Its wide dissemination and pest status has been attributed to its polyphagy and its ability to undergo both facultative diapause and seasonal migration (Devanand and Rani, 2008). The species is migratory on all continents and is a key pest on all of them (Feng *et al.*, 2005).

It is an important to be able to economically rear an important insects to study their life history, behavior, feeding habits and their susceptibility and resistance to chemical and biological pesticides.

Rearing insects on artificial diets is an expensive process, especially for developing countries where insufficient funds are available for research. As a result it is inevitable that economic threats imposed by insects to agriculture are poorly studied by John *et al.* (2003). So far various artificial diets have been developed and proposed for the maintenance and continuous rearing of economically important insects (Robert *et al.*, 2009; Ahmad and Hopkins, 1992; Cohen, 2001, 2003; McKinley, 2004; Castane and Zapata, 2005). Although, there is some success in efforts to rear successive generations of economically important insects entirely on an artificial diet, in many cases there is loss of both fitness and reproductive potential which cause longer development times and lower fecundity (Coudron *et al.*, 2002). As a result, the cost-saving ratio is diminished. Life and fecundity tables have been found to be important methods for analyzing and understanding the impact of an external factor, such as an artificial diet, upon the growth, survival, reproduction and rate of increase of an insect population (Bellows *et al.*, 1992). These tools have been used to improve rearing techniques (Abbasi *et al.*, 2007) and compare different food sources in diet (Hansen *et al.*, 1999). It is characterized as a gelling agent and provides some minerals and probably provides a stimulation of gut motility, which can be very important in terms of absorption of nutrients and effective digestion (Nene, 1996; Cohen, 2003). There are other functions of diet components such as modifiers of bioavailability, stability, palatability, emulsification and other aspects of hydrocolloid function such as viscosity, sheer strength and tensile strength (Cohen, 2003). Tapioca, prepared from the cassava plant, *Manihot esculents* Crantz7 (Euphorbiales: Euphorbiacea) has been successfully used instead of agar in plant tissue culture media (Gebre and Sathyanarayana, 2001). Tapioca can also be used as a gelling agent in media. In this study, we show that tapioca can be used in place of agar to rear *H. armigera* for up to five successive generations. All materials used in this study were fabricated locally with the purpose of determining cost effectiveness when compared to imported materials from other countries. The impact of the tapioca-based artificial diet was studied on larval development, pupal development, pupal weight, incomplete pupation, sex emergence percentage, fecundity and longevity, which were compared with results of simultaneously reared consecutive generations on the agar-based diet formula. Recently Ehric (2009) evaluated the development of control and artificial diet with natural enemies including lepidopteran pests.

Summerford (2004) recorded the development of *Spodoptera exigua* (Lepidoptera: Noctuiidae) larvae on artificial diet and cotton leaves containing the *Bacillus thuringiensis* toxin heritable variation to tolerant cry AC. Still, no more works has been done this kind of tapioca based oligidic diet in this research conducted experimental pest of *Spodoptera litura* (Hub.). Hence, we designed the following objectives such as to study the effect of tapioca based oligidic diet on the growth parameters and reproductive parameters and to identify the mortality rate and survivability of larvae and pupae of the *S. litura*.

MATERIALS AND METHODS

A colony of *S. litura* was initiated from two pairs of adults collected from boarder region of Tirunelveli and Kanyakumary district agricultural field especially cotton and caster field during the rabi season (July 15 to August 11th 2009) then reared on the agar based diet used as the control in current study (Ahmed *et al.*, 1998). Experiments were conducted on the second generation of that wild collected pair. The first experimental generation reported in results was the first third generation of that wild pair. The experimental conditions were kept at 70+5% R.H., 25 experiments were conducted on the second generation of that wild collected pair.

