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Standardizing the artificial diet and diet preference for lab rearing of *Spodoptera frugiperda* Smith, 1797 (Lepidoptera, Noctuidae)

Harshita Sharma*, Dolly Kumar
Department of Zoology, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India

Abstract
Spodoptera frugiperda Smith, 1797 (Lepidoptera, Noctuidae) was announced as an invasive species in India as recently as 2018. Commonly it is known by the name fall armyworm. It is causing nuisance because of its highly polyphagous nature. *S. frugiperda* is a pest of American origin migrating to other parts of the world including Africa and Asia. In recent years, this insect has invaded many states of India including Gujarat. Mostly chemical pesticides and a few biopesticides have been the most reliable means to control the pest. This has led to resistance development in this pest. The resistance related studies against fall armyworm has been studied in countries like the USA and Brazil. Before *Spodoptera frugiperda* becomes havoc in the agricultural fields and turns resistant to all available insecticides; evaluating the alternative methods, their efficiency to control the pest, resistance development against newer insecticides and its mechanism needs to be studied. This would require mass rearing of pests in the laboratory. Laboratory mass rearing requires ideal biotic and abiotic factors. The diet is the chief component for the rearing of pests. In this study, four artificial diets are analyzed and compared for efficient rearing of pests in the laboratory.

Keywords: diet, Gujarat, invasive, polyphagous, rearing

Introduction

Spodoptera frugiperda is a polyphagous insect and a problematic agricultural pest. *S. frugiperda* has a wide range of host crops which are of economic concern to us. This includes 353 plant species from 76 plant families having crops such as corn, tomato, millets, and potato. (Montezano *et al*, 2018) [8]. The fall armyworm, *Spodoptera frugiperda* is a lepidopteran insect, originating from America eliciting an exorbitant reproductive rate, throughout the year (Sparks, 1979) [13]. The incidences of sudden and severe outbreaks of fall armyworm populations from several West and Central African countries have been observed. This is due to the virtue of their vast dispersal and strong flying skills, while this is the first case of invasion outside the American continent (Goergen *et al*, 2016) [4]. For the first time in 2018, *Spodoptera frugiperda* has shown its occurrence in India in the state of Karnataka (Deshmukh *et al*, 2018) [3]. In China, this invasive pest was recently confirmed to be present, by using phylogenetic analysis of biological macromolecules (Jing *et al*., 2019) [6]. FAW invasion was also first reported from Gujarat maize fields in the Anand district (Sisodiya *et al*., 2018) [12]. In Karnataka, FAW infestation has already been reported on maize and paddy. Its biology has been studied there as well (Sharanabasappa *et al*, 2018) *Spodoptera frugiperda* invasion of sugarcane and other crops from Maharashtra was confirmed based on the male genital dissection of the insect (Chormule *et al*., 2019) [2]. In Rajasthan, the presence of FAW on maize has been marked (Babu *et al*., 2019) [1]. Amongst the major economically important crops produced in India, after wheat and rice, maize is the third most important cereal. An estimated loss of 200 million INR has been incurred, as reported from Mizoram in May 2019 after 122 districts with maize cultivation were infested by the fall armyworm. Further, *S. frugiperda* is shown to be resistant to commonly sprayed older classes of insecticides including carbamates, organophosphates and pyrethroids which lead to the failure of crops in Florida (Yu, 1991). The pest management due to these have become difficult since the most commonly used insecticides are unable in controlling it. Midgut and fat body tissues RNA Sequence analysis of another *Spodoptera* species i.e. *Spodoptera litura* suggested that the genes from these sites may play a substantial role in xenobiotic detoxification in these caterpillars (Li *et al*., 2019) [10].

Till now there is no certain solution for sustainable management of FAW in Africa or Asia (Padhee & Prasanna, 2019) [9]. Such a situation calls for experiments and research for alternate control methods. This will require the availability of a numerous insects which can be achieved by mass rearing. Artificial diets for insects are used as a medium for the effective rearing of pests in the lab. Studies related to di*et al*so provide knowledge about the insect's biology, behavior, and nutritional requirements and such information are fundamental for the development of efficient Integrated Pest Management (Pinto *et al*., 2019) [10]. Devising a management strategy would involve a clear understanding of physiology, for which rearing them artificially in the lab is prescribed.

The pest to be reared in the lab have specific characteristics which can be easily observed in the distinct larval and adult forms.

Materials and Methods

The laboratory rearing of insects were utilized. The FAW insects were collected in caterpillar form from nearby maize fields of Vadodara (Figure 1 A, B). They were reared on the natural diet of that generation F0. The eggs hatching from these were then kept on different diets so that when neonate emerges, can immediately feed on the diets. This was termed test generation or F1. The 1st instar larvae/ neonates of *Spodoptera frugiperda* were used from the laboratory population to see its survival on different artificial diets on larval growth and development.

