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Influence of Antibiotics on the Mortality and Bacteria Supplimented Diets on the Ovariole Number of Fruit Fly, *Bactrocera tau* (Walker) (Diptera: Tephritidae)

[3]Popi Halder, [1]Mahfuza Khan, [2]Abdullah-Al-Mahin and [3]A.J. Howlader

[1]Insect Biotechnology Division, Institute of Food and Radiation Biology,
Atomic Energy Research Establishment, Savar, GPO Box. No.3787, Dhaka-1000, Bangladesh
[2]Microbiology and Industrial Irradiation Division, Institute of Food and Radiation Biology,
Atomic Energy Research Establishment Savar, GPO Box. No.3787, Dhaka-1000, Bangladesh
[3]Department of Zoology, Faculty of Biological Sciences,
Jahangirnagar University Savar, Dhaka-1342, Bangladesh

Abstract: The pumpkin fly, *Bactrocera tau* (Walker) is considered as one of the most important pests of different fruits and vegetables world-wide. The fly usually is considered harbour a diverse community of bacteria in their digestive system which is known to play significant role on different fitness parameters. Experiments were conducted to determine the effect of antibiotics and bacteria supplemented protein and sugar diets on the mortality and ovariole numbers of adult *B. tau*. A cocktail of antibiotics and two exogenous bacterial species i.e., *Escherichia coli* and *Lactococcus lactis* was added with protein (casein:yeast extract:sugar:1:1:2) and sugar diets, separately. The suppression or elimination of gut bacteria using antibiotics showed decreased life span of nutritionally stressed i.e., sugar fed *B. tau* than those without antibiotics treatment. Adding antibiotics in protein diet did not exert any significant influence on adult longevity of male or female *B. tau.* No significant differences were recorded among ovariole number of *B. tau* fed on different bacteria added diets and only protein diet. Mean ovariole number was 45.38±1.55, 44.76±2.67 and 41.85±2.04 for *B. tau* fed on *E. coli* and *L. lactis* added protein diets and only protein diet, respectively. The results revealed the effect of bacteria in a diet dependent fashion in adult *B. tau.*

Key words: Pumpkin fly · Longevity · Adult diet · Ovary and Egg

INTRODUCTION

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. Usually these flies introduce their alimentary tract bacteria onto the host fruit surface with regurgitated fluid and subsequently into the host fruit during oviposition [1]. These symbiotes are known to have some useful functions in fruit flies like hydrolysis of proteins and detoxification of toxic substances ingested by host insect and may assist insect immunity [2, 3]. Removal of bacteria affects measurable physiological and behavioural parameters related to fly fitness [4]. Conversely, inculcation of bacteria in the diet contributes to a longer life span [5].

Ben-Yosef *et al*. [4] studied the effects of the gut microbiota on the reproductive success of medfly, *Ceratitis capitata* (Wiedemann) males and females by using antibiotics and reported that bacterial activity in the gut did not affect the fecundity of females. However, in the absence of bacteria, nutritionally stressed females laid their eggs sooner. Additionally, bacteria provided males with a significant mating advantage but only when they were not nutritionally restricted. The experiment described by Ben-Yosef *et al*. [6] also determine the effect of prolonged treatment with antibiotics on longevity of med flies that were either sugar fed or provided with a full diet. However, the bacteria associated with different tephritid fruit flies [7, 8] as well as various types of soils, plant materials and animal products [9-12] have been studied by several authors but the true biological significance of their relationship yet to elucidated properly.

Corresponding Author: Mahfuza Khan, Insect Biotechnology Division, Institute of Food and Radiation Biology, Bangladesh Atomic Energy Commission.

The pumpkin fly, *Bactrocera tau* (Walker) under the family Tephritidae is a polyphagous species has natural distribution, host range and population growth capacity similar to those of the melon fly, *B. cucurbitae* (Coquillet), one of the most destructive fruit flies in the world [13]. Despite its economic importantce comparatively less work was done on *B. tau* in terms of cytogenetical, nutritional, or microbial information which already unveiled for other fruit fly species [14-15].

The present experiment was therefore, conducted to understand the factors that could affect longevity of adult *B. tau* such as a toxic property of the antibiotics while incorporated with sugar and protein diet. The present study also design to examine whether addition of exogenous bacterial species in adult diet can enhance the production of ovariole number of *B. tau*.