Table 1: Ingredients of agar and tapioca-based diets and vitamin solution

	Weight (g)				Vitamin stock solution	
Components	Tapioca diet	Agar diet	Tapioca diet (%)	Agar diet (%)	Ingredients	Quantity (g)
Agar	0	7.1	0	4.3	Calcium pantothanate	9.5
Tapioca powder	60*	0	24*	0*	Nicotinic acid	4.0
Chickpea powder	170.4	170.4	70	919	Riboflavin	2.0
Ascorbic acid	2	2	0.8	2	Folic acid	2.0
Dried active yeast	4.7	4.7	2.3	3	Pyridoxine hydrochloride	1.5
Vitamin mixture (mg)	1.42	1.42	0.6	0.08	Vitamin B_1	0.0038
Distilled water	1.7	1.7	0.6	0.7	Sterilized distilled water	400 mL
Total	245.98	193.08	99.8	100	-	-

*Significant diet composition for longevity of *S. litura*

Ingredients of the diet: The composition of the diet is shown Table 1. The wet and dry ingredients of the diet were weighed and kept separately. The agar or tapioca was suspended in 1.5 L of water and boiled. For the tapioca based diet heat-proof mixer from Braun was used while boiling to ensure complete mixing and grinding of the Tapioca. The chickpea powder was then added to the boiled mixture and mixed, during the process the temperature of the mixture became nearly 60°C. The remaining dry and wet ingredients were then added to the mixture with thorough mixing.

Ingredients of the vitamin stock solution: Table 1 also shows the composition of the vitamin mixture. All dry ingredients were added to a flask and sterilized distilled water was gradually added with steady stirring until the entire quantity of powder had been dissolved. Water was then added until the 400 mL volume was obtained.

Egg incubation: Eggs were collected from the castor field in Kaliyakkavilai. On the layers of cotton wool oviposition pads and enclosed polyethylene bags. Eggs were allowed to develop at room temperature 30±1.5°C. After the larvae started hatching they were transferred to plastic vials containing tapioca based the diet.

Glass capsule vial technique for individual larval development: To study the larval stage a glass vial (2.5 cm in diameter and 5.5 cm in height) was used. Diet (7 mL) was placed into sterilized vials and a newly hatched first instar larva was added using a camel hairbrush. In order to provide an air exchange a sterilized cotton wool plug was used which also prevented drying of the diet before the developing larva pupated. Four replicates of 25 vials each for agar and tapioca-based diets were run simultaneously.

Adult emergence: Adult emergence was studied using plastic Petriplates (1.5 cm high with 9 cm diameter). After larvae pupated, the pupae were placed on a circular piece of blotting paper in the petridish with 1 pupa in each plate. After emergence the adults were placed in individual vials for egg lying. The details of cages have been described previously (Ahmed *et al.*, 1998). Briefly medium sized lamp glasses 10.2 cm high having 7.9 cm lower end and 6.6 cm upper end diameter were used as oviposition cages for single pairs of adults and plastic jars having 10 cm lower end and 12 cm upper end diameter were used as oviposition cages for two three pairs of adults.

Statistical analysis: The data subjected to a one way Analysis of Variance (ANOVA). Tukey-Kramer test was used for calculation of significant differences. By using SPSS (for Windows, standard version 7.5.1), $p<0.01$ value was regarded as significant. The data's were compared within generations of similar treatments.

RESULTS AND DISCUSSION

The outcome of this study explained that the tapioca is a suitable alternative for *S. litura* continuous rearing up to five generations. The results show that tapioca is a suitable alternative for *S. litura* continuous rearing up to five generations. Table 1 shows that by replacing 7.1 g of agar with 60 g of tapioca, the total concentration of each remaining ingredient changed by about 20%. In the tapioca based diet, for every gram of diet that larva consume, it acquire nearly 20% less protein than it was ingesting with the agar diet. The same reduction also occurs for the lipid content, the vitamins, etc., but the carbohydrate composition increases. The water content goes from 84% of the agar diet to about 80% of the tapioca diet, which is another potentially important change that may affect insect growth (Cohen, 2003). The nutritional importance of tapioca contributes little beyond carbohydrates. Thus, although tapioca does have a little more nutritional value than agar, it does not make up the nutritional gaps.

In order to compared the effects of diet on the biology of *H. armigera* two groups were raised on agar-based and tapioca-based artificial diets. It was found that the average larval period of the fourth and fifth generations on either agar or tapioca based diet showed significant differences with other generations (Table 2). By the fifth generation non-significant differences were observed between tapioca and agar-based diets, indicating that they were nutritionally equivalent. The fifth generation also showed a significantly longer average pupal development period on both diets.

