

International Journal of Scientific Research and Reviews

Effect of Fly Gut Bacteria Incorporated Adult Diets on the Fecundity and Longevity of *Zeugodacus cucurbitae* (Coq.) and *Bactrocera dorsalis* (Hendel) (Diptera:Tephritidae)

Mahfuza Khan^{1*}, Tasmina Tonu² and Kajla Seheli¹

¹Insect Biotechnology Division, Institute of Food and Radiation Biology, Atomic Energy Research Establishment, Ganak bari, Savar, Dhaka-1349, Bangladesh

²Department of Zoology, Faculty of Life and Earth Science, Jagannath University Dhaka, Bangladesh

ABSTRACT

The Melon fly, *Zeugodacus cucurbitae* (Coq.) and Oriental fruit fly, *Bactrocera dorsalis* (Hendel) are agriculturally important tephritid fruit flies. The probiotic use of insect gut bacterial community known to have significant positive impact on the overall fitness of different tephritid fruit flies in support of Sterile Insect Technique (SIT). In the present study the influence of fly's gut-bacteria enriched adult diets, i.e., *Serratia* and *Erwinia* spp. added protein and sugar diets and their corresponding controls were evaluated on the ovariole number, fecundity, and mortality of *Z. cucurbitae* and *B. dorsalis*. The experimental results showed that *Erwinia* sp. and *Serratia* spp. enriched protein diets have no significant effect on the ovariole number of *Z. cucurbitae* and *B. dorsalis* after 14 days of adult emergence compared to those fed on only protein diet. No egg production was also recorded for both the fly species fed on only sugar diet at this age. Whereas delayed ovarian development and egg production was observed in *Z. cucurbitae* and *B. dorsalis* on 24 and 29 days, respectively while fed on *Serratia* sp. enriched sugar diet. The response of *Z. cucurbitae* in terms of egg production to bacteria enriched sugar diet was greater than *B. dorsalis*. In laboratory small cage experiment the mean mortality of *Z. cucurbitae* and *B. dorsalis* fed on *Erwinia* sp. and *Serratia* sp. enriched sugar diets were higher than bacteria enriched protein diets. Protein fed flies survival longer than protein deprived i.e., only sugar fed flies. Almost similar results were observed under semi-field cage experiment.

KEYWORDS: Tephritid fruit flies, adult diet, gut bacteria, ovariole number, longevity.

*Corresponding author

Mahfuza Khan

Director and Chief Scientific Officer,
Institute of Food and Radiation Biology (IFRB)
Atomic Energy Research Establishment (AERE),
Ganak bari, Savar, Dhaka-1349, Bangladesh. **E-mail:** mahfuza79@gmail.com.

INTRODUCTION

The Melon fly, *Zeugodacus (Bactrocera) cucurbitae* (Coq.) and Oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) are important polyphagous pests of horticultural products worldwide. In Bangladesh *Z. cucurbitae* and *B. dorsalis* are considered economically most important species of different fruits and vegetables causing 10-30% losses of annual production¹. The control strategies remain exclusively based on insecticides, despite the awareness of a need for the use of more environment friendly control methods as Sterile Insect Technique (SIT). SIT is one of the proven control methods against many insect pests including fruit flies under the Area-Wide Integrated Pest Management (AW-IPM) Programme. In SIT the insects are irradiated to render them sterile prior to release in the field, where the sterile males copulate with wild females of the target pest population and are unable to produce viable offspring². Over time and with repeated releases of sterile flies, the pest population is suppressed or may be eradicated within the release area. Low cost mass rearing and production of high quality target insects are one of the major components of SIT field application programme. Recent studies showed that target specific manipulation of insect gut bacteria can have significant positive impact on the overall fitness of SIT specific insects. By impacting on host nutrition, physiology, metabolism, and immunity, gut microbiota can profoundly influence various aspects of insect health, fitness and behaviour^{3, 4}, and thus the quality of mass-reared flies for the SIT.

The Tephritidae is large family that includes many fruit pests and these are usually adopted for housing large quantities of bacteria in their digestive tract^{5, 6, 7, 8}. Twenty different strains of bacteria from laboratory reared *B. dorsalis* and 23 strains from wild adults, characterized as members of family Enterobacteriaceae have been reported by Jang and Nishijima⁹. The most common bacteria associated with *Bactrocera* flies were *Citrobacter freundii*, *Enterobacter agglomerans*, *Enterobacter cloacae*, *Klebsiella oxytoca* and *Kluyvera* spp.^{9, 10}. These bacteria were collectively referred as “Fruit fly type” bacteria. However, knowing the intestinal bacteria is important in the context of developing our understanding of symbiotic relationships, multitrophic interactions between insects and plant or animal host and in the developing new strategies for controlling insect pests³. Gut microbiota are not only a source of nutrients but can also assist the host insect in nutrient acquisition, allocation, assimilation, and detoxification, modulate the foraging behavior^{11, 12, 13, 14, 15, 16}. A reduction in gut microbial diversity may lower microbial colonization resistance, thereby allowing pathogenic microorganisms to establish in the gut¹⁷. de Vargas *et al.*¹⁸ reported that the gut microbiota may have alternated between mutualism/commensalism and parasitism in response to changes in their host’s diet. The addition of probiotic bacteria may be a means of restoring or improving gut bacteria to positively influence fly production and performance. Recent years many

