



Successful rearing of *Bactrocera dorsalis* on a semi-solid artificial diet

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ABSTRACT

The fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) is an economically important polyphagous pest and has been adjudged as one of the five most devastating fruit flies throughout the world. These flies are highly polyphagous, multivoltine, fertile, and mobile which makes it very difficult to manage these insect-pests. Finding a simple, cheap, and appropriate rearing approach of *B. dorsalis* is an urgent need to support research activities for management of this pest. This investigation is meant to evaluate various artificial diets and to find out the most suitable artificial diet for mass rearing of this pest. In the present studies, five different artificial diets were used for the rearing of *B. dorsalis* at Punjab Agricultural University, Ludhiana during 2018–19. A semi-solid artificial diet consisting of 1000 ml water, 40 g dry yeast, 120 g sucrose, 1 g sorbic acid, 6.6 g ascorbic acid, 10 g agar, 10 g soya peptone, 0.1 g ampicillin, and 0.03 g tetracycline was considered most suitable for successful rearing of *B. dorsalis*. The biological parameters of *B. dorsalis* were used to validate the diet quality and rearing procedure. This diet supported the development of *B. dorsalis* efficiently and was used further for evaluation of RNA interference (RNAi) studies. This artificial diet and suggested rearing procedure will help to maintain culture of *B. dorsalis* and further help to study biology and management of other insect-pests species as well.

Keywords: Biological attributes, Diet development, Pest management, Rearing in laboratory, RNA interference

The fruit fly, *Bactrocera dorsalis* (Hendel) is the member of order Diptera and belongs to family Tephritidae. Around 50-60% annual yield losses were reported due to infestation of fruit flies on different hosts in India (Narayanan and Batra 1960). *Bactrocera dorsalis* has adjudged to be one of the five most devastating and invasive fruit flies in the world that infests varied range of vegetables and fruits (Singh and Sharma 2013a, Singh and Sharma 2013b, Singh 2020). It is a serious pest of a varied range of fruit crops in the Indian subcontinent (Kamala Jayanthi *et al.* 2011).

In addition to direct damage to fruits, it could also confine the free trade and export of fresh horticultural products to huge remunerative markets (Ole-MoiYoi and Lux 2004). Management of these fruit flies is a challenging task as they are extremely polyphagous, multivoltine, adults have high mobility and fecundity, and all the life stages are unexposed except adults (Sharma *et al.* 2011, Singh and Kaur 2016, Singh 2020). The possible enhancement in the rearing techniques of *B. dorsalis* is a pre-requisite for

an effective study of the genetics or management of this pest through novel biotechnological strategies like RNA interference (RNAi). Accessibility of an efficient laboratory rearing technique is required for the advancement of pest control approaches for fruit flies. Mass rearing of fruit flies and their adaptation in the laboratory can be expensive and challenging process (Parker 2005). The main reason behind the lack of investigations on tephritids is due to complications related to the culturing of these insect-pests (Kamala Jayanthi and Verghese 2002). The understanding of the biology of target species is vital for the accomplishment of mass rearing (Claderaj *et al.* 2014). Any variation in the quality of diet in the course of various development stages can have a significant effect on their biology (Nestel *et al.* 2016). The objective of our study is to evaluate various artificial diets and to find out the most suitable artificial diet for mass rearing of *B. dorsalis*.

MATERIALS AND METHODS

Punjab lies in the northwestern India with a total area of 50362 square km (19445 square miles). The geography and subtropical latitudinal location of Punjab has led to large variations in temperature from month to month. The maximum temperature usually occur in mid-May and June followed by rains in July-August. This investigation was conducted during 2018–19 at Punjab Agricultural University, Ludhiana (30.9010° N, 75.8071° E), Punjab, India.

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Collection of Bactrocera dorsalis: Guava fruits infested with *B. dorsalis* were collected from the orchards/Fruit Research Farm, Punjab Agricultural University, Ludhiana, Punjab, India. The experiment was carried out under the regimes of 32.5 ± 1.6 °C at $80.5 \pm 3.8\%$ RH with the 14 h light/10 h dark period cycle. Two hundred pairs of *B. dorsalis* as a stock culture were sexed and released into cages containing guava fruits for oviposition.

