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Role of Baker's Yeast on Weight, Larval Performance, Duration of Pupation, Oviposition and Longevity of *Drosophila melanogaster* Exposed to UV Induced Stress.

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Keywords: *Drosophila melanogaster, Baker's yeast, dietary yeast, UV induced stress,ad libitum(100%), yeast restriction(50%), yeast free diet(0%), Rava media.*

Abstract:*Drosophila* is a holometabolous insect with well definedlarval, pupaland adult stage.The larval period being a non reproductive stage is marked by rapid growth and moulting to successive instars. The feeding habit of larva serves to obtain adequate energy stores primarily in the larval fat body, to fuel them through non feeding pupal stage. The larva being voracious eaters feed continuously on the yeast growing in the media. Any variation in the concentration of yeast in the media may affect the fly lifecycle. In this study we analysed the role of dietary yeast(baker's yeast) on the larval weight, larval performance, moulting, duration of pupation, geotaxis, egglaying in females and overall longevity in adult flies. We also studied the effect of yeast diet on UV induced stress withstanding capacity on the larva and adult flies. We observed that dietary yeast inturn affects the life cycle trait of *Drosophila*.Our data suggests that the larva raised in restricted dietary yeast(50%) showed increased larval performance, the average weight of the larva and adult flies were found to be more in comparison to the larva raised in ad libitum(100%) and yeast free diet(0%).The negative geotaxis in the flies in 50% media were also found to be more in comparison to the other two. The average time taken for pupation were least in ad libitum followed by the 50% and yeast free. The larva showed more stress with standing capacity when compared to the larva raised in ad libitum and the media with yeast free diet respectively. The average longevity was observed to be more in the flies raised in yeast free diet even after UV induced stress.

Introduction:

Diet plays an important role in the development of organism. The quality and quantity of food determines the fitness and overall survivability of the organism. Food must be consumed in sufficient amounts and contain essential nutrients to avoid cellular dysfunction and exposure to pathologies[1].Yeasts are considered as a major food source for the majority of species of Drosophila in both adult and larval stages2. Yeast not only acts as a valuable source of nutrition, it also interacts with the host immune system. Yeasts affect

several aspects of *Drosophila* physiology, behavior, and immunity. For example, particular yeast species affect larval development time and influence adult body weight[3].Any decrease in thequantity of yeast may lead to delayed eclosion from pupation and the flies may have decreased body weight[4].the poor yeast diet may also lead to decreased fertility[5]A yeast free diet is also associated with increased life span[6]. We evaluated the effect of Dietary yeast, Baker's yeast on larval, pupal and adult

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life trait which includes larval weight, larval performance, duration of pupation, adult fly weight. activity, egg laying capacity and longevity of adult fly. We also did a comparative study between the larva exposed to UV rays at different time period from 1-5 min respectively and evaluated the same parametersin both larva ,pupa and adult fly.

Materials and Methodology

Media Preparation:

To determine the role of yeast diet in development and UV induced stress in *Drosophila melanogaster*, the flies were cultured in three different bottles containing Rava(semolina) media with three different yeast concentration.

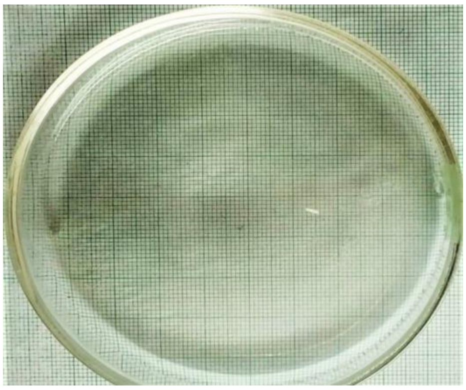


Figure 1 larval crawling assay/Performance assay

Rava media was prepared by using100g /l of jaggery to the 1000ml filtered water and dissolved completely. 100g rava and 10g agar was added to this slurry and is heated with continuous stirring until it starts boiling.. The temperature of the media was then brought about to 50^{o} C by gradual cooling and 5mL of Propionic Acid was added to the media and mixed well.40 mL of media were transferred to the cultured bottle. The bottles were sealed using cotton plug. Following day , to the culture bottles three different concentration of yeast was added to each of the bottlesi.e10g baker's yeast /L, (100% or ad libitum), 5g/L. (restricted yeast conc. Or 50%) and no yeast(yeast free ,0% yeast concentration).The following day, flies were introduced to the bottles ,.in the ratio 1 male: 5 females .The media with the flies were maintained at 24^{0}c.