advances have made on the quantitative studies on the microbial communities of different fruit fly spp. but very few studies where the bacterial supplements (probiotics) were added to tephritid larval or adult diets to evaluate their benefits^{19, 20, 21, 22, 23, 24}. Ben-Ami *et al.*¹⁹ reported that regenerating the original microbiota could result in enhanced competitiveness of the sterile med fly, *Ceratitidis capitata* (Wiedemann). Hamden *et al.*²⁰ used gut bacteria spp. viz., *K. pneumoniae*, *C. freundii* and *Enterobacter* spp. from *C. capitata* as a probiotic in the larval diet and improved adult fitness. Likewise, the addition of *Enterobacter* sp. to larval diet also reported to led significantly enhanced fitness and sexual performance of the laboratory-raised *C. capitata*²¹ and *Z. cucurbitae*²⁵. The addition of bacteria from the Enterobacteriaceae family to the larval diets used to rear the ‘Vienna 8’ genetic sexing strain (GSS) of *C. capitata* used in SIT also increased pupal weight, longevity, morphometric traits (greater head width, abdomen, and thorax length), mating competitiveness under laboratory conditions, and spermatozoa storage in females²¹. Although few research works were conducted on the isolation and characterization of gut bacterial community of *B. dorsalis*^{8, 26, 27, 28}, but very little is known about the application of a probiotic/diet supplements to mass rearing and enhanced performance of male *Z. cucurbitae* and *B. dorsalis* in support of SIT.

The aim of the present research work was therefore to determine the influence of fruit fly’s gut bacterial spp. incorporated adult diets on different biological parameters viz., ovariole number, fecundity of *Z. cucurbitae* and *B. dorsalis* under controlled laboratory condition. The longevity of above mentioned fly species was also conducted and recorded under both the laboratory condition and semi-field cage trials fed on fly’s gut bacterial spp. incorporated adult diets. The experimental results would help to detect the potential of gut bacterial spp. of fruit flies those may help to improve different fitness parameters of *Z. cucurbitae* and *B. dorsalis* in support of SIT application.

MATERIALS AND METHODS

Insect rearing and isolation of gut bacterial spp.:

Rearing of *Z. cucurbitae* and *B. dorsalis* were maintained in the laboratory of Insect Biotechnology Division (IBD), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka for more than 50 generations. *Z. cucurbitae* was reared on natural host (sweet gourd) and also on bran-based diet. *B. dorsalis* was maintained on natural host (banana) and on artificial liquid larval diet²⁹. About 5,000 adult flies were maintained in steel framed cages (76.2 x 66 x 76.2 cm, H x L x W) covered with wired net. The flies were supplied with protein based diets both in the liquid and dry form viz., (i) baking yeast:sugar: water at 1:3:4 ratio, and (ii) casein: yeast extract: sugar at 1:1:2 ratio. Water was supplied in a conical flask soaked

with cotton ball. The temperature and the relative humidity of the rearing room were maintained at $27\pm 1^{\circ}\text{C}$ and $75\pm 5\%$, respectively.

The isolation and identification of mid-gut bacterial community of *Bactrocera* species^{28, 30} was performed in the laboratory of Microbiology and Industrial Irradiation Division (MIID), IFRB, AERE, Savar. Two previously identified gut bacterial species of adult fly viz., *Erwinia* and *Serratia* spp. were randomly selected and were sub cultured on LB-agar and incubated for 18-24 h at 37°C . The bacterial isolates were resuspended in to 2 ml of 0.85% saline. The cells were then centrifuged at 6000 rpm for 10 min. The cell pellet was subsequently suspended in a measured volume of 0.85% saline and the turbidity was adjusted to 3.8×10^6 CFU/ml. Cell suspensions were mixed with protein and sugar diets.

The diet treatments used were: i. Only protein diet (yeast extract: casein: sugar, 1:1:2); ii. Isolates of *Erwinia* sp. (3.8×10^6 CFU/ml) in 0.8% saline water, and protein diet (2ml/40gm); iii. Isolates of *Serratia* sp. (3.8×10^6 CFU/ml) and protein diet (2ml/40gm); iv. Only sugar diet (20% sugar solution); v. Isolates of *Erwinia* sp. (3.8×10^6 CFU/ml) and sugar diet (2ml/40ml); vi. Isolates of *Serratia* sp. (3.8×10^6 CFU/ml) and sugar diet (2ml/40ml). Probiotic protein diets were given on small watch glass and changed every two days. Water was supplied as common drinking source for all protein diets. Sugar diet supplied in small plastic container soaked into cotton wick.

Determination of ovariole number of Z. cucurbitae and B. dorsalis fed on bacteria added protein and sugar diets:

Newly emerged 30 male and 30 female adult *Z. cucurbitae* and *B. dorsalis* were housed in small rearing cages (8x6x12 cm) and supplied with six diet treatments mentioned earlier. Three replicates were maintained for each diet treatment. The ovary of 14 days old *Z. cucurbitae* and *B. dorsalis* were dissected in 0.85% sodium chloride (NaCl) solution under stereo microscope. Total number of eggs per ovary were counted and recorded. The ovariole development of only sugar fed and bacteria added sugar fed 14 days old adult flies was also determined. In mature flies, there is normally only one mature oocyte (egg with shell) per ovariole that is ready for laying.