MATERIALS AND METHODS

Fruit Fly Rearing: Adult *B. tau* culture originated in 2010 from infested Luffa (*Luffacy lindsica* (L.)) collected from Atomic Energy Research Establishment (AERE) campus. Rearing of *B. tau* was maintained in the laboratory of Insect Biotechnology Division (IBD), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh for more than 30 generations using natural hosts. About 3,000 adult flies were maintained in steel framed cages (76.2 x 66 x 76.2 cm) 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 socked with cotton ball. The temperature and the relative humidity of the rearing room maintained at 27±1°C 75±5% and 14h light:10h dark cycle.

Effect of Antibiotics on the Mortality of Adult Male and Female *B. tau*: In the present experiment cocktail of different antibiotics was used to suppress the gut bacterial community of *B. tau*. Antibiotics *viz.*, flucloxacillin (500mg) and fimoxyl (500mg) was purchased from the pharmacy of local market (Dhaka, Bangladesh). Sugar solution (20%) containing 10 µg/ml flucloxacillin (same group of ciprofloxacin) and 200 µg/ml fimoxyl (instead of piperacillin) was provided. The antibiotic cocktail (10 µg/ml ciprofloxacin and 200 µg/ml piperacillin) was reported to effectively clear the gut of bacteria of *C. capitata* [4]. Experimental flies were obtained as pupae from the stock rearing of *B. tau* from the laboratory of IBD, IFRB, Savar, Dhaka. 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 20% sugar solution was provided. This period served to filter out weak individuals who did not survive eclosion and allowed the intestinal microbiota to establish in the gut [16]. Subsequently, four groups (either males or females) of 50 individuals were confined in small rearing cages and each was subjected to one of the following four dietary regimes: i. sugar (S), ii. sugar and antibiotics (SA), iii. protein diet (casin:yeast extarc:sugar,1:1:2) (P) and iv. protein diet and antibiotics (PA). Diets were presented to the flies as solutions socked in small cotton ball and placed on small glass watch. Treatment period lasted for a maximum of 30 days. During this time the daily mortality in each cage was recorded, dead flies were removed from the cages and diet solutions were replaced every 24 h. All experiments were conducted under controlled laboratory condition. Three replicates were run, for both males and females, each using flies of different cohorts.

Influence of Bacteria Supplemented Adult Diets on the Ovariole Number of *B. tau*

Collection of Bacterial Culture: The isolates of bacteria, *Escherichia coli* and *Lactococcus lactis* were obtained from the laboratory of Microbiology and Industrial Irradiation Division, IFRB, AERE. *E. coli* is a gram-negative, rod-shaped bacterium. *L.lactis* is a gram-positive bacterium used extensively in the production of butter, milk and cheese.

Bacterial Culture for Feeding Test of Adult *B. tau*: *E. coli* was inoculated in 100ml nutrient broth in 250ml Erlenmeyer flask and incubated overnight at 37°C. *L. lactis* was inoculated overnight at 30°C. Cell cultures were then transferred and centrifuged at 4000g for 15 minutes and washed twice with 0.01M sodium phosphate buffer. Finally the cell pellets were suspended with 0.9% Nacl solution and aseptically transferred in test tubes. The cell suspensions were then mixed with protein diet for feeding of newly emerged *B. tau*.

Determination of Ovariole Number of *B. tau*: In the present study newly emerged *B. tau* were given access to six diet treatments *viz*: (i) Only protein diet (yeast extract:casin:sugar, 1:1:2); (ii) Isolates of *E. coli* and protein diet (3.8×10^{-6} CFU/gm); (iii) Isolates of *L. lactis* and protein diet (3.8×10^{-6} CFU/gm); (iv) Only sugar diet (20% sugar solution); (v) Isolates of *E. coli* and sugar diet (3.8×10^{-6} CFU/ml); (vi) Isolates of *L. lactis* and sugar diet (3.8×10^{-6} CFU/ml).

Newly emerged 30 males and 30 females adult *B. tau* were housed in small rearing cages and supplied with different bacteria added protein diets. Three replicates were maintained for each diet treatment. Control batch without addition of bacteria in protein diet was also maintained. On day 14 of adult emergence females were collected and kept in freeze for 10 minutes. Ovary of flies was dissected under stereo microscope and total number of eggs per ovary were counted and recorded. Ovariole development of only sugar fed and bacteria added sugar fed 14 days old adult *B. tau* was also determined.