Artificial diets: Composition and preparation: In the present study, five artificial diets with different compositions were used for the rearing of fruit fly, *B. dorsalis* (Table 1). For Diet A, yeast extract was added to distilled water followed by the addition of sucrose and honey. In Diet B, yeast extract was added to distilled water followed by addition of powdered sugar and honey. In Diet C, addition of agar to boiling water was followed by brisk stirring with plastic rod. Then, yeast extract along with powdered sugar and honey was added after cooling down of media. For Diet D, agar was added to the boiling water followed by sucrose and soya peptone. Then, yeast extract was added after cooling down of media. In Diet E, distilled water was added into a pan placed on the hot plate and heated to the boiling point. Agar was added into the solution followed by sucrose and soya peptone. Media was then kept as such to cool down followed by addition of yeast extract. Sorbic acid along with ascorbic acid was then added. Antibiotics (Ampicillin and Tetracycline) were added to distilled water and then mixed with the cooled medium.

Rearing of B. dorsalis on artificial diets: Maggots were collected from the *B. dorsalis* infested guava fruits and released on the petri dishes (9 cm diameter) containing 40 g of an artificial diet. Five artificial diets were used for the rearing of *B. dorsalis*. There were five replications for each of the artificial diet. A study on artificial diet aspect was undertaken on maggot to adult stage transitory of *B. dorsalis*. The guava fruits infested with *B. dorsalis* were

placed in glass jars at room temperature. After two days, the maggots were taken from infested fruits and released on an artificial diet in plastic vials. The vials were covered with the white muslin cloth to avoid the escape of the maggots. After feeding for a few days, maggots developed to the pupal stage. The pupae were removed from vials and placed in cages. The artificial diet in petri dishes along with non-infested guava fruits was also placed in cages. After a few days, adults emerged and started feeding on artificial diet in cages. The sexually mature mated females of *B. dorsalis* successfully oviposit on these fruits.

Estimating biological parameters: Biological parameters were estimated during the rearing of *B. dorsalis* on the different semi-solid artificial diets. All quality parameters including survival were estimated and performed under laboratory conditions, i.e. 32.5 ± 1.6 °C at $80.5 \pm 3.8\%$ RH with 14 h light/10 h dark period.

Statistical analysis: Individuals treated and all biological parameters of treated individuals were subjected to a completely randomized block (CRD) analysis by computer software CPCS1 (Cheema and Singh 1990). Mean \pm SE was calculated for the particular parameters and overall survival was calculated as per cent age. Critical difference was calculated for all the parameters at P=0.05.

RESULTS AND DISCUSSION

Five artificial diets were used for rearing and after evaluation; selection of the most suitable diet was done. Results (Table 2) for selection of most suitable artificial diet after conducting statistical analysis are presented. The diet found to be most suitable was based on overall development and survival rate for the rearing of fruit fly, *B. dorsalis* (Fig 1). The results indicated that the duration of maggot stage was less with the highest pupae recovery. The pupal period on Diet E was least combined with maximum number of adult emerged. The maximum sex ratio was recorded in Diet E with the maximum per cent of survival rate. The present results show that Diet E is most suitable for the rearing of *B. dorsalis* as compared to other diets (Fig 1).

Diet E was considered most suitable for rearing purpose and the probable reason behind this could be the presence of yeast, sucrose, ascorbic acid, and antibiotics. Proteins are indispensable for cellular growth of life. The yeast-fermented products are the main ingredient of nutrition/protein in the diet used for mass-rearing of larval and adult stages of fruit flies (Rohlf and Hoffmeister 2005). Yeast has a direct effect on the pivotal fertility and fecundity of the adult fruit flies. Sucrose was reported as the main source of energy in herbivorous insects and it also acts as the most important nourishing stimulant particularly during early larval development. Yeast and sucrose favor development of an insect, hence, they acts as vital nourishing constituents in diets used for the mass rearing of fruit flies (Moadeli *et al.* 2018). Yeast : sucrose (Y:S) mixtures are fundamental ingredients for the mass-rearing of fruit fly, *B. dorsalis*. It was found that comparatively high Y : S ratios diets for feeding of fruit flies, from 3:1 to 100:0, had significantly

Table 1 Composition of various artificial diets used for rearing of fruit fly, *B. dorsalis*

Ingredient	Artificial diets				
	Diet A	Diet B	Diet C	Diet D	Diet E
Distilled water	250 ml	250 ml	500 ml	1000 ml	1000 ml
Yeast extract	30 g	25 g	25 g	40 g	40 g
Sucrose	80 g	-	-	120 g	120 g
Sugar	-	75 g	50 g	-	-
Honey	30 ml	25 ml	25 ml	-	-
Agar	-	-	5 g	10 g	10 g
Soya peptone	-	-	-	10 g	10 g
Sorbic acid	-	-	-	1 g	1 g
Ascorbic acid	-	-	-	6.6 g	6.6 g
Ampicillin	-	-	-	-	0.1 g
Tetracycline	-	-	-	-	0.03 g

-, No value available.

