EFFECT OF NATURAL AND ARTIFICIAL DIETS ON THE LIFE HISTORY PARAMETERS OF MELON-FRUIT FLY, *Bactrocera cucurbitae* (DIPTERA: TEPHRITIDAE)

P.H. RANAWEERA[1], L.D. GALANIHA[1], M. RANATHUNGA[1], K.M.D.W. P. NISHANTHA[1], M. SIRIWARDANA[1] and L. NUGALIYADDE[2]

[1]*Horticultural Crop Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka*[2] *, Sri Lanka Organizations of Agriculture Professionals, Formerly Faculty of Agriculture, university of Ruhuna , Sri Lanka*

ABSTRACT

Mass rearing of insects for sterilization and release requires protocols for the production of insects that are behaviorally and physiologically similar to those of the natural population. In this study, five larval diets (Thailand , Mauritius, Standard and Liquid and NPQS) were tested to determine the efficient medium for mass rearing of melon fruit fly (*Bactrocera cucurbitae*), as compared to four natural foods; Pumpkin, Bitter gourd, Snake gourd and Cucumber. The tested natural and artificial diets except liquid diet found to provide favorable conditions for egg hatchability (range 61.3 – 90.5%). The percentage pupal recovery in natural and artificial diets (except standard and liquid diets) tested ranged from 71 – 97% and was not significantly different to each other. The larval period in artificial diets was longer (7.8a) as compared to natural diets (4.8b). High male to female ratio was observed on Thailand diet (2.5: 1) compared to other diets which recorded from 1.5 to 1 male: 1 female. High perish ability nature of natural diets can be used as initial stock for culturing *B. cucurbitae*. NPQS diet and Thailand diets are selected on the basis of the cost factor for the use of mass rearing of *B. cucurbitae* to introduce Sterile Insect Technology (SIT) program in Sri Lanka.

Keywords: Artificial and Natural Diet, *Bactrocera cucurbitae*, Mass Rearing

INTRODUCTION

The tephrid fruit flies of the genus Bactrocera, with more than 500 species currently described are important pests of reproductive stages of a number of fruit and vegetable crops. These fruit flies are among the most economically important pest species in the world (Kumar *et al.*, 2011, Bandara and Billa, 2015, Vargas *et al.*, 2015). Among the known melon-fruit flies, *Bactrocera cucurbitae* (Coquillett) is considered as the major threat to cucurbits resulting significant yield losses and quality losses inappropriate for local and export market. The magnitude of losses varies from species to species which may range from 30 to 100% depending on the cucurbit species and season (Dhillon *et al.*, 2005).

Department of Agriculture, initiated an island wide fruit and melon fly control programme in 2014 to minimize the damage caused by these insects and to produce quality assured, insecticide residue free fruits and cucurbits for export and local market and to enhance farmer income (Annon,2014). Recommended methods of pre-harvest control programme for fruit and melon flies include; use of protein bait, male annihilation with pheromones, biological control, fruit bagging, early harvesting, and orchard sanitation. Application of Sterile Insect Technology (SIT) as a component of IPM is new to Sri Lanka but, implementation of such technology programme is important because it is considered as an ecologically safe procedure. Benedict and Robinson (2003) reported that irradiation is the most practical way to sterilize insects. In this technique, sterile males are released in the fields for mating with females to produce sterile eggs and minimize population growth. Eradication of *B. cucurbitae* has already been achieved in Japan through sterile-male release in Kikaijma Islands in 1985, Amami-oshima in 1987, Tokunoshima, and the Okierabu-jima and Yoron-jima Islands in 1989 (Sekiguchui, 1990). The success of SIT depends on the ability to establish a cost effective mass rearing method. Hence, efforts were made to determine the effect of available natural and artificial diets on the life history parameters of *B. cucurbitae* such as; fecundity, growth and development of larvae, pupae and Male: Female ratio to identify the best diet for mass rearing of *B. cucurbitae* to enable initiate a SIT in Sri Lanka.