Preparation of Crawling Assay.

0.25g of Agar-Agar Bactowas dissolved in 25ml of distilled water. the mixture was heated and poured in a standard sized Petri plate (100mm x 15mm). The gelwas solidified. The solidified agar was used to evaluate the larval crawling activity / performance assay.

Larval Crawling Assay

5 sets comprising ten one day old third instar larvae were selected each from the ad libitum, restricted yeast and yeast free media.. The larvae were gently cleaned with distilled water using a brush. Each time, one larva was placed on the Petri plate containing crawling assay, placed on the standard millimetre graph sheet (25cm x 20cm). The larva was allowed to crawl and the number of grids it moved was counted. Since each grid contains ten divisions, once larva completes one grid, it is counted as 1cm. The locomotory activity of each larva was noted for 60 seconds. The above procedure was repeated thrice with the same larva and the average grids covered by the larva were noted.The above steps was repeated for all the larvae after different doses of UV- irradiation and the results were recorded and analyzed for differences. The larval crawling assay was performed both before and after the UV exposure of the larvae and at 24 hours' intervals until it undergoes pupation and the observations were recorded accordingly.

Exposure to UV Radiation

One set comprising of ten third instar larvaefrom each of the three different media were exposed to UVfor a period of 1 min. After which they were transferred to the new vials with the respective media.

The experiment was repeated for 2.3.4. and 5 min. Every time 3 new sets of larva from the respective media were taken and the observation were noted.

Study of Developmental Duration for Each Phase

Time duration required for every larva raised in different dietary yeast concentration to undergo pupation were recorded (in hours) and analyzed for differences. In the same manner, time duration required for pupation, were also recorded (in days) and analyzed for differences.

Weight of Larvae and Flies

One larva from every set of 10 from each concentration of yeastwere taken and weighed using the laboratory weighing balance. The weight of each larva was recorded.

Similarly, the weights were recorded for the flies emerged from every set of 10 larva.

Negative Geotaxis Assay

For negative geotaxis study of the flies, 25mL measuring cylinders were used. Using a strip of paper, 15cm scale was marked and attached to every cylinder.

The flies emerged from every batch of 10 from each concentration of yeastwere taken within the cylinders and their movement against gravity (upwards climbing) was measured at the same time. The speed of negative geotaxis was measured for 5 seconds for each batch of flies.

Figure 2 Drosophila Adult showing negative geotaxis

Assessment of Longevity

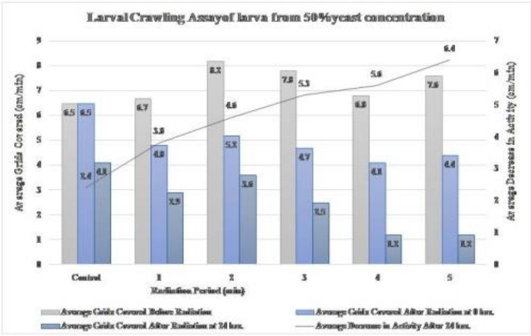
For every flies, emerged from each concentration of yeast, duration of longevity (from eclosion till death) were recorded (in days) and analyzed for differences.