Table 2 Survival and development of fruit fly, *B. dorsalis* on different artificial diets

Treatment	Number of maggots released (n)	Duration of maggot stage (days) (Mean±SE)*	Number of pupae developed (n)	Duration of pupal stage (days) (Mean±SE)*	Number of adults emerged (n)	Sex ratio (♂ : ♀)*	Adult longevity (days) (Mean±SE)*	Survival (%)*
Diet A	100	15 ±0.81	60	13±0.38	40	0.74:1	3±0.38	40 (39.14)
Diet B	100	15 ±0.58	34	14±0.51	23	0.77:1	4 ±0.51	23 (28.50)
Diet C	100	14±1.03	52	12±0.45	44	0.84:1	5±0.84	44 (41.49)
Diet D	100	12±0.58	49	10 ±0.25	47	0.88:1	6±0.58	47 (43.13)
Diet E	100	11±0.58	75	10±0.38	64	0.94:1	7±0.58	64 (54.96)
CD (P=0.05)		2.18	4.0	1.18	4.64		1.76	2.02

*Mean of 5 replications; Figures in parentheses are arc sine $\sqrt{\text{percentage}}$ transformed values. Significant at P=0.05.

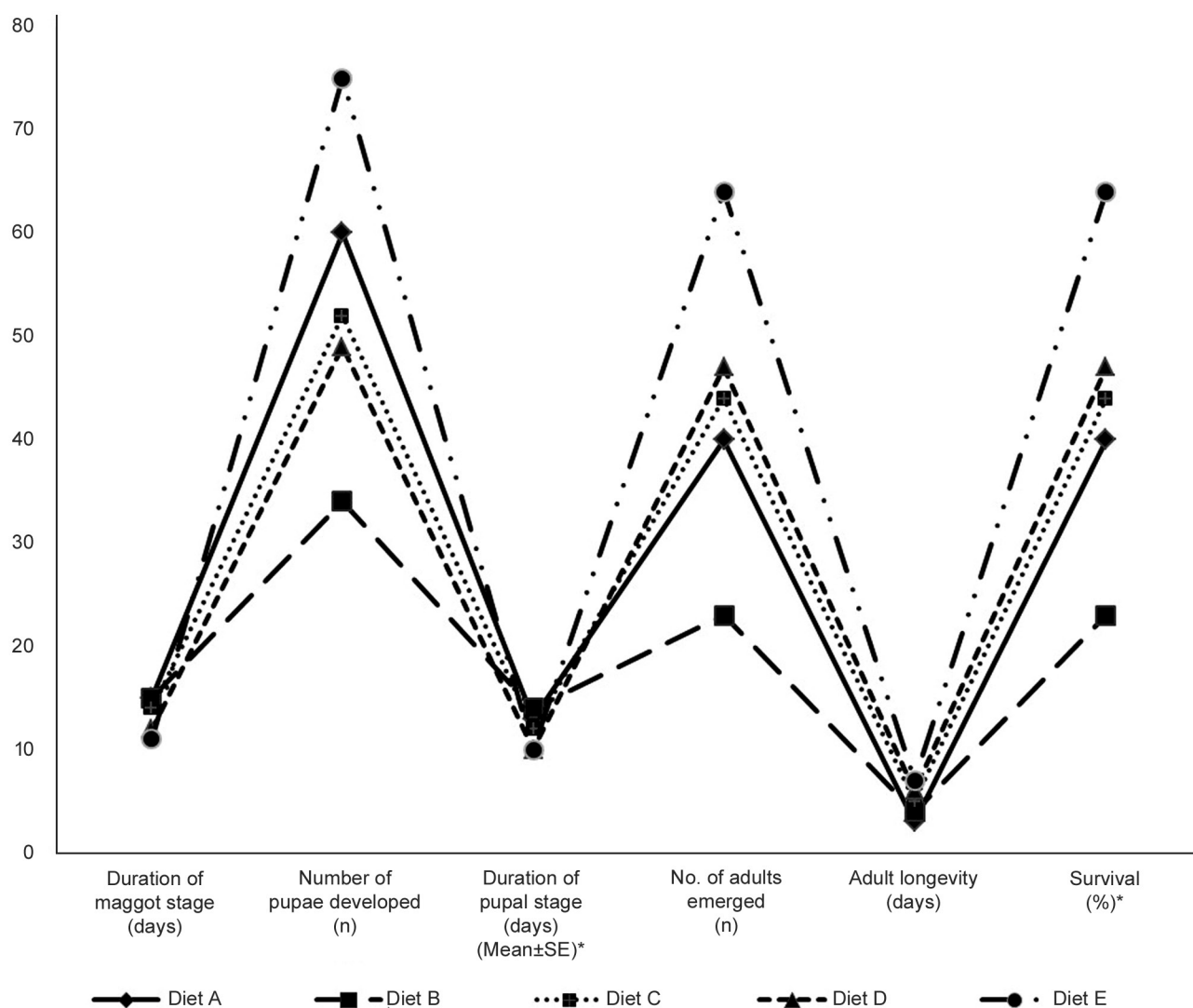


Fig 1 Survival and development of fruit fly, *B. dorsalis* on different artificial diets.

reduced production of egg. It was also reported that flies could not stay alive and even died before oviposition at some levels of relatively high Y : S ratios diets. Presence of the excess of protein in diets might be toxic and influence metabolic and physiological processes (Piper and Partridge 2007). The adult diets comprising of 1:1 and 1:3 Y:S

ratios resulted in maximum production of eggs and the development of larger ovaries of *B. dorsalis* (Hou *et al.* 2020). An agar based gel diet for larval stage has overcome some limitations of liquid and solid diets of *B. dorsalis*, and permitted higher rate of pupal production, pupal weight, emergence of adults, and short developmental period than

the both liquid and solid diet, and act as a potential media for a mass-rearing of *B. dorsalis* at large scale. So, agar-based diet plays an important role in successful rearing of *B. dorsalis* (Hou *et al.* 2020).

Ascorbic acid was incorporated in almost all the artificial diets used for rearing of insects due to its antioxidant properties. Presence of ascorbic acid in artificial diets might have resulted in highest rate of pupation and antibiotics suppressed microbial infections. The results of an investigation demonstrated that sucrose, brewer's yeast along with an ascorbic acid provide an adequate nutrition for normal growth of insect, survival, and sustained completion of multiple life cycles in insects (Wang *et al.