MATERIALS AND METHOD

Experiments were conducted at the Insectory, Horticultural Research and Development Institute, Gannoruwa, Peradeniya from 2015 to 2016. During the study period, the average air temperature and Relative Humidity (RH) in the insectory ranged between 27-29 C0 and 70-77%, respectively, and the photoperiod was maintained at Light: Dark - 9: 15h. B. *cucurbitae* eggs for the experiments were obtained from egg-laying devices kept in B. *cucurbitae* rearing cages in the insectory (Figure 1).

Natural Diet:

Fruit pieces, weighing approximately 250g of fresh Snake gourd (*Trichosanthes cucummerina*), Bitter gourd (Momordica charantia), Pumpkin (*Cucurbita* spp.) and Cucumber (*Cucumis sativus*) were used as natural diets. B. cucurbitae eggs were inoculated on pieces of cucurbits at the rate of 15 eggs/ fruit (Figure 2). These fruit pieces were kept in separate wire mesh cages and recorded the incubation period and hatching percentage. Mature larvae emerged from fruit were counted and then, kept on sterilized sand (sterilized at 120 C0 for 2h) for pupation. Pupae were sieved with a plastic mesh (18 meshes) and weighed. Emerging adults were sexed and counted and transferred to net cages for egg laying. The adult flies were fed with protein hydrolysate, and a mixture of sugar and water (1:1 v/v) soaked in cotton. The above experiment was replicated five times per natural diet.

Artificial Diets:

Four popular larval diets (Thailand, Mauritius, Standard and Liquid Diet) and a locally formulated diet in National Plant Quarantine Service (NPQS) were used for the test (Table 1). These larval diets were poured separately into 9 cm diameter Petri Dishes at the rate of 50g/ dish. These artificial diets were covered with wet tissue papers and inoculated with *B. cucurbitae eggs* at the rate of 15 / dish (Figure 3). These Petri dishes were individually kept inside plastic cups covered with nylon net to protect from desiccation. The experiment was replicated five times. The incubation period and hatching percentage of eggs and larval duration in each artificial diet were recorded. The procedure adopted under natural diet was followed to determine the pupal weight, pupal duration and adult emergence and sex ratio. The emerging adult flies were fed with protein hydrolysate, a mixture of sugar and water (1:1 v/v) soaked in cotton.

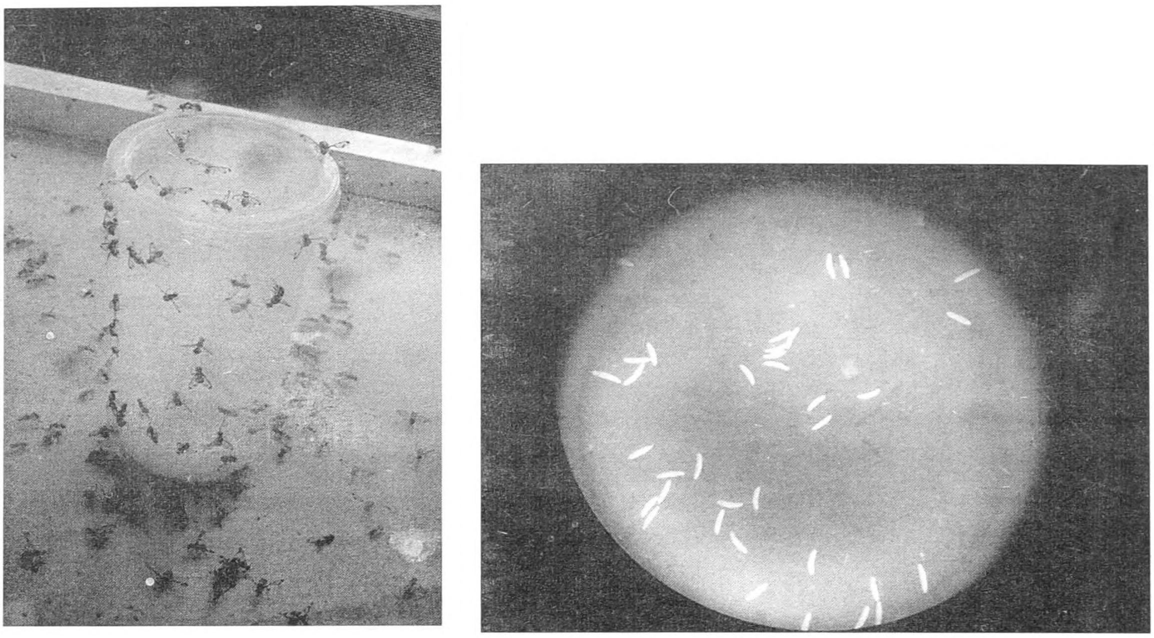

Figure 1. Artificial egg laying device kept in insect-cages and eggs of *B. cucurbitae* (10X10)