Determination of egg per female per day of Z. cucurbitae and B. dorsalis fed on bacteria enriched protein and sugar diets:

Four sets of 50 pupae of *Z. cucurbitae* and *B. dorsalis* were collected from stock culture and placed in plastic Petri dishes (55 mm). Each Petri dish was then placed individually inside four small rearing cages and provided with four different types of adult diets viz, only protein, *Erwinia* sp. + protein, only sugar, and *Erwinia* sp. + sugar diet. A covered plastic container of water with cotton wick was provided inside each cage. Fourteen days after adult emergence, 10 male and 10 female *Z.*

cucurbitae and *B. dorsalis* from each rearing cage were again placed into small rearing cages (6x6x10 cm). The flies were provided with four different types of diets as mentioned above and water was supplied via a cotton wick inserted into a plastic vial (5 ml). Small pieces of sweet gourd for *Z. cucurbitae* and banana for *B. dorsalis* were placed inside the cages for egg collection for 24 hours on 24, 29, and 33 days after adult emergence. Fecundity was determined by counting total number of larvae produced by ten pairs of *Z. cucurbitae* and *B. dorsalis*. The experiment was repeated three times with three replicates for each diet treatment. Similar procedure was followed with *Serratia* sp. incorporated protein and sugar diets with their respective controls.

Determination of the longevity of Z. cucurbitae and B. dorsalis fed on bacteria enriched protein and sugar diets under laboratory condition:

In the present trial, experimental flies were obtained as pupae from the stock rearing of IBD, IFRB. Following eclosion, flies were separated by sex and housed in small rearing cages (12x 8x 6 cm), in groups of 200–300 individuals for 72 h, during which sucrose and water were provided. This period served to filter out weak individuals who did not survive eclosion³¹. Subsequently, four sets of 50 male and 50 female *Z. cucurbitae* and *B. dorsalis* were confined in small rearing cages and each was subjected to one of the following four dietary regimes: (i) only sugar diet, (ii) sugar + bacteria enriched diet, (iii) only protein diet, and (iv) protein + bacteria enriched diet. Bacteria species viz., *Erwinia* and *Serratia* spp. were used in the experiment. Bacteria enriched sugar diet was soaked in small cotton ball and placed on small glass watch. Whereas bacteria added protein diets were given directly on small watch glasses. Diet treatments were replaced every 24 h up to 20 days. During this time the daily mortality in each cage was recorded, dead flies were removed from the cages. Three replicates were maintained for each diet treatment. All experiments were conducted in a controlled laboratory condition.

Determination of the longevity of Z. cucurbitae and B. dorsalis fed on bacteria enriched protein and sugar diets under semi-field cage trials:

In the present experiment pupae of *Z. cucurbitae* and *B. dorsalis* were collected from stock culture maintained in the laboratory of IBD. Four diet treatments of *Erwinia* and *Serratia* spp. added protein and sugar diets and their respective controls were provided to newly eclosed 500 pairs adult *Z. cucurbitae* and 500 pairs adult *B. dorsalis* into a semi-field cage (25 x 10 x 12.5 cm, L x W x H) separately with ornamental and guava plants, respectively. Water was provided in a plastic container with a cotton wick and bacteria enriched diets were provided in small watch glasses. Both the diet and water were changed every two days. Cages were checked daily for mortality up to 20 days and data were recorded.

STATISTICAL ANALYSIS

Data for the ovariole number, egg/female/day, and longevity of *Z. cucurbitae* and *B. dorsalis* on different diet treatments were analyzed using Analysis of Variance (ANOVA) and Tukey's family error rate was performed using Statistical Software -Minitab USA (version-17). Graphs were created in Microsoft Excel 2007.

RESULTS AND DISCUSSION

Determination of the fecundity and longevity of Z. cucurbitae and B. dorsalis fed on probiotic adult diets:

Mean Ovariole Number of Z. cucurbitae and B. dorsalis:

The influence of gut-bacteria bacteria spp., *Serratia* and *Erwinia* enriched protein diets and only protein adult diet on the ovariole number of *Z. cucurbitae* and *B. dorsalis* is presented in Figure 1. No significant differences ($P>0.05$) were recorded among ovariole numbers of *Z. cucurbitae* ($P=0.36$, $F=1.03$, d.f.= 2, 45) and *B. dorsalis* ($P=0.33$, $F=1.14$, d.f.=2, 45) fed on *Serratia* and *Erwinia* spp. enriched protein diets, and only protein diet. Mean ovariole number per ovary were recorded as 28.8 ± 3.06 and 29.8 ± 3.61 , 30.87 ± 2.49 and 33.75 ± 2.69 , and 27.12 ± 3.1 and 29 ± 2.89 , respectively for above mentioned diet treatments of *Z. cucurbitae*. In *B. dorsalis* it was 38 ± 3.24 and 38.3 ± 3.16 , 43.12 ± 2.79 and 40.25 ± 1.85 , and 41.62 ± 1.87 and 40.25 ± 2.45 , respectively fed on *Erwinia* and *Serratia* spp. incorporated protein diets and only protein diet. No ovariole development was recorded for only sugar and bacteria added sugar fed 14 days old *Z. cucurbitae* and *B. dorsalis*. Mean ovariole number is significantly higher for *B. dorsalis* than *Z. cucurbitae* on above mentioned diet treatments.