* 2013). Wang and his co-workers (2014) also supported that incorporation of different antimicrobial compounds was a breakthrough in the artificial diet. The addition of antimicrobial compounds in the artificial diet of insects efficiently prohibited contamination by insecticidal pathogens for the period of 8-10 days. A study confirmed the readily suppression of the bacteria with the use of an appropriate amount of antimicrobial compounds. The incorporation of antimicrobial compounds is essential to inhibit or suppress the growth of microbes in artificial diets. Also, good sanitary conditions in the laboratory and sterilization of appliances and diets are necessary to avoid microbial infection. Due to the presence of these vital nutrients in an appropriate amount accompanied by antibiotics, diet E efficiently supported successful rearing of *B. dorsalis*.

However, there is an urgent need to further investigate correlation among these biological parameters of *B. dorsalis*. This study provides a proof of concept of feasibility to silence two potential genes by feeding bacteria expressing dsRNA based on this diet in all developmental stages of *B. dorsalis* to step further to perform RNAi based pest control (Mohanpuria *et al.* 2021). For this purpose; molecular, biochemistry and entomological tools can be used in combination which will provide a new outline for investigations on management of *B. dorsalis*. Thorough knowledge of physiology and biology of this pest is of utmost importance for these kinds of research studies.

The objective of this research was to determine most suitable artificial diet that resulted in enhanced larval performance and survival for successful mass rearing of *B. dorsalis*. A semi-solid artificial diet consisting of 1000 ml water, 40 g dry yeast, 120 g sucrose, 1 g sorbic acid, 6.6 g ascorbic acid, 10 g agar, 10 g soya peptone, 0.1 g ampicillin, and 0.03 g tetracycline was found to be most suitable diet for rearing of *B. dorsalis*. This artificial diet and suggested rearing technology will help to maintain culture of *B. dorsalis* in laboratories and further help to study biology. Though, this research was conducted in the laboratory at a small scale, we believe that the present data will provide significant source of reference information for successful rearing of *B. dorsalis* at large-scale. This technique can be utilized as an expedient technology for rearing of fruit fly, *B. dorsalis* for novel biotechnological and molecular approaches.

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