Egg laying capacity. (Oviposition)

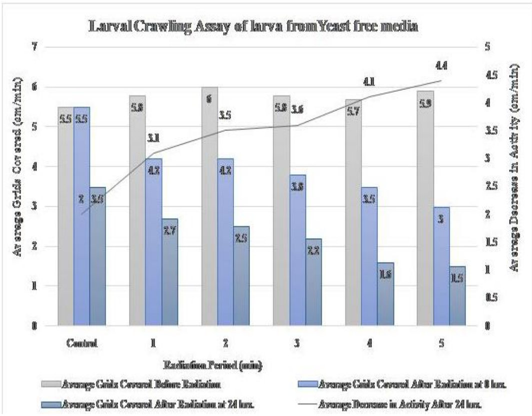
The total numbers of eggs layed by the flies which were exposed to different duration of UV exposure during larval stages were noted down. The parent flies were transferred separately to new culture media of respective yeast supplement. The number of larvae appeared in each container for every respective batch were recorded, which is directly proportional to the number of eggs laid at a time. Thus, the number of eggs produced by each batch of all the three media were recorded and analyzed for differences.

Result:

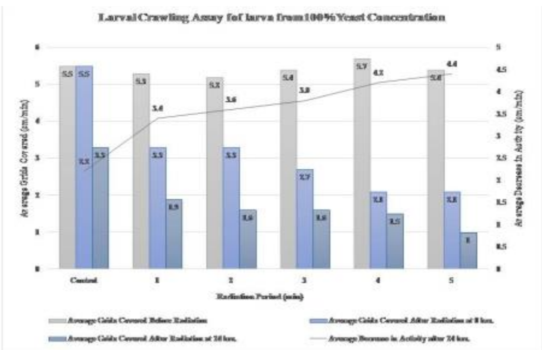
Larval Activity



Graph 1:The average grid covered by the larvae of yeast free media before radiation was 5.5/min. which was reduced to 3.3grids/min. soon after UV exposure and to 2.2 grids/min. after 24 hrs



Graph 2:The average grid covered by the larvae of yeast restricted (50%) media before radiation was 6.5/min. which was reduced to 4.1grids/min. soon after UV exposure and to 2.4 grids/min after 24 hrs



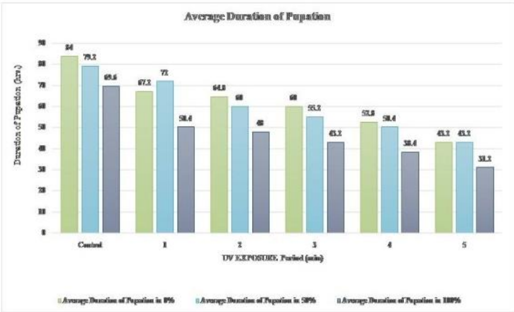
Grph3:The average grid covered by the larvae raised in ad libitum(100%) yeast before radiation was 5.5/min. which was reduced to 4.1grids/min. soon after UV exposure and to 2.4 grids/min after 24 h

In the data, it has been observed that in 50% yeast diet the larvae were more activethan that of 0% and 100%, which is supposed to be proportional to their metabolic rate in non-radiated condition (control). Under UV induced stress,these larvae showed sharp decrease in their activity within 24 hrs. When compared to that of control. Most significantly, reduction in the larval activity was much higher (6.4 cm/min) in test larvae of 50% yeast diet than that of 0% and 100%. In our study, the graph is showing a steeper reduction of larval activity in case of 50% test larvae than 0% or 100% test larvae. This observation helped us to interpret that proper diet helps in better growth of the larvae and make them more susceptible to radiation stress at the same time.

Duration of Pupation

The larvae raised in 100% yeast diet achieved pupation early by an average of 69.6 hrs .The pupae eclosed by4 days after pupation.While the larvae of 50% yeast diet pupated by an average of 79.2 hrs and lasted for 3.7 days. The larvae raised in yeast free diet showed delayed pupation when compared to the other two. It took 84 hrs to pupate and lasted for 4.5 days before eclosion.