Egg/Female/Day of Z. cucurbitae and B. dorsalis:

Mean egg/female/day of *Z. cucurbitae* and *B. dorsalis* fed on *Serratia* and *Erwinia* spp. enriched protein and sugar diets and only protein on 24, 29 and 33 days after adult emergence was presented in Figures 2-3. In case of *Z. cucurbitae* both *Serratia* and *Erwinia* spp. enriched sugar diets trigger egg production at later stage of life than usual time (14 days of adult emergence in our lab). First egg was collected on 24 and 29 days fed on *Serratia* and *Erwinia* spp. enriched sugar diets, respectively.

In case of *B. dorsalis* no egg production was recorded on *Erwinia* sp. enriched sugar diets up to 33 days of adult emergence. Later egg production was recorded on 50 days of adult emergence. Whereas first egg production was recorded on 29th day on *Serratia* sp. enriched sugar diet.

The effect of gut bacteria added sugar diets in terms of mean egg/female/day on later stages of life was higher on *Z. cucurbitae* than *B. dorsalis*. In the present experiment no egg was observed to lay by *Z. cucurbitae* and *B. dorsalis* fed on only sugar fed diet up to 33 days of observation.

Longevity of Z. cucurbitae and B. dorsalis on probiotic diet under laboratory condition:

Percentage mortality of adult *Z. cucurbitae* and *B. dorsalis* fed on *Erwinia* and *Serratia* spp. enriched protein and sugar diets, and only protein and sugar diets up to 20 days under laboratory condition are shown in Figure 4. Bacteria enriched protein fed flies mortality is lower than those fed on bacteria enriched sugar diet. Comparatively higher mortality was recorded for *Erwinia* and *Serratia* spp. enriched sugar diets than only sugar diet fed *Z. cucurbitae* and *B. dorsalis*.

Determination of the survival of Z. cucurbitae and B. dorsalis fed on probiotic adult diets under semi-field cage trials:

Percentage (%) mortality of *Z. cucurbitae* and *B. dorsalis* fed on *Serratia* and *Erwinia* spp. enriched protein and sugar diets, and only protein and sugar diets up to 20 days under semi-field cage trials are presented in Figure 5. No significant differences ($P>0.05$) were recorded for *Z. cucurbitae* fed on *Serratia* and *Erwinia* spp. enriched protein diets and only protein. Comparatively higher mortality was recorded for *B. cucurbitae* fed on *Serratia* sp. enriched sugar diets which is also higher than control sugar fed flies. Percentage mortality of control *Z. cucurbitae* on *Serratia* and *Erwinia* spp. enriched protein and sugar diets, and only protein and sugar diets was 58.5, 50.1 80.2, 57.5, 48.6, and 62%, respectively (Fig. 5).

Percentage mortality of *B. dorsalis* fed on *Serratia* and *Erwinia* spp. enriched protein and sugar diets, and only protein and sugar diets up to 20 days under semi-field cage trials are shown in Figure 5. Comparatively higher mortality was recorded for *B. dorsalis* fed on *Serratia* and *Erwinia* spp. enriched sugar diet and only sugar diet than both bacteria spp. added protein diet and only protein diet.

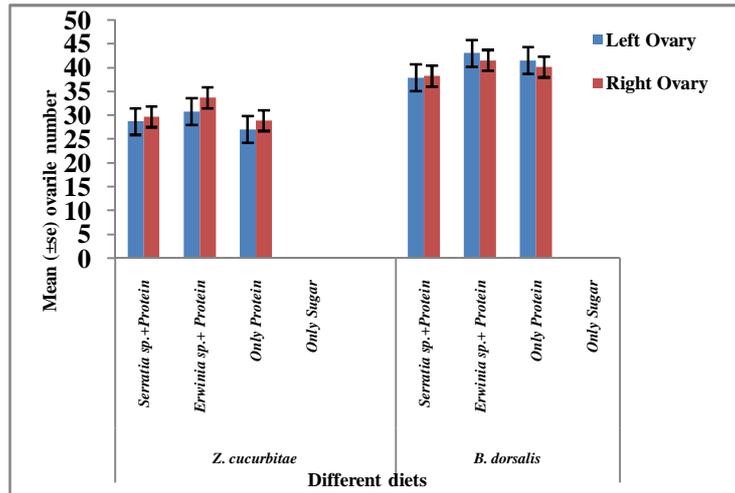


Figure 1: variole number of fourteen days adult *Z. cucurbitae* and *B. dorsalis* fed on *Serratia* and *Erwinia* spp. incorporated protein and sugar diets and controls

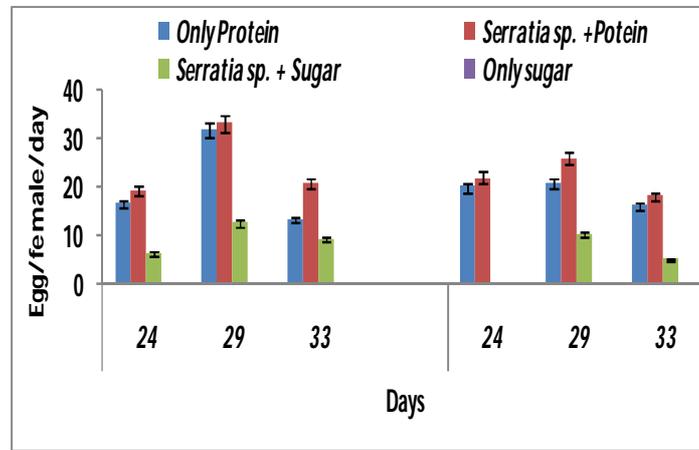


Figure 2: Eggs/female/day of *Z. cucurbitae* and *B. dorsalis* fed on *Serratia* sp. enriched protein and sugar diets and controls

