

Effect of Dietary Yeast on the Growth and Performance of *Drosophila melanogaster*

Raji Sukumar*, Athira M. Sarath, Ruchita Aggarwal, Deepika U

Department of Zoology and Genetics, The Oxford College of Science, Bengaluru, India

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ABSTRACT: Yeast plays a vital role in the development of *Drosophila melanogaster*. It acts as a nutrition source and the variation of its quality and quantity can affect the behavior of *Drosophila melanogaster*. The yeast species provide the nutrients that cannot be prepared by the *D. melanogaster*. Here we tested the effect of yeast in the diet of *D. melanogaster* larvae by checking the larval weight, size, developmental time and by the larval assays. Our data indicates that yeast plays a major role in the development and performance of *Drosophila*.

INTRODUCTION

Drosophila melanogaster, used as the animal model system in biological studies. It depends on the yeast that grows in the medium for survival.¹ Larvae and adult *Drosophila* species feed on the food sources containing yeast. The breeding cycle of *Drosophila* takes place on decayed and fermented fruits and vegetables.² *Drosophila* use yeast that grown on these fermented food for the survival because they are auxotrophic for sterol, which is required in order to complete its life cycle.³ Sterol is essential for maintain the permeability and fluidity of the cell membrane and also serve as a precursor for the steroid hormone biosynthesis.⁴

Effect of yeast on the growth of *Drosophila melanogaster* was examined by using *Sacharomyces cerevisiae*, heat dried yeast, inactivated yeast and *Candida albicans*. The variation in the duration of life cycle, difference in the size and weight of larvae when compared with the control and the third instar larvae were subjected to crawling assay, negative geotaxis and protein and lipid assay in order to find the role of these dietary yeast.⁵

*rajishibu12@gmail.com

MATERIALS AND METHODS

Drosophila melanogaster egg collection

D. melanogaster strains were cultured in 50 ml glass vials containing semolina, palm sugar and yeast and maintained at 25. Young emerging flies were separated from the bottle and allowed to mate. After 2-3 days adults were transferred to new vials for egg laying. Females produce the greatest number of eggs in the late afternoon and evening. The media along with eggs were then dipped in 20ml of 29% sucrose solution. The eggs were gently washed in to the sucrose solution with a small brush and poured into a 100ml beaker (without media). After a few minutes all eggs floated onto the surface and using a small pointed brush it was transferred to respective media.⁶

Yeast strain and culture

Saccharomyces cerevisiae culture maintained in the Department of Zoology and Genetics and *Candida albicans* maintained in Department of Microbiology of The Oxford College of Science was used for the studies. Different types of media was prepared with yeast slurry, powdered dry yeast, inactivated yeast and *Candida* sp. respectively and one plain media without adding yeast.

LARVAL SIZE AND WEIGHT DETERMINATION

The third instar larvae from all the five different vials were collected for determining its size. The size of the larvae was measured under a light microscope using eyepiece in such a way that the graduations sketched on the ocular is visible when an observation is made using the microscope. A stage micrometer was used to calibrate the ocular micrometer. A stage micrometer is simply a microscope slide with a scale etched on the surface. The stage micrometer was placed on the stage of a microscope and the graduations were focused using low power objectives. The larvae being observed was positioned in such a way that the ocular micrometer was able to measure length of the larvae in arbitrary components. The difference in the size of larvae with different yeast statuses were noted with respect to the control.

The third instar larvae from each vial were collected to measure its wet weight on a precision balance. The weight of the larvae was noted and compared with the control.