The results in Table 3 indicate that larval mortality varied considerably during the generations, with little significant differences between diets, the variation in morality from 1st to 5th generation may be linked to the factors vis-à-vis larval entanglement in the cotton plug, or injury from infestation or fungal contamination. Previously, it is reported 2.7% mortality in 1st generation *H. armigera*, which were reared on bean powder diet.

By the fourth and fifth generations pupal mortality was not significantly different between diets (Table 3). The highest mortality of 19.75% was recorded for the third generation fed the agar-based

Table 2: Oligidic diet and its effect of average larval and pupal periods of larvae in *S. litura*

Diet	Generation	Ave. larval period	Ave. pupal period
Agar	1a	16.10*	10.14±0.41
Tapioca	1b	13.25**	10.11±0.53
Agar	2a	15.06*	11.45±0.68
Tapioca	2b	13.30**	09.50±0.81
Agar	3a	11.04*	10.10±0.71
Tapioca	3b	16.63*	09.80±0.63
Agar	4a	12.37[is]	09.64±0.34
Tapioca	4b	15.30*	09.5±60.52
Agar	5a	15.25[is]	15.88±0.39[is]
Tapioca	5b	14.38[is]	15.44±0.67[is]
CV	-	14.11	7.27%
LSD	-	16.03	1.178

*Shows the 5% level of significant $p<0.01$, **Shows the highly significant at 5% level of $p<0.01$. [is]Indicated in-significant at $p<0.05$ level

Table 3: Oligidic diet and its influence of pupation and pupal mortality (%) of *S. litura*

Diet	Generation	Larval mortality	Pupal mortality	Pupation
		(%)		
Agar	1a	5.00±0.91C^{is}	8.00±0.94^{is}	76.0±2.6*
Tapioca	1b	4.04±1.0C^{is}	6.00±1.1*	81.0±3.8^{is}
Agar	2a	16.00±2.1BC*	11.00±0.88*	62.0±3.2*
Tapioca	2b	14.00±1.2BC*	4.00±1.2*	65.71±3.5*^{s}
Agar	3a	30.65±2.2A*	19.75±0.56*	34.0±2.8*
Tapioca	3b	36.00±2.0A*	7.00±1.01^{is}	51.0±2.67*
Agar	4a	41.50±1.45A^{is}	3.50±0.91*	49.0±3.8^{is}
Tapioca	4b	37.25±2A*	7.00±0.87^{is}	46.5±3.5^{is}
Agar	5a	26.00±2.3AB^{is}	4.00±1.0*	58.0±4.0*
Tapioca	5b	15.43±1.8C	4.00±0.89^{is}	67.0±3.2*
CV (%)		37.97	62.04	17.73
LSD		12.33	6.459	15.29

*Shows the 5% level of significant $p<0.01$, ^{is}Shows the in-significant level of the mortality of pupae, larvae and pupation

diet. The maximum pupal mortality was attributed to incomplete chitinization of the first three abdominal segments on the ventral surface (cause unknown) and other microbial contamination. The fifth generation of *S. litura* reared on a bean powder diet exhibited high larval mortality of 41.5±1.45 and this value was linked with incomplete chitinization of segments of pupa (Howell, 1972; Ahmad *et al.*, 1998).

Percent pupation varied considerably over the 5 generations with no significant differences between diets, except for the third generation. Maximum pupation of 81% was observed for the first generation on tapioca-based diet while its minimum value was 34% for the third generation on the agar-based diet (Table 3). Significant differences were observed in percent pupating for the third generation on the agar-based diet when compared with other generations on the same diet. From the overall statistical value represented the agar maintained the second and third generation of larvae and pupae were expressed the significant response when compared with rest of the experimental categories. When *H. zea* was reared on bean and wheat-soy blend diet, the pupation achieved was 86.2 and 92%, respectively (Burton and Perkins, 1972). Moreover, many authors were suggested about this kind of observation whereas, maximum pupation was 83.7% for the first lab generation and 61.64% for the first field generation for *H. armigera* (Ahamad *et al.*, 1998). The sorghum stem borer, Chilo zonellus, was successfully reared on Kabuli gram diet (Dang *et al.*, 1970), with 75% pupation, which is similar to 79% pupation for the 1st generation reared on the tapioca-based diet (Table 3).