Usually, in poly-phagous tephritid fruit flies the paired ovaries consist of about 30-40 polytrophic ovarioles [17]. In mature flies, there is normally only one mature oocyte (egg with shell) per ovariole that is ready for laying, although there may be oocytes in various earlier stag.

Statistical Analysis: Data for adult longevity of *B. tau* against protein and sugar diets with and without antibiotics treatment and the ovariole number of *B. tau* fed on different bacteria treated diets were analyzed using Analysis of Variance (ANOVA) (Statistical software Minitab-version-16).

RESULTS AND DISCUSSION

The mortality rates of adult *B. tau* was compared between antibiotic-treated and non-treated flies in both nutritionally stressed (fed on sugar only) and insects provided with protein diets (casein, yeast extract and sugar) (Fig. 1 and 2). The experimental result showed that feeding on antibiotics significantly reduced the longevity of both sugar fed male and female *B. tau.* Almost 100% percentage mortality was recorded for male and female *B. tau* within 30 days when fed on antibiotics added sugar solution. Whereas 88% male and 67% female flies were found dead when fed only sugar solution within the experimental time (i.e., 30 days). In this case only sugar fed male mortality was higher than female *B. tau* (Fig. 1).

Adding antibiotics on the protein diet on the other hand reduced the percentage mortality of both male (26%) and female (35%) *B. tau* compared to that of only protein fed male (32%) and female (48.73%) flies, respectively. More percentage mortality of female than male was recorded in only protein fed *B. tau* (Fig. 2). The present experimental result revealed that while antibiotics were added in sugar diet percentage mortality of both the male and female *B. tau* increases. In contrast feeding antibiotics added protein diet seems to decrease the percentage mortality of both the sexes of *B. tau*.

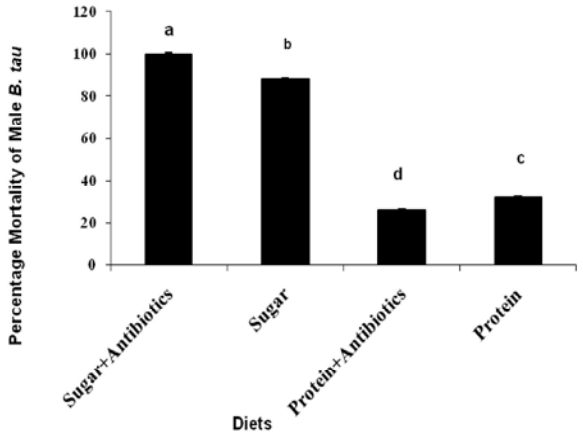

Fig. 1: Percentage (%) mortality of adult male *B. tau* over 30 days fed on sugar+antibiotics, only sugar, protein+antibiotics and only protein diets, respectively.

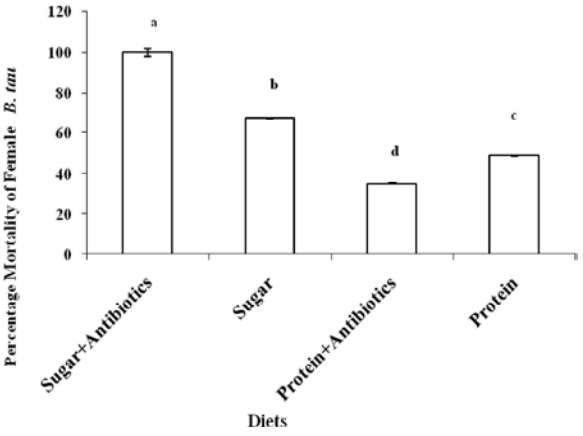

Fig. 2: Percentage (%) mortality of adult female *B. tau* over 30 days fed on sugar+antibiotics, only sugar, protein+antibiotics and only protein diets, respectively.

