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Original Research Article

Evaluation of New Larval Diet in Rearing of Kala-azar Vector, *Phlebotomus argentipes* (Dipteral: Psychodidae)

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A B S T R A C T

Phlebotomus argentipes is an established vector for Visceral Leishmaniasis in Indian subcontinent. Its colonization is imperative in research. The conventional food comprising; Rabbit faeces/chow grinded mixed with sand are being used as larval food in the laboratory. Processing of rabbit faeces along with Rabbit chow is suffocating along with high mites infestation & lower nutritive value to larvae. In the current study, new composition of food having Carbohydrate, Proteins & fat rich grains of rice (*Oryza sativa*), peanut (*Arachis hypogaea*) and soya been (*Glycine max*) were used in equal quantity and compared with conventional food. Artifical food has shortened the larval development time up to 3-5 days ($P<0.05$) with increased body mass and without any manifestation of mites compare to conventional food. This study will be of immense help in generating healthy sand flies.

Keywords

Artifical Food, Nutrients, Phlebotomus argentipes, Sand fly

Introduction

Phlebotomus argentipes is the established vector for Visceral Leishmaniasis in Indian sub-continent (Swaminath et al., 1942). Its prefers moist and humid indoor places like cattle shed, house with mud plaster and places with thick shrub vegetations in peridomestic area. Both male and female feed on plant juice. Females also feed on blood of various vertebrate hosts such as human, cattle, buffalo, dog and goat for maturation of follicles (Garlapati et al., 2012). Sand fly colonization is an important as well as critical determinant for understanding to the vector potential, pathogen transmission and vector management, etc. experimentally. Rearing of sand flies is an exigent job and initiation of colony is more difficult than its maintenance (Young et al., 1981). This is because of their undefined habitat, gaps in the knowledge of their breeding ecology and lack of information on their nutritional requirement during their life cycle. Sand fly rearing requires consistent care and proper management of artificial environmental conditions similar to nature and food sources. Difficulties in rearing of sand fly were also highlighted by (Killick-Kendrick et al., 1991). Conventionally, the rabbit faeces and rabbit chows are used as a food to provide nutrition to the growing larvae (Dougherty et al., 1995) which some time proved catastrophic for the handler (by inhalation of dust particles, skin allergy, eye infection etc) as well as it retards the growth of larvae resulting high percentage of larval

mortality. However, various artificial diets and supplements influenced insect growth and defined ease in laboratory settings. Usually eggs of *P. argentipes* hatches in one to two weeks depending on the environmental condition (26-28^{o}C and 80-100% Relative Humidity). A larva of sand fly is usually of 3 to 6 mm long and passes four larval stages to reach pupal stage. Life cycle from egg hatching to adult emergence takes 4 to 6 weeks. Larvae can use any kind of organic food resources, in absence of their preferred food resource. Proper artificial diet and optimum environmental conditions are vital for successful mass rearing. Rabbit faeces and other conventional fermented foods have unpleasant odour, unhygienic and variable in nutrient composition and quality. In contradiction to rabbit faeces, artificial diets are odourless, less prone to harmful bacteria and mites invasion, consistent in quality and easily available for regular use (Rock et al., 1964, Howell et al., 1967).Usually in most laboratories, larvae food is composted rabbit faeces and rabbit chows. Most Sand fly species larvae don't prefer autoclaved food (Volf et al., 2011). The decomposed organic vegetables or dry bovine faeces have shown good potential as a diet for larval development (Young 1981). Liver powder in larval food had shown good results for development of larvae (Killick-Kendrick et al., 1977). The modified high nutritive supplements as beef liver powder to the conventional dried mixture of rabbit faces and rabbit chow food was found potential in keeping the larvae healthy (Modi et al., 1983). So, there is a need of cheap and easily accessible food of high nutritive value, which is being provided in this present study where, natural diet was developed for *P. argentipes* larvae by using plant based ingredients to resolve the problems associated with the conventional diet.

Materials and Methods

The study was compared with a food having rabbit faeces and Rabbit chows and artificial food containing Rice (*Oryza sativa*), peanut (*Arachis hypogaea*) and soya been (*Glycine max*) having high nutritive value with rich protein contents. The protein content and other nutrient content of these foods were based on the guidelines of National Instituite of Nutrition (NIN), India. Fresh grains of all three components washed with distilled water, air dried and grinded to make fine powder and stored in air tight vials at room temperature for further use. The required amount of food was used after hatching of eggs for up to 6th generation to larvae in rearing Hilton pots time to time till getting pupal instar.

Results and Discussion

The effect of larval food on duration of life table and status of different larval stages with physical conditions of *P. argentipes* was compared with the conventional food having rabbit faeces, sand and chows. The average developmental period of larvae from the first instar to fourth instar in experimental group was 23-25 days up to 6th generation than 28 days in control($p<0.01$)(Fig-1). The food choice of Sand fly larvae in field is not well defined but in laboratory, rabbit faeces are generally used as source of nutrient. Rabbit faeces are inconsistent of nutrient as the nutrific value of rabbit excreta depend on the metabolism of rabbit and the type of food given to them. In typical animal house, rabbits are provided with cabbage (*Brassica rapa*), cucumber (*Cucumis sativa*) and gram (*Cicer arietinum*).