LARVAL GRID ASSAY

The third instar larvae from each of the five different media was collected and washed with de-ionized water. The individual larvae were then transferred into a 15cm petri dish (containing 2% agarose) over graph paper with a 0.2cm grid. The number of grid lines crossed by the larvae in 1 minute was counted. The experiment was repeated with all the larvae from different and compared with the control⁷

NEGATIVE GEOTAXIS

Drosophilamelanogaster are negatively geotactic, i.e. flies move opposite to the earth's gravitational force when disturbed⁸. For the negative geotaxis 4-5 flies from each vial were transferred into a climbing apparatus. The climbing apparatus was prepared by taking a measuring cylinder calibrated with a vertical distance of 15cm

from the bottom. The mouth of the cylinder was properly closed using aluminium foil. The flies from each different media is transferred into this cylinder without anesthetizing. The flies were allowed to acclimatize with the new environment for 1 minute before the experiment was started. After 1 minute, the flies were gently tapped down to the bottom of the cylinder and the number of flies that climbed above the 15cm mark in 1 minute was noted. The experiment was repeated with flies from all the different media and compared with the control.

RESULTS AND DISCUSSION

EFFECT OF YEAST ON DEVELOPMENTAL TIME AND SIZE

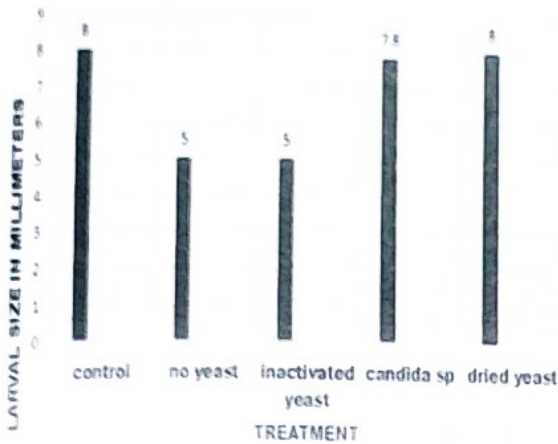
There was a significant effect of diet on the developmental time of *Drosophilamelanogaster*. Flies raised on cream of wheat agar media took 218.5 hours for the development(control). The flies raised on media containing inactivated yeast took 260.2 hours, those raised on media containing dried yeast took 222 hours and those raised on media without yeast took 294 hours and the larvae were very small when compared to the control (Table 1). There was no significant difference in the developmental time of flies raised in media containing candida sp.

Table 1

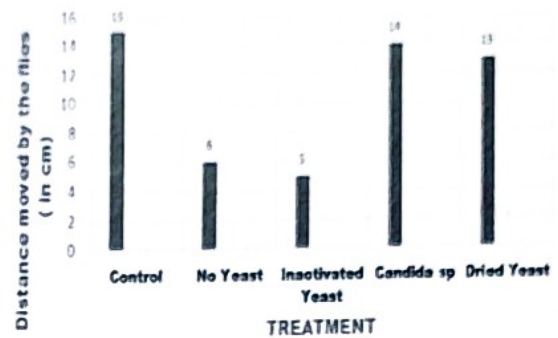
S.no	Hours of development
Control	218.5
Dry Yeast	222
Candida sps	220
With out Yeast	294
Inactivates yeast	260.2

Larvae grown on control, dry yeast and candida inoculated showed no significant difference in the size, whereas the size of the larvae was drastically reduced in the case of media without yeast and inactivated yeast.

EFFECT OF YEAST ON SIZE OF LARVAE



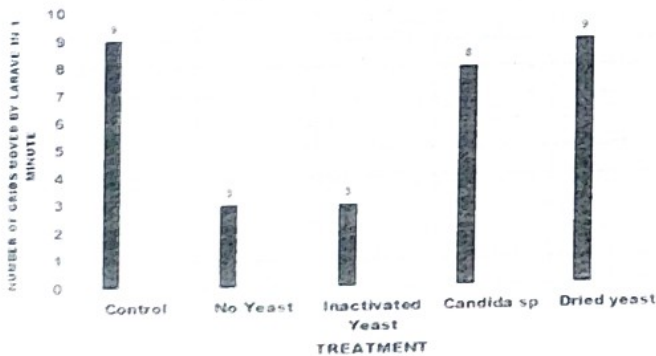
NEGATIVE GEOTAXIS ASSAY



LARVAL GRID ASSAY

Result of this assay showed that there is significant difference in their crawling ability between control and the test larvae. Among the test larvae, the one grown in dried yeast media showed maximum activity and those grown in media without yeast and media containing inactivated yeast showed minimum activity.