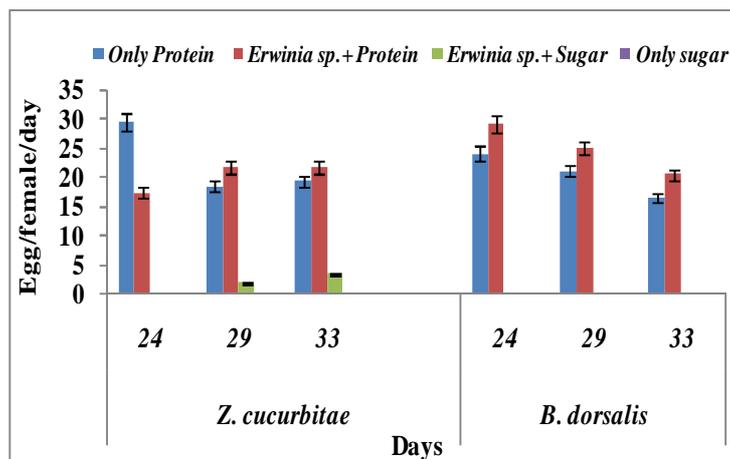


Figure 3: Eggs/female/day of *Z. cucurbitae* and *B. dorsalis* fed on *Erwinia* sp. enriched protein and sugar diets and controls

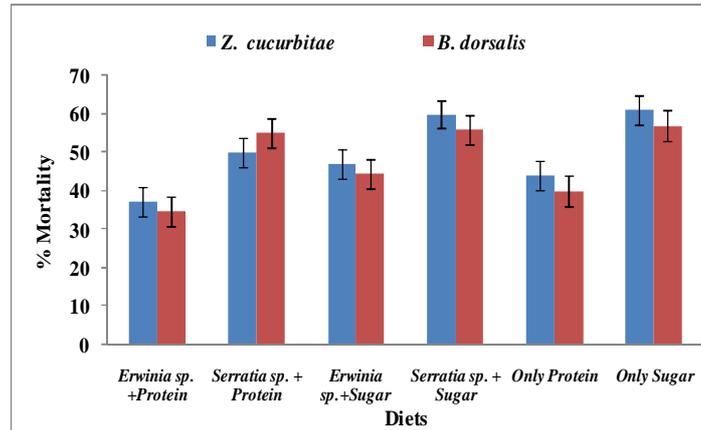


Figure 4: Percentage (%) mortality of adult *Z. cucurbitae* and *B. dorsalis* fed on *Erwinia* and *Serratia* spp. incorporated protein and sugar diets and controls under laboratory condition

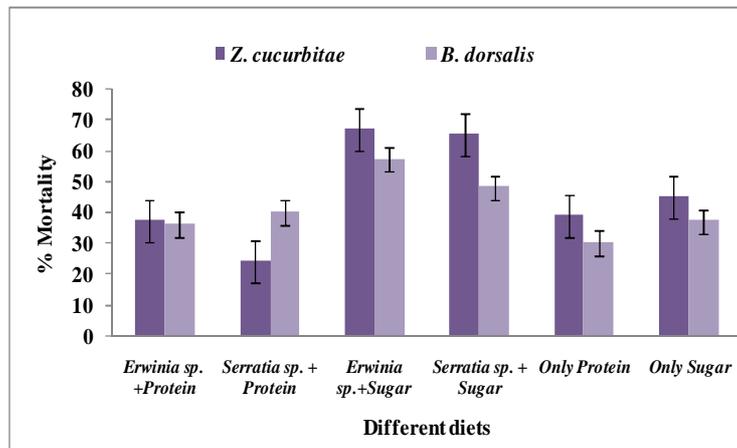


Figure 5: Percentage (%) mortality of adult *Z. cucurbitae* and *B. dorsalis* fed on *Erwinia* and *Serratia* spp. incorporated protein and sugar diets and controls under semi-field cage trials

In many Tephritid species proteinaceous component is required in the diet for sexual maturation and oogenesis of adult female fly^{32, 33, 34}. Drew *et al.*³¹ established that bacteria of the gram negative family Enterobacteriaceae could serve as an attractant and proteinaceous food for adult Queensland fruit fly, *Bactrocera tryoni* (Froggatt) from a long term laboratory culture. The authors noted that diets of bacteria, sugar and water gave equal longevity and increased fecundity in *B. tryoni* compared with the conventional diet of autolyzed brewer's yeast, sugar and water. In contrast Meats *et al.*³⁵ noted that *B. tryoni* could not produce eggs or mature oocytes on a bacterial diet above the level attained with access to culture medium without bacteria. Khan *et al.*³⁰ also reported that *B. tau* fed on *Proteus rettgeri* and *Klebsiella oxytoca* added protein diets and only protein diet did not show significant influence on mean ovariole number. Halder *et al.*³⁶ used exogenous bacteria species e.g., *Escherichia coli* and *Lactobacillus lactis* and also showed that bacteria added protein diet had no effect on ovariole number of *B. tau*. The present findings is in

agreement with the above mentioned findings^{30, 36} and showed no significant differences ($P>0.05$) among ovariole numbers of *Z. cucurbitae* and *B. dorsalis* fed on *Erwinia* and *Serratia* spp. enriched protein diets, and only protein diet after 14 days of adult emergence (Fig. 1).