Experiment was set-up to see the food preference of fall armyworm. It was a preliminary study to understand the diet preference in presence of different host crop ingredients in the artificial diets on which fall armyworm causes infection was done. For checking the preference, four diets containing varying flours viz. Maize, Soya, Chickpea, and Jowar were kept equidistant from one another and from the center. In the center, 10 neonates were released, and the position was noticed after 1 hour and 24 hours, respectively.

To check the effect of different diets on the survival of FAW, four diets were made (Table 1). The Artificial Diet was inspired from a diet, was later modified for effective rearing of another *Spodoptera* species- *Spodoptera litura* (Gupta *et al*, 2005) [5]. In culture trays rearing was done. Diets were changed as and when required. Temperature and humidity were maintained by the BOD incubator. For diet optimization, temperature and humidity are kept constant at 26±2˚C and 70±10% with a 12:12 D: L photoperiod. Photography and percent survival was recorded. Observations were recorded till adult emergence. Data were analyzed through graphs made in GraphPad Prism version 9.3.1

Diet Preparation Process

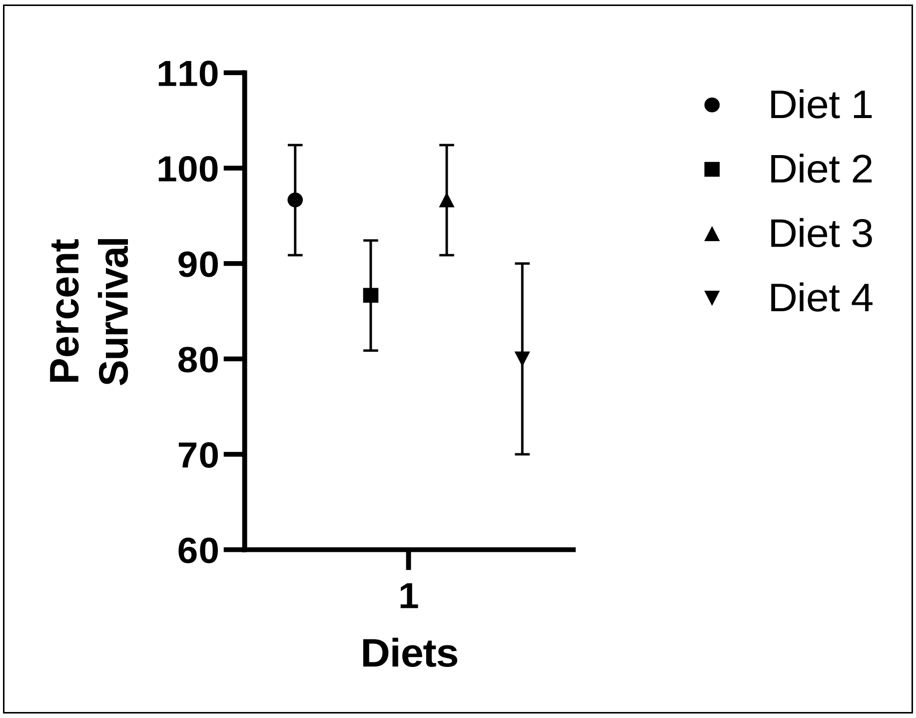
All the ingredients in the diet are mixed in water. The water content of the diet is divided into two parts. Example- For a 500 ml diet, 250 ml is taken for each part. In the first part, water is boiled till the bubbles come, then agar is added, and it is boiled and stirred continuously till it has a thick consistency, yeast is then added and boiled for a minute. In the second part, the rest of the ingredients of the diet is added with liquid components like becosule. Formaldehyde is added initially and solid ingredients like flour later. It is mixed with the help of a hand blender. Then the first part is added and blended properly. The fresh diet is prepared which is poured into the ketchup bottles, and some portions may also be poured into the wide trays for making diet pieces (Figure 2 A). The 'semisolid diet' after cooling down gets solidified which can be then used by transferring insects of any stage to it and covering the latter with stickers (transparent covers which stick at the edges and provide scope for air exchange through its tiny holes) or lids to avoid insects moving out from the trays. The diet can be used for 2-3 days when an early instar is released (Figure 2 B). The excreta can be seen and mark as indicator of good feeding (Figure 2 C).