Figure 2. Testing the life cycle of *B. cucurbitae* on natural diets

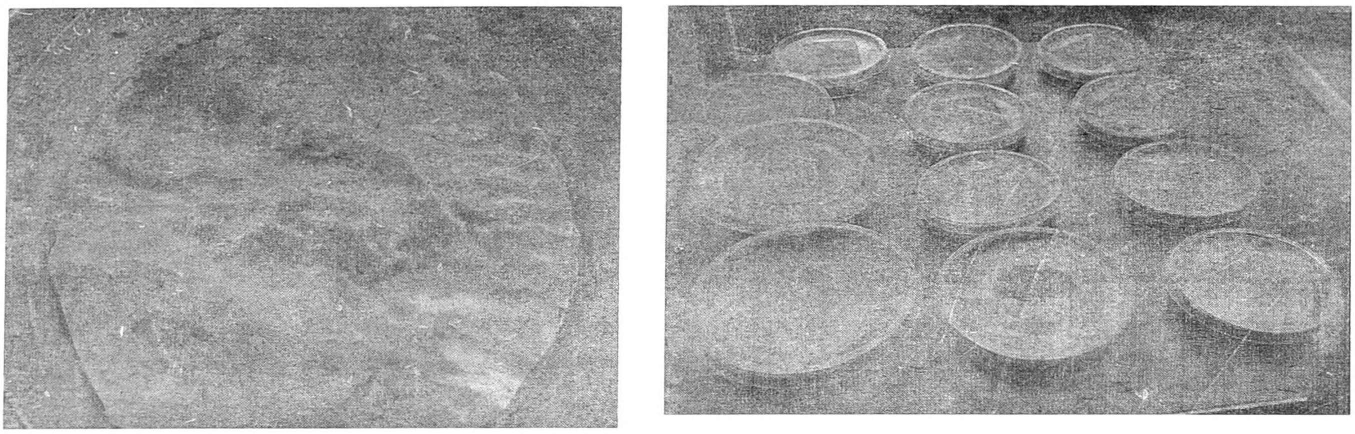

Figure 3. Testing of life cycle of B. *cucurbitae* on artificial diets; eggs are placed on wet tissue papers laid over artificial diets in Petri dishes for incubation and larval development: the Petri dishes are placed inside plastic cups covered with a nylon mesh

Evaluation of Biological parameters:

Using the standard procedures (Chang *et al.*, 2007) the following biological parameters of *B.cucurbitae* reared on natural and artificial diets were assessed; egg hatchability and incubation period, larval and pupal durations, pupal weight, percentage of pupal yield from larvae, percentage adult yield from pupae. All the percentage values were check for normality and log-transformed. The data of all tested parameters were analyzed using ANOVA Statistical Analytical Software (SAS) Version 8.1.

Table 1. Ingredients of the larval diets (for 1 liter) it's pH value and Cost/ Liter

Ingredient	Artificial Larval Diets				
	NPQS	**Mauritius**	**Thailand**	**Standard**	**Liquid**
Sugar (g)	50	82.8	120	162	121.8
Brewer's Yeast (g)	35	40	36	80	204
Wheat Bran (g)	175	351.4	260	242	-
Wheat Germ Oil (ml)	-	-	-	-	2
C. HCl (ml)	1	8	2	-	-
M.P.H. Benzoate (g)	0.75	1	1	-	2
Sodium benzoate (g)	0.75	2	1	5	2
Tissue Paper (g)	25	-	-	-	-
Water (ml)	650	650	580	505	1000
Citric Acid (g)	-	-	-	6	23.1
pH	4.5-4.9	5	5	4.8	3.5
Cost/ L (SLR)	1,621/	2,595/	1,854/	3,319/	8,436/

RESULTS AND DISCUSSION

Egg hatchability of B. *cucurbitae* was not significantly different from natural and artificial diets (Table 2). However, all the diets (natural and artificial) except liquid diet found to provide equally favorable conditions for egg development and these value were in par with those observations reported by Samalo *et al.* (1991). The incubation period on artificial diets was lower than on natural diet but was not significantly different.