The present experimental results on egg/female/day of *Z. cucurbitae* and *B. dorsalis* fed on different bacteria added adult diets and only protein diet partially in agreement with the findings of Khan et al.³⁰ in case of *B. tau* and showed no significant differences (Figs. 2-3). However, in the present study interestingly, *Z. cucurbitae* and *B. dorsalis* showed delayed ovariole development and egg production after 29 and 24 days, respectively, while fed on bacteria, *Serratia* sp. enriched sugar diet. In case of *Erwinia* sp. enriched sugar diet first egg production was recorded on 29 and 50 days of adult emergence for *Z. cucurbitae* and *B. dorsalis*, respectively. Whereas no egg production was recorded for the fly species fed on only sugar diet up to 33 days of observation. The present findings partially in contrast with the observation of Ben-yosef et al.³⁷ who noted that female *C. capitata* feeding on full diet produce significantly more eggs than females on the sugar diet, but the presence of bacteria does not affect numbers of egg produced. Ben-Yosef et al.³⁸ also evaluate the presence of bacteria in female olive flies, and monitored fecundity-an indirect measure of fitness. The authors reported that bacteria did not affect fecundity when females were fed a nutritionally poor diet of sucrose, or a protein-rich, nutritionally complete diet. However, when females were fed a diet containing non-essential amino acids as the sole source of amino nitrogen, egg production was significantly enhanced in the presence of bacteria. Bacteria were able to compensate for the skewed amino acid composition of the diet and may be indispensable for wild adult olive flies that subsist mainly on nitrogen-poor resources such as honeydew.

Longevity however, could also greatly depend on the diet. Qualitative or quantitative changes in the species composition of the gut bacterial community, induced by the diet, could have been the cause of a different net effect on the longevity of the host fly³⁸. Thus, the gut microbiota may have alternated between mutualism/commensalism and parasitism in response to changes in their host's diet¹⁸. The lack of protein in the diet had a prolonging effect on longevity, but only when flies were deprived of their gut microbiota. However, in the presence of bacteria this effect was reversed in males who lived longer when fed the full diet. Niyazi et al.²⁴ reported a significant benefit of probiotic post teneral diets on aspects of behavioural ecology in sterile male *C. capitata*. Access to protein in the adult diet has been reported to increase the longevity of *B. tryoni*³⁹ and *Anastrepha serpentina* (Widemann)⁴⁰, while the response of *C. capitata* to dietary protein is variable. Recently, Shuttleworth et al.⁴¹ tested several individual bacteria that had been previously isolated and characterized from the gut of wild *B. tryoni* larvae e.g., *Asaia* sp., *Enterobacter* sp., *Lactobacillus* sp., *Leuconostoc* sp. on the fitness parameters viz., adult survival in field cages, laboratory mate

selection of bacteria supplemented males by bacteria non-supplemented females, and laboratory locomotor activity of adult flies. They observed that none of the bacterial probiotic treatments significantly differed to the control for field survival, mate selection or locomotor activity of adult *B. tryoni*, while bacterial probiotics fed to the larval stage of *B. tryoni*. In the present study the mortality of bacteria enriched protein fed adult *Z. cucurbitae* and *B. dorsalis* was lower than those fed on bacteria enriched sugar diet. Comparatively higher mortality was recorded for *Erwinia* and *Serratia* spp. enriched sugar diets than only sugar fed diet. *Z. cucurbitae* and *B. dorsalis* fed on protein diet survive better than those fed on only sugar diet (Figs. 4-5). The inconsistency among the results of different investigations by different authors may be due to differences of the effects of different bacterial species or different strains of the same species on different insect hosts. The exact contributions and costs of bacteria to tephritids, and the conditions whereby they are evident, need to be worked out in further experiments.

CONCLUSION

The present study revealed the fact that the use of selected gut bacteria, *Erwinia* and *Serratia* spp. in protein and sugar diets did not exert significant influence on the ovariole number and egg/female/day of *Z. cucurbitae* and *B. dorsalis*. But, both *Z. cucurbitae* and *B. dorsalis* showed delayed ovariole development and egg production at 24 and 29 days on *Serratia* spp. enriched sugar diet, and 29-50 days while fed on *Erwinia* added sugar diet, respectively. Whereas no egg production was recorded for both the fly species fed on only sugar diet during this time indicated the influence of bacteria on fruit fly might be diet dependent as well as on physiological/developmental stage of flies. The response of *Z. cucurbitae* in terms of egg production to bacteria enriched sugar diets is sharper than *B. dorsalis*. Mean mortality of *Z. cucurbitae* and *B. dorsalis* fed on *Erwinia* and *Serratia* spp. enriched sugar diet was higher than bacteria enriched protein diets under laboratory condition. Protein fed flies survive longer than protein deprived i.e., only sugar fed fly. Almost similar results also observed in semi-field cage experiment. In future more beneficial gut microbial community could be exploited from wild and mass reared fruit flies to produce high quality sterile flies with enhanced fitness e.g., mating competitiveness, longevity etc., for field application of SIT. Moreover, studies related to approaches as comparative genomics and real-time PCR can be performed to understand the molecular study of gut bacterial community and their interactions with fruit flies.

ACKNOWLEDGEMENTS

The present study was part of International Atomic Energy Agency (IAEA) Co-ordinated Research Project (via IAEA CRP No. 17011/ R2 to Dr. Mahfuza Khan).