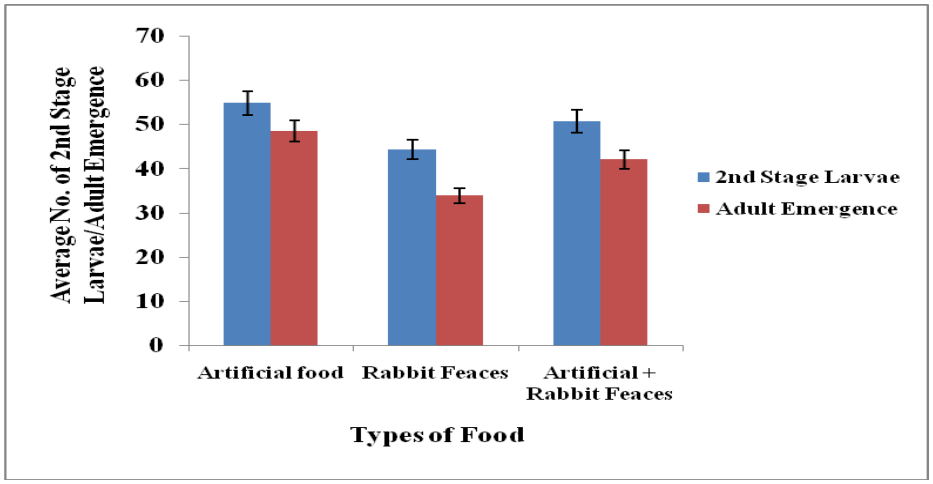
Table.1 Comparison of life cycle with artificial diet and Conventional food

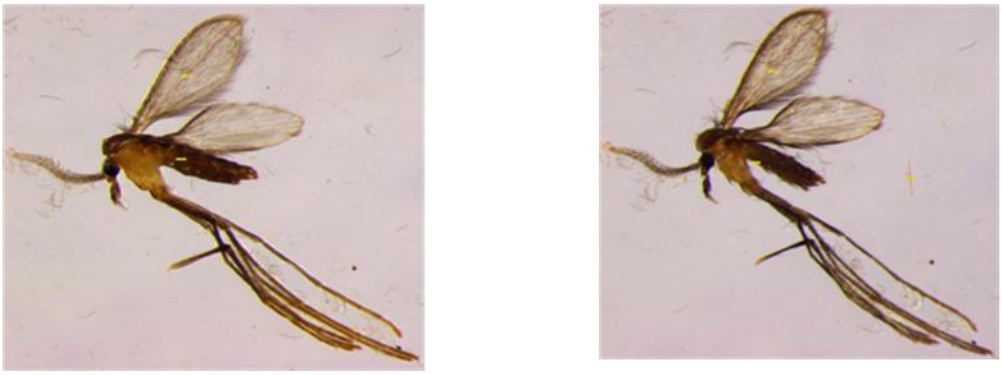
	1st Generation		2nd Generation		3rd Generation		4th Generation		5st Generation		6st Generation	
Types of Food	Artificial Food	Conventional	Artificial Food	Conventional	Artificial Food	Conventional	Artificial Food	Conventional	Artificial Food	Conventional	Artificial Food	Conventional
No. of Days upto pupal stage	23	28	25	28	24	28	23	30	25	30	24	28

Table.2 Nutrient content of food material used in artificial food (derived from National Institute of Nutrition, India)

Major Nutrient (in 100 gms)	**Soya bean** (*Glycine max)*	**Pea Nuts** (*Arachis hypogaea)*	**Rice** (*Oryza sativa)*	**Mixed food Used in Test**
	Nutrient in gms			
Protein	42.3	25.3	6.4	0.072
Fat	19.5	40.1	0.4	0.06
Fiber	3.7	3.1	0.2	0.007
Carbohydrate	20.9	26.1	79	0.13

Fig.1 Effect of Food on larval development and adult emergence





A B

Fig-2 : Adult Sand fly emerged from treated larvae (A), Adult Sand fly emerged from Control (B)

Major nutrients of food might be absorbed in the body of rabbit after feeding of this food. The faeces must be having low nutrients causing low survival rate of larvae. It is already reported that Insect's larvae require greater amount of protein during their growth (Canato et al., 1998, Souza et al., 1999). The rich source of proteins was used in larval development of *Lutzomiya longipalpis*, resulted decrease in larval mortality and increase in the number of adult emergence (DADD et al., 1985). The food having high calorific value/nutrients plays a crucial role in proper development and maintenance of life table. Adult and immature stages of insects require carbohydrate, protein, lipid, minerals and vitamins in order to proper development (DADD at al., 1985). So to improve the nutrition and protein content of *P. argentipes* larvae food, high protein rich artificial food was utilized by combining soyabean, pea nuts & rice in this study. These are easily available at house hold level and cost effective than fish chaws and liver powder. In this study it is observed that the survival of larvae, which was fed on artificial diet, was increased by 28.57 % in compare to control fed on rabbit faeces & Rabbit Chows (Fig-1). Normally the development of sand fly larvae takes 28-29 days depending on temperature (26-27^{o}C) and humidity (82-100%). On the diet developed in this study; *P. argentipes* life cycle comprising of larvae to adult was shorter than the normal, lasting on an average of 23-25 days upto 6th generation from 28 days ($P<0.05$) (Table-1). This artificial diet contains balanced nutrients which is vital for cellular processes and can be utilized by any insects as rich nutritional source of food (Table-2). Since, the food for larvae is also utilized by mites coming from faeces and ground dust. Their growth reduced in this developed diet compare able to control. This might be due to use of rabbit faeces, which are not sterile and contaminations free. The food developed in this study having high protein diet was found more nutritionally rich, in terms of minimizing larval death and higher emergence of adult as compared to pre existing larval diet, ultimately leading to healthier Sand flies for colonization (fig-2).

It can be concluded that the natural herbal composition of food was found having high nutritive value for the fecundity and growth of sand fly larvae for healthier colonization.

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