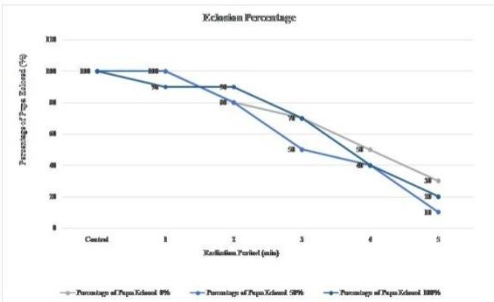
Our observation suggests that larvae raised in yeast free diet showed a lengthy pupation period. The average of which was of 84 hrswhen compared to the larva of 50% and 100%yeast dietwhichwere observed to be 79.2 and 69.6 hrs respectively. .Theaverage duration to pupate decreased with the increased period of UV exposure among the larvae of all the three different yeast concentration.However there was a delay in eclosion among all the three categories with increased UV exposure.



Graph 4: Yeast free larva showed delayed pupation whereas the larvae from ad libitumpupated early. The same phenomenon was observed in the larvae exposed to UV from the same media.

Pupal Mortality

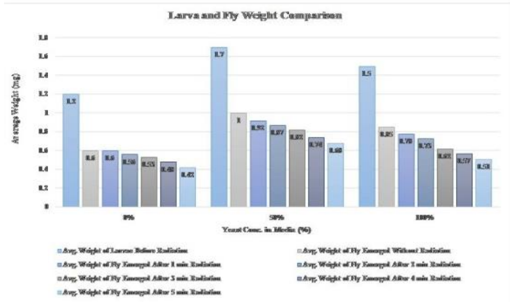
The data showed that mortality increased with the increasing dose of radiation and it was more in case of larvae of 50% yeast diet (90%), which is almost close to that of 100% yeast diet (80%) and in case of larvae of 0% yeast diet it was comparatively less (70%).



Graph 5:All the control pupa eclosed. the pupal mortality increase with increased UV exposure

Weight Comparison of Flies

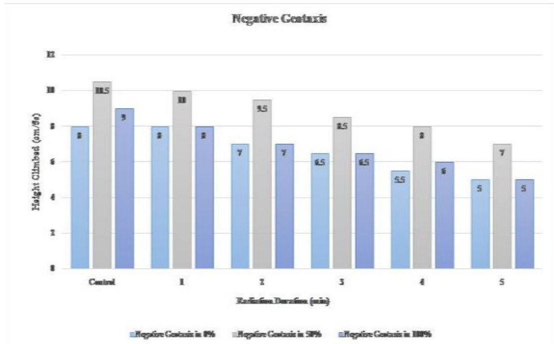
Weight, size, and activity of the larvae and the flies are directly proportional to their diet. From the dataobserved the larvae and flies raised in 50% yeast diets showed the average weight of - 1.7mg in larvae and 1.0mg in flies.Followed by ad libitum (1.5mg in larvae and 0.85mg in flies)and yeast free(1.2mg larval weight and 0.6mg in flies) .The data also suggests that the flies emerging from the UV exposed larvae tend to have lower body weight . The body weight decreases with increase in UV exposure time. The observation was same for all the flies of three different media. However, the flies belonging to yeast free media showed less variation in body weight. The control fly weighed 0.6mg where as the fly emerging from the larva after 5 min. Uv exposure weighed 0.42 mg. The control fly of 50%yeast media weighed 1mg and the test fly weighed 0.68. Whereas in 100% yeast media the control fly weighed 0.85mg and the test fly 0.51mg.



Graph 6:The larvae and the flies raised in 50% yeast diet showed more weight. The weight of the flies decreased with increase in UV exposure.