Table 1: Different diet compositions in gm or ml for *Spodoptera frugiperda* (in 1 L H_2O diet)

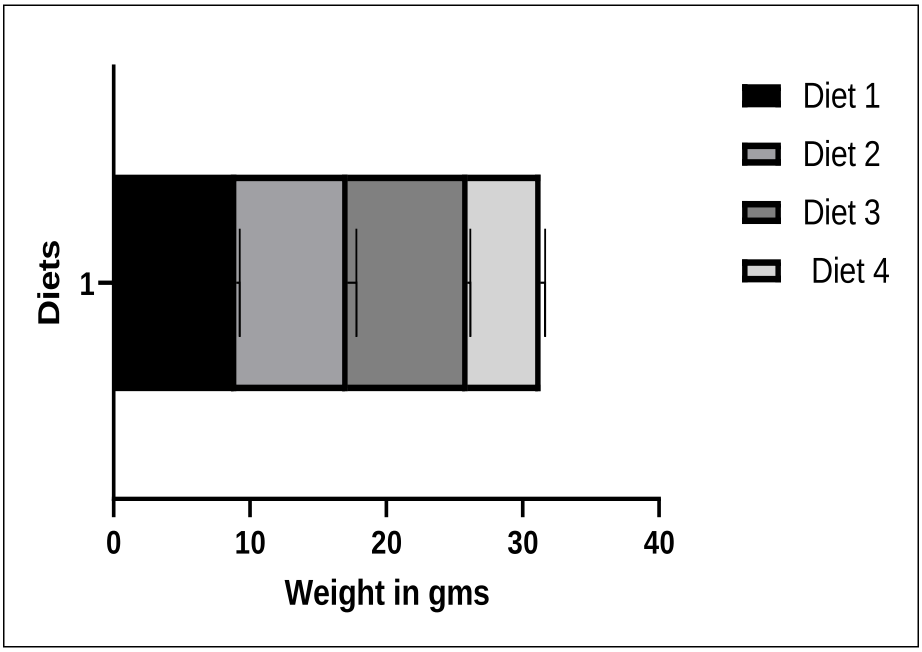
S/N.	Ingredients	Diet 1	Diet 2	Diet 3	Diet 4
1	Wheat germ	60g	60g	60g	60g
2	Sucrose	36.36g	36.36g	36.36g	36.36g
3	Yeast	53g	53g	53g	53g
4	Agar	20g	20g	20g	20g
5	Sorbic acid	1.7g	1.7g	1.7g	1.7g
6	Ascorbic acid	5.3g	5.3g	5.3g	5.3g
7	Methyl-p-hydroxy-benzoate	3.3g	3.3g	3.3g	3.3g
8	Formaldehyde	13.5ml	13.5ml	13.5ml	13.5ml
9	Becosule	12ml	12ml	12ml	12ml
10	Propionic acid	2ml	2ml	2ml	2ml
11	Maize flour	160g	-	-	-
12	Soya flour	-	160g	-	-
13	Chickpea flour	-	-	160g	-
14	Jowar (Sorghum) flour	-	-	-	160g

Results

The observations for various diets on the survival of *Spodoptera frugiperda* are shown in the graph where all diets were found to be efficient for the rearing of FAW (Graph 1). However, two diets were found to be better among all of them. The difference in weight between the early instar (2nd) and late instar (5th) was checked to evaluate the diet on which better feeding occurred (Graph 2). Larval Growth Index serves as a good formula that takes into consideration both survival and the number of days required to complete the life stage. LGI was also checked for all the diets (Table 2).



Graph 1: Survival of FAW on various diets



Graph 2: Increase in weight (Early and late instar)

Table 2: Larval growth index for *Spodoptera frugiperda*

Diet	Percent survival	Avg. Larval period	Larval Growth Index
1	96.87	14.61	6.63
2	87.5	15.67	5.58
3	96.87	14.52	6.67
4	81.25	16.15	5.03

Larval Growth Index (LGI) = Percent pupation/ Larval periods (days)

Experiments for food preference showed fondness for the maize-based artificial diet. Maximum of the neonates were observed moving toward maize diet as soon as kept in the center and after an hour's observation, most of them were observed on a maize-based diet. After 1 hr, 7 of 10 FAW of them were found on the maize diet. Post 24 hrs, their position remained at the same position with 7 of them on maize based diet, 2 on chickpea based diet while 1 found near the soya based diet. This reveals the fall armyworm's feeding preference for maize.

Spodoptera frugiperda, percent survival was found to be 96.87, 87.5, 96.87, and 81.25 % for diets 1, 2, 3, and 4 respectively (Graph 1). Larval Growth Index was found to be 6.63, 5.58, 6.67, and 5.03 for diets 1, 2, 3, and 4 respectively (Table 2). LGI is in the order: diet3>diet1>diet2>diet4. Increase in weight while growing on different diets was found to be for diet1>diet3>diet2>diet4. The maximum increase in weight was found to be in diet 1 and nearly similar in diet 3 (Graph 2).