The percentage pupal recovery in natural and artificial diets (except standard and liquid diets) tested ranged from 71 – 97% and was not significantly different to each other (Table 3.)

Table 2. Egg hatchability and incubation period of B. cucurbitae on natural and artificial diets (n= 45) (HORDI, 2015)

Diet	Hatchability (%)	Incubation period (d)
Pumpkin	82.4 a	5.4 a
Bitter gourd	77.8 a	5.4 a
Snake gourd	90.5 a	5.4 a
Cucumber	70.1 a	5.4 a
Mauritius Diet	82.0 a	3.6 a
Thailand Diet	61.3 a	3.6 a
NPQS Diet	77.5 a	3.8 a
Standard Diet.	82 a	3.6 a
Liquid Diet	Not hatched	-
CV%	10.9	4.4

P>0.05; Means followed by the same letter(s) are not significantly different at 5% the probability level.

Table 3. Pupal recovery and Larval duration of B. cucurbitae in tested natural and artificial diets (n = 45) (HORDI, 2015)

Diets	Pupal recovery (%)	Larval duration (Days)
Pumpkin	81.2 a	4.8b
Bitter gourd	80.7 a	4.8 b
Snake gourd	76.6 a	4.8 b
Cucumber	81.4 a	4.8 b
Mauritius Diet	71.5 a	7.8 a
Thailand Diet	80.2 a	7.8 a
NPQS Diet	97.1 a	7.2 ab
Standard Diet.	0	0
Liquid Diet	0	0
CV%	8.3	27.2

P>0.05; Means followed by the same letter(s) are not significantly different at 5% DMRT

The larval duration in artificial diets were longer (7.8a) as compared to natural diets (4.8b) indicating the favorable nature of the nutritional and allelochemical environment of the natural food for the larval development.

Average weight of 100 pupae (g) and adult emergence (pupal survival) of B. cucurbitae did not differ significantly when larvae were fed on natural and artificial diets and lowest pupal weight and % of adult emergence was observed when fed in bitter gourd (Table 4). Leppla *et al.* (1989) reported no significant difference among the life cycle parameters of the fruit fly species C. capitata, when reared on natural and artificial diets up to the 3rd generation. We observed that the pupal duration of the larvae reared on artificial diet was not significantly different with those reared on natural diet. Similarly, Hollingsworth *et al.* (1997) reported that the temperature and the food source (host) influence the pupal period from 7-13 days.

Table 4. Mean weight of 100 pupae, percentage adult emergence (Pupal survival) and Pupal stadium, of B. cucurbitae in natural and artificial diets (n= 45) (HORDI, 2015)

Diets	Pupal weight X 100 (g)	% of adult emergence	Pupal duration (Days)
Pumpkin	1.3 ab	82.7 a	7 a
Bitter gourd	0.7 b	59.9 a	7 a
Snake gourd	1.3 ab	81.4 a	7 a
Cucumber	1.8 a	85.8 a	7 a
Mauritius Diet	1.5 ab	77.5 a	6.4a
Thailand Diet	1.3ab	83.5 a	6.4 a
NPQS Diet	2.2 a	87.8 a	5.6 a
Standard Diet.	0		
Liquid Diet (Chang mtd)	0		
CV%	22.9	7.2	23.5

P>0.05; Means in a column followed with the same letter(s) are not significantly different at 5% DMRT

High male to female ratio was observed in Thailand diet fed insects (2.5: 1) compared to other diets which recorded from 1.5 to 1 male per 1 female (Table 5).

We observed that eggs did not hatch when incubated in liquid diet and larvae did not survive in standard diet, therefore these two diets considered as poor performers in B. *cucurbitae* rearing. The rest of the artificial diets performed similarly in terms of hatchability, incubation period, pupal recovery and pupal weight, pupal duration and adult emergence except larval duration. Therefore, NPQS diet, Mauritious diet and Thailand diet can be use for mass rearing as larval diets. After considering the cost factor and high male ratio a decision was taken to use NPQS diet or Thailand diet in mass rearing of B. cucurbitae to introduce SIT program in Sri Lanka.