LARVAL GRID ASSAY



NEGATIVE GEOTAXIS ASSAY

There was a significant decline in the physical activity of the flies which was cultured in different yeast containing media. The flies grown in media containing inactivated yeast showed minimum physical activity (5cm) and that grown in *Candida* sp showed maximum activity (14 cm).

DISCUSSION

The results of the current study emphasized on the importance of dietary yeast to *Drosophila melanogaster* in the larval development and other physical and physiological activities.⁹ The larvae grown on media without yeast showed a significant decrease in the weight. In this study the role of yeast on the development of larvae was checked by performing larval grid assay wherein it was clearly seen that the larvae grown in the presence of yeast showed the maximum activity (Simmons, H. Fred. The activeness or locomotory activity was checked by conducting negative geotaxis wherein it was seen that the flies grown in cream of wheat agar media containing candida species move 14cm in the assay¹⁰. The suitability of single yeast species for the growth of *Drosophila* showed a positive effect on the larval fitness traits.¹¹ There was a significant difference in all the assays conducted on the larvae grown on media without yeast and the media with inactivated yeast when compared with larvae grown on media with *Saccharomyces cerevisiae* and *Candida* species¹². Based on the results of our study, further detailed research is needed to understand the interaction of yeast and *Drosophila*.

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REFERENCES

1. Dermerec. (1950). "Biology of Drosophila". New York: John Wiley and Sons, Inc.
2. Begon. M. (1982). "Yeast and Drosophila". The Genetics and Biology of Drosophila (ed. by M Ashburner, HL Carson & JN Thompson Jr). pp.345-384. Academic Press, London, UK.
3. Anagnostou, Christiana, Dorsch, Monika, Rohlf, Marko. (2010). "Influence of dietary yeast on *Drosophila melanogaster* life history traits". DOI: 10.1111/J.1570-7458.2010.
4. Gantner P.F. (2006). "Yeast and invertebrate associations". The Yeast Handbook: Biodiversity and Ecophysiology of Yeasts. (ed. by CARosa & GPeter). pp.303-370. Springer, Berlin, Germany.
5. Vega, F. E. Dowd, P. F. (2004). "The role of yeasts as insect endosymbionts". Insect-Fungal Associations: Ecology and Evolution (ed. by FE Vega & M Blackwell). pp. 211-243. Oxford University Press, New York, NY USA.
7. Nichols, C.D., Beemel, J., Pandey, U.B. (2012). "Methods to Assay Drosophila Behavior". J. Vis. Exp. (61). e3795. doi:10.3791/3795.
8. Jiménez Padilla, Yanira. (2016). "Effects of gut-associated yeasts on *Drosophila melanogaster* performance". Electronic Thesis and Dissertation Repository. 4285
9. Hoang et al. (2015). "Interactions between *Drosophila* and its natural yeast symbionts—Is *Saccharomyces cerevisiae* a good model for studying the fly-yeast relationship?". PeerJ3: e1116. DOI:10.7717/peerj.1116.
11. Grangeteau, Cedric, Yahou, Fairouz, Everaerts, Claude, Dupont, Sebastien, Farine, Jean-Pierre, Beney, Laurent, Ferveur, Jean-Francois (2018). "Yeast quality in juvenile diet affects *Drosophila melanogaster* adult life traits". Scientific Reports. 13070. volume 8. issue 1. DOI: 10.1038/s41598-018-31561-9
12. Sun, X., Heckscher, E.S. (2016). "Using Linear Agarose Channels to Study *Drosophila* Larval Crawling Behavior". J. Vis. Exp. (117). e54892, doi:10.3791/54892.