REFERENCES

1. Naqvi MH. "Management and quality assurance of fruits and vegetables for export needs for product to market approach". In: Islam MS and Khan SA (eds.). Proceedings of the First National Seminar on the Use of Irradiation for Quarantine Treatment of Fresh Fruits and Vegetables. Dhaka, Bangladesh; 2005; 4–24.
2. Knippling EF. Possibilities of insect control or eradication through the use of sexually sterile males. *J. Econ. Entomol.* 1955; 48:459–62.
3. Dillon RJ, Dillon VM. The gut bacteria of insects: nonpathogenic interactions. *Annu Rev Entomol.* 2004; 49:71–92.
4. Engel P, Moran NA. The gut microbiota of insects – diversity in structure and function. *FEMS Microbiol Rev.* 2013; 37:699–735.
5. Prabhakar, CS, Sood, P, Kanwar, SS, Sharma, PN, Kumar, A Mehta, PK. Isolation and characterization of gut bacteria of fruit fly, *Bactrocera tau* (Walker). *Phytopara.* 2013; 41:193-201.
6. Sood P, Nath A. Bacteria associated with *Bactrocera* sp. (Diptera: Tephritidae) – isolation and identification. *J. Pest Manage.* 2002; 10(1): 1-9.
7. Sood, P, Prabhakar, CS. Molecular diversity and antibiotic sensitivity of gut bacterial symbionts of fruit fly, *Bactrocera tau* Walker. *J. Biol. Control.* 2009; 23(3): 213-220.
8. Wang H, Jin L, Zhang, H, Comparison of the diversity of the bacterial communities in the intestinal tract of adult *Bactrocera dorsalis* from three different populations. *J. Appl. Microbiol.* 2011; 110: 1390–1401.
9. Jang, E B, Nishijima KA, Identification and attractancy of bacteria associated with *Dacus dorsalis* (Diptera: Tephritidae). *Environ. Entomol.* 1990; 19:1726–1731.
10. Lloyd AC, Drew RAI, Teakle DS, Hayward AC. Bacteria associated with some *Dacus* species (Diptera, Tephritidae) and their host fruit in Queensland. *Aust J Biol. Sci.* 1986;39:361–368.
11. Ben-Yosef M, Pasternak Z, Jurkevitch E, Yuval B. Symbiotic bacteria enable olive flies (*Bactrocera oleae*) to exploit intractable sources of nitrogen. *J. Evol. Biol.* 2014; 27:2695–705.

12. Ben-Yosef M, Pasternak Z, Jurkevitch E, Yuval B. Symbiotic bacteria enable olive fly larvae to overcome host defences. *R Soc Open Sci.* 2015; 2:150–70.
13. Douglas AE. The microbial dimension in insect nutritional ecology. *Funct. Ecol.* 2009; 23:38–47.
14. Ceja-Navarro JA, Vega FE, Karaoz U, Hao Z, Jenkins S, Lim HC, Kosina P, Infante F, Northen TR, Brodie EL. “Gut microbiota mediate caffeine detoxification in the primary insect pest of coffee” [online]. 2015 [cited 2015 July 14] Available from: <https://doi.org/10.1038/ncomms8618/6>: 7618.
15. Piper MDW, Blanc E, Leitao-Goncalves R, Yang MY, He XL, Linford NJ, Hoddinott MP, Hopfen C, Soultoukis GA, Niemeyer C, et al. A holidic medium for *Drosophila melanogaster*. *Nat Methods.* 2014; 11:100–105.
16. Akami M, Andongma AA, Zhengzhong C, Nan J, Khaeso K, Jurkevitch E, Niu C-Y, Yuval B. “Intestinal bacteria modulate the foraging behavior of the oriental fruit fly *Bactrocera dorsalis* (Diptera: Tephritidae)” [online]. 2019 [cited 2019 January 16] Available from: [https://doi.org/10.1371/journal.pone.0210109/14\(1\):](https://doi.org/10.1371/journal.pone.0210109/14(1):) e0210109.
17. Chang JY, Antonopoulos DA, Kalra A, Tonelli A, Khalife WT, Schmidt TM, Young VB. Decreased diversity of the fecal microbiome in recurrent *Clostridium difficile*—associated diarrhea. *J Infect Dis.* 2008; 197:435–8.
18. de-Vries, EJ, Jacobs G, Sabelis MW, Menken SBJ, Brecu-wer, JAJ. Diet dependent effects of gut bacteria on their insect host; the symbiosis of *Erwinia* sp. and western flower thrips. *British Biol. Sci.* 2004; 271: 2171-2178.
19. Ben-Ami E, Yuval B, Jurkevitch E. Manipulation of the microbiota of massreared Mediterranean fruit flies *Ceratitis capitata* (Diptera: Tephritidae) improves sterile male sexual performance. *ISME J.* 2010; 4:28–37.
20. Hamden H, Guerfali MM, Fadhl S, Saidi M, Chevrier C. Fitness improvement of mass-reared sterile males of *Ceratitis capitata* (Vienna 8 strain) (Diptera: Tephritidae) after gut enrichment with probiotics. *J Econ Entomol.* 2013;106: 641–7.
21. Augustinos AA, Kyritsis GA, Papadopoulos NT, Abd-Alla AMM, Cáceres C, Bourtzis K. “Exploitation of the medfly gut microbiota for the enhancement of sterile insect technique: use of *Enterobacter* sp. in larval diet-based probiotic applications” [online]. 2015 [cited 2015 September 1] Available from:10:e0136459. <https://doi.org/10.1371/journal.pone.0136459>.
22. Gavriel S, Jurkevitch E, Gazit Y, Yuval B. Bacterially enriched diet improves sexual performance of sterile male Mediterranean fruit flies. *J. Appl. Entomol.* 2011; 135:564–573.