Table 5. Male Female Ratio of B. cucurbitae when fed on natural and artificial diets

Diet	Male: Female
Pumpkin	1: 1.1
Bitter gourd	1:1
Snake gourd	1:1
Cucumber	1.1 :1
Mauritius Diet	1.4:1
Thailand Diet	2.5:1
NPQS Diet	1.5:1

CONCLUSIONS

The life history parameters of *B.cucurbitae* larvae reared on artificiall diet and natural diets did not show significant differences except in larval duration. Egg hatchability, pupal recovery and % of adult emergence did not significantly differ in tested natural diets over the artificial diets. Due to higher perishability nature of natural diets, Snake gourd (*Trichosanthes cucummerina*), Bitter gourd (*Momordica charantia*), Pumpkin (*Cucurbita* spp.) and Cucumber (*Cucumis sativus*) can be used as initial stock for culturing of *B. cucurbitae*. Among the tested artificial diets, NPQS, and Thailand diets found to be the best in terms of the low cost of the diet. Based on the cost factor NPQS diet and Thailand diet were selected as the most suitable artificial diets for mass rearing of *B. cucurbitae* to introduce SIT program in Sri Lanka.

ACKNOWLEDGEMENTS

Authors would like to acknowledge the financial support extended by the Director General of Agriculture, Dr. R.R. Wijekoon for conducting area wide Melon fruit fly control programe and all the Directorates in HORDI, SCPPC, Gannoruwa for their encouragement; all officers in NPQS for providing egg laying cups and wheat bran.

REFERENCES

Anon, 2014, Seasonal Reports, Horticultural Crop Research and Development Institute, Department of Agriculture, Sri Lanka

Bandara, K.A.N.P. and M.K. Billa. 2015. Parasitoids/predators and alternative hosts of economically important fruit fly pests of low, mid and high elevations of Sri Lanka. Annals of Sri Lanka Department of Agriculture.17: 303-309 .Department of Agriculture, Sri Lanka.

Benedict M.Q. and A.S. Robinson. 2003. The first release of transgenic mosquitoes: an argument for the sterile insect trchnique. trends Parasitol. 19:349-355.

Chang, C.L,C. Caceres and Ekesi, S. 2007. Life history parameters of Ceratitis capitata (Diptera: Tephritidae) reared on liquid diets, Annals of Entomological Society of America 100, 900-906.

Dhillon, M.K., R. Singh, J.S. Naresh and H.C. Sharma. 2005. The melon fruit fly Bactrocera cucurbitae: a review of its biology and management.16pp. Journal of Insect Science 5:40, available online: insectscience.org/5.40.

Hollingsworth R., M. Vagalo and F. Tsatsia. 1997. Biology of melon fly with special reference to the Solomon Islands. In: (Eds. A.J. Allwood and R.A.I. Drew Management of fruit flies in the Pacific. Proceedings of Australian Country Industrial Agricultural Research.76:140-144.

Kumar P., Abubakar, Alma Cinda, Ketelaar and T.W. Shanmugam. 2011. Field exercise guide on fruit fly integrated pest management. Asian fruit fly IPM project, Asia Institute of Technology, Bangkok, Thailand.

Leppla, N. C. 1989. Laboratory colonization of fruit flies, In: (Eds. Robinson, A.S. and Hooper, G.) Fruit Flies: their Biology, Natural Enemies and Control, Elsevier, Amsterdam, 3B:91-104.

Samalo A.P., Beshara R.C. and Satpathy C.R.1991. Studies on comparative biology of the melon fruit fly, Dacus cucurbitae Coq. Orissa Journal of Agricultural research,4:1-2.

Sekiguchui Y. 1990. Eradication of the melon fly (Dacus cucurbitae) from Amani Islands of Japan. Quarterly Newsletter-Asia and Pacific Plant Protection Community. 33:19–20.

Vargas R.I., J.C. Pinero and L. Leblanc. 2015. An Overview of pest species of Bactrocera fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the pacific region. Insects 6: 297- 319