Negative Geotaxis

The data obtained from the observation showed us that negative geotaxis was much higher in control flies of 50% yeast diet (10.5 cm/5s)when compared with100%(9.0cm/5s) and 0%(8cm/5s) respectively. Climbing against the gravity for flies is also correlated with their fitness and in this case, the data suggest that flies of 50% yeast diet were more fit than that of others. The observation remained same with the test flies of 50% yeast media. The average height covered by the test flies from 5 min UV exposure was 7.0 cm/5s



Graph 7: Negative Geotaxis is seen more in Adult flies belonging to 50% yeast concentration. Reduced activity is seen after UV exposure

Oviposition

We analyzed the oviposition ability of control and test flies. The female flies from each of the respective media were

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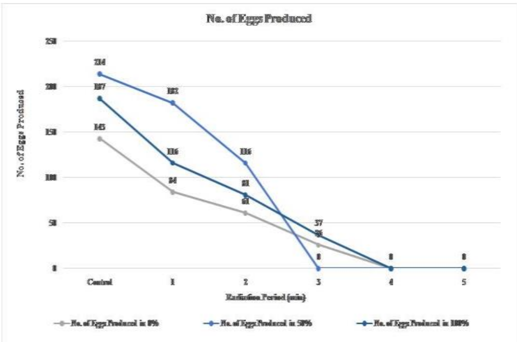
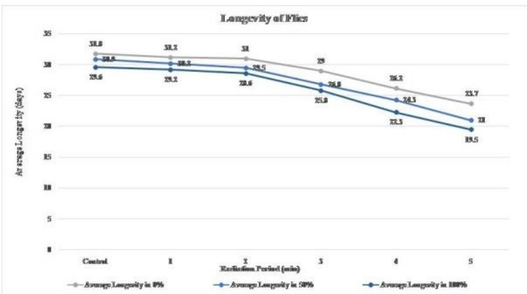
transferred to the separate vials and were allowed to lay eggs. The numbers of egg laid were counted. The data collected suggests that the flies raised at 50% yeast concentration laid 214 eggs, while the flies from 100% laid 187 eggs. The flies from the yeast free diet laid least number of 143 eggs. The flies of yeast free diet showed delayed egg laying.The test flies also, the number of eggs produced was more in case of flies of 50% yeast diet than that of others. Decrease in Oviposition was seen in flies emerged from the larva of 2min. UV exposure.However the flies obtained from the larva of 3-5 min. UV exposure were infertile.



Graph 8: Oviposition is seen more in flies of 50% and least in the flies from yeast free media.. Which again

Longevity

Data observed for the longevity of the flies showed us that control flies of 0% had more longevity (31.8 days) than that of the others. The observation in case of the test flies have been reduced with respective doses of radiation though, in all radiation dose, flies of 0% yeast diet showed more longevity (31.2-23.7 days) than that of others.



Discussion:

Yeast quality and quantity in the juvenile diet influencesthe preimaginal and post imaginal development in Drosophila[7,8]. Yeast is also known to support larval development[9]. Our study suggests that the quantity of yeastalways works as a contributing factor for weight, size, and activity of the larvae and flies.Yeast when provided in precise quantity during the larval diet benefits more when compared to ad libitum concentration of yeast.

The above point is supported by our data and also by the previous report. The larvae raised in media with 50% yeast concentration showed increased weight and larval performance. The larval performance was measured using larval crawling assay. The average duration of pupation was found to be less when compared to that of pupa of yeast free and ad libitum media.

The average weight of fly was found to be more in flies of yeast restricted (50%) media. The flies showed increased geotaxis when compared to the flies of other two media. However the average longevity was found to be more among the flies raised in yeast free media .this data is supported by very few reports[11,12,13,14]as against many which suggested that yeast poor diet leads to decrease in survival rate[10].Under UV induced stress , amarked decrease in larval performance was observed with the larva raised in yeast restricted media(50%) followed by yeast free and ad libitum.

We also observed a delay in duration between larva to pupa and pupa to adult among the larva of yeast free media. The result appears to be associated with iow yeast diet[14].the duration to pupate decreased with increased UV exposure. This phenomenon was observed among all the three categories of larva. The Uv exposed larva showed delayed eclosion .

The pupal mortality increased with increaeased UV exposure. The pupal ortality was highest among 50% yeast diet larva followed by 1005 yeast diet larva. The pupal mortality was observed to be less among yeast free pupa. the reason for less pupal mortality among yeast free pupa was not clear. Hence further study is required to ascertain the same.