23. Sacchetti P, Ghiardi B, Granchietti A, Stefanini FM, Belcari A. Development of probiotic diets for the olive fly: evaluation of their effects on fly longevity and fecundity. *Ann Appl. Biol.* 2014;164:138–150.
24. Niyazi N, Lauzon CR, Shelly TE. Effect of probiotic adult diets on fitness components of sterile male Mediterranean Fruit flies (Diptera: Tephritidae) under laboratory and field case conditions. *J. Econ. Entomol.* 2004; 97:1570-1580
25. Yao, M, Zhang PC, Xiaohong G, Dan W, Qinge J. Enhanced fitness of a *Bactrocera cucurbitae* genetic sexing strain based on the addition of gut-isolated probiotics (*Enterobacter spec.*) to the larval diet. *Entomol. Exper. Appl.* 2017; DOI: 10.1111/eea.12529.
26. Shi Z, Wang L, Zhang H. Low diversity bacterial community and the trapping activity of metabolites from cultivable bacteria species in the female reproductive system of the Oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae). *Inter. J. Mol. Sci.* 2012; 13: 6266–78.
27. Stathopoulou P, Asimakis, ED, Khan M, Caceres C, Bourtzis K, George T. Irradiation effect on the structure of bacterial communities associated with the oriental fruit fly, *Bactrocera dorsalis*. *Entomol. Exp. Appl.* 2019; DOI: 10.1111/eea.12770.
28. Pramanik MK, Mahin AA, Khan M, Miah A. B. Isolation and Identification of Mid-Gut Bacterial Community of *Bactrocera dorsalis* (Hendel) (Diptera:Tephritidae). *Res. J. Microbiol.* 2014; 9: 278–286.
29. Khan, M, Hossain M.A., Shakil A.K., Islam M.S. and C. L. Chang. Development of liquid larval diet with modified rearing system for *Bactrocera dorsalis* (Diptera: Tephritidae) for the application of sterile insect technique. *ARNP J. Agric. Biol. Sci.* 2011; 6: 52-57.
30. Khan M, Mahin AA, Pramanik MK, Akter H. Identification of Gut Bacterial Community and Their Effect on the Fecundity of Pumpkin Fly, *Bactrocera tau* (Walker). *J. Entomol.* 2014; 10: 3923.
31. Lauzon, CR. “Symbiotic relationships of Tephritids”. In: Bourtzis K and Miller, TA (eds.) *Insect symbiosis*. CRC, Boca Raton, FL; 2003; 115–129.
32. Drew, RAI, Courtice, AC, Teakle, DS. 1983. Bacteria is a source of food for adult fruit flies (Diptera: Tephritidae). *Oecol.* 1983; 60: 274-284.
33. Carey JR, Liedo P, Harshman L, Lin X, Muller HG, Partridge L, Wang JL. Food pulses increase longevity and induce cyclical egg production in Mediterranean fruit flies. *Funct. Ecol* 2000; 16: 313-325.

37. Meats, A, Leighton, SM. Protein consumption by mated, unmated, sterile and fertile adults of the Queensland fruit fly, *Bactrocera tryoni* and its relation to egg production. *Physiol. Entomol.* 2004; 29: 176-182.
38. Meats A, Streamer K, Gilchrist AS. Bacteria as food had no effect on fecundity during domestication of the fruit fly, *Bactrocera tryoni*. *J. Appl. Entomol.* 2009; 33: 633-639.
39. Halder P, Khan M, Mahin AA, Howlader AJ. Influence of antibiotics on the mortality and bacteria supplemented diets on the ovariole number of fruit fly, *Bactrocera tau* (Walker) (Diptera:Tephritidae). *Aca. J. Entomol.* 2014; 6(1): 27-32.
40. Ben-Yosef M, Behar A, Jurkevitch E, Yuval B. Bacteria–diet interactions affect longevity the medfly – *Ceratitis capitata*. *J. Appl. Entomol.* 2008; 132: 690- 694.
41. Ben-Yosef, M, Aharon Y, Jurkevitch E, Yuval B. Symbiotic bacteria affect olive fly fitness in a diet-dependent fashion. *Proc. Biol. Sc.* 2010; 277 (1687): 1545-1552.
42. Perez-Staples D, Harmer AMT, Collins SR, Taylor PW. Potential for pre-release diet supplements to increase the sexual performance and longevity of male Queensland fruit flies. *Agric. Forest Entomol.* 2008; 10: 255-262.
43. Jacome I, Aluja M, Liedo P, Nestel D. The influence of adult diet and age on lipid reserves in the tropical fruit fly *Anastrepha serpentina* (Diptera: Tephritidae). *J. Insect Physiol.* 1995; 41: 1079-1086.
44. Shuttleworth LA, Khan M-A-M, Collins D, Osborne T, Reynolds OL. “Wild bacterial probiotics fed to larvae of mass-reared Queensland fruit fly [*Bactrocera tryoni* (Froggatt)] do not impact long-term survival, mate selection or locomotor activity” [online]. 2019 [cited 2019 March 8] Available from:doi:10.1111/1744-7917.12670.