No significant (P>0.05) differences were recorded among ovariole number of *B. tau* fed on bacteria, *E. cloi* and *L. lactis* added protein diets and only protein diet. The mean number of ovariole of left and right ovary of *B. tau* fed on above mentioned diets were shown in (Fig. 3). Highest and lowest mean (±se) ovariole number recoded as (45.38±1.55) and (41.85±2.04) for *E. coli* added protein diet and only protein diet fed *B. tau,* respectively. Individually ovariole number ranged from 15 to 30 per ovary depending on diet treatments of adult *B. tau.* Mean ovariole number ranged from 20±7.1 to 22±7.6 per ovary of adult *B. tau* fed on different adult diets. No ovariole development was recorded for only sugar and bacteria added sugar fed 14 days old *B. tau*.

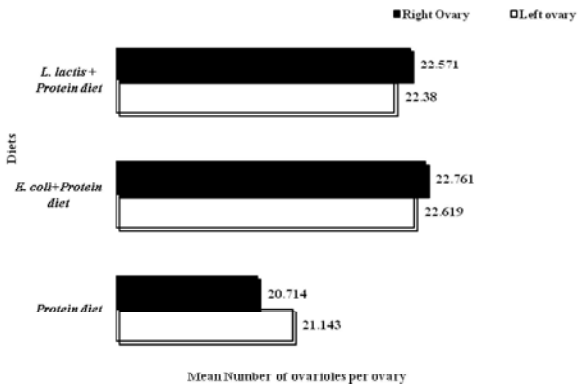

Fig. 3: Mean ovariole number of left and right ovary of fourteen days old adult *B. tau* fed on *E.coli*, *L. lactis* added protein diets and only protein diet, respectively.

Insects have been recorded to gain important benefits from their bacterial companions e.g. nutrient provisioning, enhanced protection against pathogens, synthesis of pheromonal components [18, 19]. The present experimental findings on the effect of antibiotics on the longevity of *B. tau* was in contrast with the observation of Ben-Yosef *et al.* [4] noted the increased life expectancy of *C. capitata* on antibiotic added sugar diet than only sugar diet. The same authors also reported adding antibiotics to protein diet did not affect the longevity of *C. capitata* which is also different from present longevity study of *B. tau* on antibiotics added protein diet (Fig. 1 and 2).

De Vries *et al*. [20] noted the gut microbiota may have alternated between mutualism/commensalism and parasitism in response to changes in their host's diet. Longevity however, could also greatly depend on the diet, which in turn may have determined the ability of the flies to resist antagonistic bacterial activity in the gut. Insects regularly confront and neutralize pathogens through their immune system. Dietary protein in particular, may be essential for maintaining a functional immune response [21, 22]. However, starvation or dietary restriction usually result in a reduced immunity and render the insect more susceptible to attack [23]. Therefore, the exact contributions and costs of bacteria to tephritids and the conditions whereby they are evident, need to explore further [4].

In adult females of many tephridid species proteinaceous component is required in the diet for sexual maturation and oogenesis [24-26]. Oogenesis is known to reduced or prevented by dietary restriction. Dietary bacteria could serve as source of protein [27] and some of them have been shown to fix nitrogen [28]. Drew *et al*. [27] showed that for adult female Queensland fruit fly, *Bactrocera tryoni* (Froggatt), *Klebsiella oxytoca* could serve as proteinaceous food and the fecundity of flies fed such food was equal (if not greater) than that of flies fed the usual proteinaceous diet of yeast autolysate. In contrast clearing the gut bacteria of fertile *C. capitata* females had no effect on fecundity [4]. In the present study *B. tau* fed on *E. coli*, *L. lactis* added protein diet and only protein diet did not show significant influence of bacteria on mean ovariole number.The present findings is partially in agreement with the findings of Meats *et al*. [29] who reported 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. While considering flies from several generations, similarly there was no evidence that bacterial supplementation to a diet that include a paste of sucrose and yeast autoly-yeast was more beneficial than when the paste was the sole source of proteinaceous food. The inconsistency among the results of different investigations may be due to differences between the effects of different bacterial species or different strains of the same species to different fruit flies. The results of the present investigation may differ from that found by Drew *et al*. [27] and others for the above mentioned reasons. Moreover, bacteria species used in the present study were exogenous and did not belong to gut bacterial community of *B. tau*.

Therefore, future research should be directed towards the identification of gut bacterial community of *B. tau* and use of beneficial bacterial species on different fitness parameters i.e., longevity, fecundity and mating competitiveness of *B. tau* in both the laboratory and semi-field cage experiment. Molecular diversity of gut bacteria of *B. tau* and related fruit fly species may also be the area to investigate further.

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