The incidence of incomplete pupation varied insignificant over 5 generations, except for the 3rd generation Table 4). Meanwhile, results clearly showed the maximum percentage of incomplete pupation observed on agar fed third generation *S. litura* (25.25±1.59). Another fourth generation of experiment indicated minimum percentage of incomplete pupation has been formed. Because of that reason was to the tapioca based diet was almost naturally available nutrient enriched matters were present. Entanglement of larva in cotton plugs was major reason causing incomplete pupation. The average pupal weights were also not significantly different between generations.

The present adult emergence was significantly higher for the first generation on both type of diets but varied considerably during the fifth generations noticed on the Table 5. Pupal and adult few abnormalities were observed for any generation. The minimum adult emergence was male

Table 4: Impacts of Oligidic diet and its formation of pupation from the larvae of *S. litura*

Diet	Generation	Incomplete pupation (%)	Pupal weight (Average)
Agar	1	14.00±2.1*	0.3680±0.012*
Tapioca	1	13.50±1.8*	0.3685±0.02*
Agar	2	14.00±1.54*	0.3350±0.013*
Tapioca	2	16.00±2.0*	0.3110±0.009
Agar	3	25.25±1.59**	0.4275±0.02**
Tapioca	3	11.00±2.01**	0.3075±0.015**
Agar	4	10.25±3.0	0.3275±0.012
Tapioca	4	9.04±2.3^{is}	0.3300±0.021^{is}
Agar	5	12.00±2.1^{is}	0.3400±0.03^{is}
Tapioca	5	10.00±1.98^{ns}	0.3175±0.018^{is}
CV (%)		68.11	4.17
LSD		10.82	0.04588

^{is}In-significant, ^{ns}Non-significant, *Shows the 5% level of significant p<0.01, **Shows the 5% level of highly significant p<0.001

Table 5: Effect of Oligidic diet and total adult emergence (Male and Female) of *S. litura*

Diet	Generation	Male emergence	Female emergence	Emergence
		(%)		
Agar	1	37.00±2.1*	39.00±4.0*	76.0±6.1*
Tapioca	1	42.00±3.0*	39.00±3.87*	77.0±5.6
Agar	2	20.00±2.8	23.00±3.8*	35.0±6.3
Tapioca	2	29.00±3.0	29.00±2.9	58.0±5.4
Agar	3	7.00±1.99	10.00±4.0	18.0±3.8
Tapioca	3	16.00±2.3	23.00±4.1*	31.0±5.0
Agar	4	26.38±3.1	16.25±2.89	44.6±4.8
Tapioca	4	25.38±3.3*	21.25±4.3	45.6±4.2
Agar	5	27.00±4.0	23.00±3.6*	57.0±3.9
Tapioca	5	29.00±3.8*	25.00±4.1	53.0±5.0
CV (%)		3.96	36.15	24.54
LSD		14.67	13.57	15.73

*Shows the 5% level of significant p<0.01, ^{is}Shows the insignificant level of emergence

16.0±2.3% and female 23.0±4.1% for the third generation of the agar diet which was significantly different from those fed the tapioca based diet. Eventhough, highest adults emergence seen at first generation such as 42.0±3.0 and 39.0±3.87% males and females, respectively. Male and female emergence varied during the generations, but by the fourth and fifth generations were indicated significantly not different (Table 5). Eventhough, remaining generation such as rate first, second generation of male and females of the *S. litura* percentage of emerging rate was significantly higher when compared to that of control category.

Longevity of males and females was not significantly affected by diet with the exception of the second generation (Table 6). Regardless the reproductive parameters like sex ration and fecundity rate was significantly increased the fecundity rate observed from the first and fifth generation on agar and tapioca based diet also noted significantly seen the female and male biased sex ratio in the agar based diet on the agar and tapica based diet during the first third generation respectively. The number of eggs laid was not significantly affected by diet. However, the number of eggs laid by the females laid by females fed the tapioca diet was always higher than those fed agar based diet. The number of eggs laid generally increased for use those fed the tapioca diet. A correlation

Table 6: Oligidic diet and effect of longevity, reproductive parameters on adult *S. litura*