Figures

Fig 1: Maize field in Vadodara A. Maize field in Waghodia region of Vadodara B. FAW larva damaging the maize plant

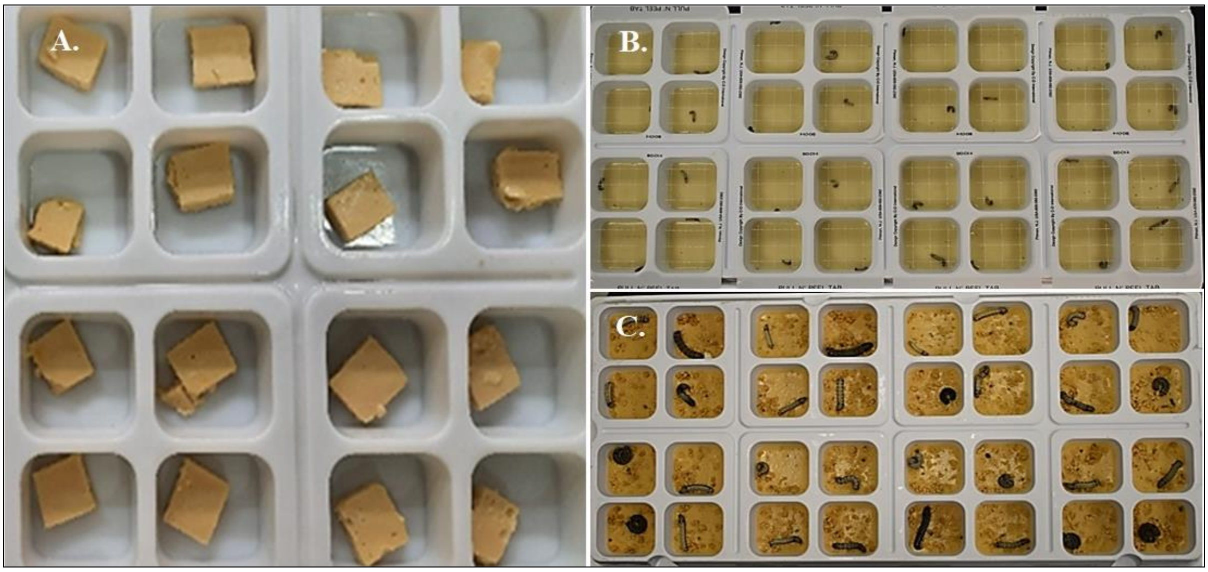


Fig 2: Artificial diet for lab rearing A. Solidified diet pieces B. Early instar on fresh diet B. Late instar on diet (excreta visible)

Conclusion

A survey of some of the agricultural fields in and around Vadodara revealed the infestation of crops by insect pests and heavy infestation of the crops by several Lepidopteran pests like *Helicoverpa armigera, Spodoptera litura, Earias insulana, Aphis gossypii* and *Plutella xylostella*. In maize fields, a severe infestation of *Spodoptera frugiperda* was observed. To optimize the artificial diet for *Spodoptera frugiperda,* the 4 diets made were having high percent survival. In diet 1, major components were maize flour, wheat germ, yeast, agar, sorbic acid, ascorbic acid, methyl-p-hydroxybenzoate, formaldehyde, becosule, and propionic acid. Other diets contained all ingredients similar to Diet 1 except maize flour replaced by soya flour in diet 2, chickpea flour in diet 3, and jowar flour in diet 4. From all four diets, the maximum percent survival was in diet 1 and diet 3. However, the larval growth index which takes into account both percent survival and the number of days required by the larva to turn pupa was highest for diet 3. Diet 1 and diet 3 came out to be the best diets. The components in diets 1 & 3 are having all the components necessary for the satisfactory growth of larva. R.K. Seth & V.P. Sharma 2002 from Delhi University also evaluated the growth of *Spodoptera litura* on different diets. They observed survival of pupae upto 78-81% on a chickpea-based diet. In the present work, we found the larval survival on the diets in between 81-96% for *Spodoptera frugiperda*. The various components of the diet have different nutritional values. Some of these such as chickpea, and wheat germ serves as the main carbohydrate provider in the diet while formaldehyde, methyl-p-hydroxybenzoate, and sorbic acid serve as antimicrobials, and the yeast, becosule provide vitamins in the diet. Chickpea-based artificial diet seems best for the laboratory rearing, where different lepidopteran pests are being worked on. We have tried the chickpea-based diet on *Spodoptera litura and Helicopverpa armigera* also along with *Spodoptera frugiperda*, and it has been working well for all of them.

Discussion

To test for any pesticide efficiency or to conduct any experimental work on the insect pests, we require huge numbers of the pest in the laboratory. Such a study would help to get a good and effective culture of the insect pests in the lab. Especially the Lepidopteran pests like Fall armyworm. With this, we can open up for all those research works that would study the rearing methods for insect pests in a laboratory.

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