		Longevity of adults		Reproductive parameters	
Diet	Generation	Male	Female	Fecundity eggs laid/female	Hatching (%)
Agar	1	14.14±1.23A	15.51±1.12*	215.0±30.1*	56.88±2.3
Tapioca	1	13.98±1.3*	17.03±1.45	206.0±26.7^{is}	59.01±3.0
Agar	2	15.96±2.0^{is}	12.29±1.56*	192.0±40.4^{is}	53.25±3.1
Tapioca	2	16.89±2.3^{is}	17.69±1.9^{is}	231.5±49.1^{is}	54.25±2.9
Agar	3	14.45±2.8*	13.73±1.5	169.8±50.2^{is}	49.75±2.4
Tapioca	3	14.50±2.1*	16.65±1.8**	162.3±53^{is}	50.25±4.0
Agar	4	16.20±1.7^{is}	15.13±1.6^{is}	168.3±34.6	57.00±4.2
Tapioca	4	17.13±1.5*	17.02±1.4*	297.1±45.3*	60.75±3.5
Agar	5	16.12±1.0^{is}	15.50±1.9^{is}	148.8±43.6*	55.25±4.0
Tapioca	5	13.06±2.0^{is}	16.03±1.98*	234.2±50.1*	56.50±2.9
CV (%)		23.59	17.41	41.47	15.31
LSD		4.711	3.752	114.7	12.28

Means followed by the same letter(s) are not significantly different ($p < 0.01$). *Shows the 5% level of significant $p < 0.05$, **Shows the 5% level of highly significant $p < 0.001$, ^{is}Shows the insignificant level of the longevity for male and female (adults)

between the length of life (life span) and fecundity rate was also showed in Table 6. However, the percentage of egg hatching rate was not significantly affected by diet. The vigor and viability of the insects was normal up to eight generations (Data was not shown). When the *S. litura* treated with tapioca and agar based diet maximum numbers of eggs were laid fed with tapioca feed (297.1±45.3) than the agar diet. This kind of similar observation has been made by Rao *et al.* (1993), Brunner and Lampson (1997) and Greenberg *et al.* (2001) for third generation in other type of lepidopteran pests such as *H. armigera*. Again Burton in 1970 published when rearing the *H. zea* on a corn-soybean meal based diet and recorded an average oviposition of 406 eggs for mated females. Burton and Perkins (1972) and Portilla *et al.* (2004) found highest number 190 eggs from a female the same species of pest reared on wheat-soy blend diet. Egg production or fecundity rate was affected by biotic and abiotic factors like temperature, which was probably due to inhibition of mating and oviposition activity.

The quantitative experiment of eggs were laid by female insect through out the generation mainly depends upon the feeding material like beef liver (Yves and Patrick, 2004) contributed the consistent findings on the predatory species of *Orius laevigatus* and other influenced the factors like climatic factor also biotic factors by Rakotoarivelo *et al.* (2009). These authors also have been suggested that the increased egg deposition multiple mated females may be related with metabolically variation as well as hormonal effects on egg production. It has been observed that repeatedly mated females had short lives (Robert *et al.*, 2009). Male mating frequency in *S. litura* had a dominating effect on fecundity of the paired females and fecundity also affected the life span of adult females (Merkx-Jacques and Bede, 2005).

Many researches suggested that the agar is the most expensive ingredient of insect rearing diet and consequently its substitution would have the greater effect. The average cost estimated for production of one pupa was Rs. 0.9 on the tapioca based diet, while the cost on the agar based diet amount is Rs. 1.24. This was reduced the price for tapioca-based diet by 3.13 fold. Then the 10% sucrose solution was used to feed adults that cost Rs. 5.0 for one generation. Previously, Abbasi *et al.* (2007) described that the approximate cost to produce *H. zea*/*H. virescens* pupa was equivalent to Rs. 0.95 using an agar based diet (Racioppi *et al.*, 2004) recorded when produced pupa on a modified agar- based diet for Rs. 1.4. Hence, the diet developed was more efficient and feasible for short and long term mass rearing of tobacco army worm of *S. litura*. The tapioca-based diet developed for rearing *Spodoptera litura*, maintained this insect for up to five generations with no loss of vigor or viability. This diet would, therefore, have potential l to be used as an artificial diet for rearing several other economically important Lepidopteran pests. This is a good beginning for a new diet for this polyphagous insect.

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