The adult fly weight raised in 50%yeast diet showed more weight when compared to 100% and yeast free media. the flies eclosed after UV exposure showed marked decrease in the body weight. the negative geotaxis ability was found to be more in yeast restricted larvae.The result was found to be same in UV exposed larvae belonging to yeast restricted media.

Our observation shows the oviposition in female raised in 50%yeast concentration media was found to be more when compared to the larvae of 100% and yeast free media.

In our study, the most arguable observation has been the longevity of the flies. The longevity of flies from yeast free media was observed to be more when compared to the larvae from 50% and 100% yeast media[7].The longevity of flies from UV exposed larvae from yeast free media was also observed to be more when compared with the other two categories of flies. The reasons for increased longevity in yeast free raised flies remain unclear. One way of resisting UV induced stress is by activation HSP 26 and HSP27 pathway. These protein will increase stress resistance and inturn increases lifespan by 30%[15, 16.]. But the role of yeast free diet in activation of these two proteins remains unclear.

Conclusion

Yeast provided at optimum quantity play an important role in fitness and longevity in Drosophila. The larvae raised in optimum quantity of yeast show more larval and adult weight. They also show increase larval and adult performance. Their fitness can also be observed in oviposition. The larvae when exposed to UV induced stress,regardless of the yeast concentration in the media show early pupation and delayed eclosion. These may be one of the method adopted by them as stress resistance mechanism. The increased longevity of flies raised in yeast free media remains unclear. Further study is required to understand this mechanism.

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TABLES

Yeast Conc. In Media (%)	Average Weight of Larvae (mg)	Average grid covered at 0hrs	Average grid covered after 24hrs	Average decrease in activity	Average time taken to pupate
0% (Yeast free)	1.20	5.5	3.5	2.0	84.0
50% (yeast restricted)	1.70	6.5	4.1	2.4	79.2
100% (Ad libitum)	1.50	5.5	3.3	2.2	69.6

Table 1.1- Growth and Development of *Drosophila* in Different concentration of Yeast Diet

Yeast Conc. In Media (%)	Average Duration of Eclosion (days)	Percentage of Pupa Eclosed (%)	Average Weight of Fly (mg)	Negative Geotaxis (cm/5s)	No. of Eggs Produced	Average Longevity (days)
0% (Yeast free)	4.5	100	0.60	8.0	143	31.8
50% (yeast restricted)	3.7	100	1.00	10.5	214	30.9
100% (Ad libitum)	4.0	100	0.85	9.0	187	29.6

Table 1.2-Duration of pupation, weight of flies , egg laying ability of *Drosophila* flies raised in different concentration of dietary yeast

Table 2.1- Larval performance and duration of pupation among Larva raised in Yeast free Diet

Yeast Conc. In Media (%)	Radiation Period (min)	Average Weight of Larvae before Radiation (mg)	Average Grids Covered (cm/min)	Average Grids Covered at 0 hrs.	Average Grids Covered at 24 hrs.	Average Decrease in Activity after 24 hrs. (cm/min)	Average time taken to pupate
0% (Yeast free)	1.0	1.20	5.8	4.2	2.7	3.1	67.2
	2.0	1.20	6.0	4.2	2.5	3.5	64.8
	3.0	1.20	5.8	3.8	2.2	3.6	60.0
	4.0	1.20	5.7	3.5	1.6	4.1	52.8
	5.0	1.20	5.9	3.0	1.5	4.4	43.2

Table 2.2- Growth and Development of *Drosophila* lines of Yeast free Diet under UV Induced Stress

Yeast Conc. In Media (%)	Radiation Period (min)	Average Duration of Eclosion (days)	Percentage of Pupa Eclosed (%)	Male-Female Ratio (M:F)	Average Weight of Fly (mg)	Negative Geotaxis (cm/5s)	No. of Eggs Produced	Average Longevity (days)
0%	1.0	5.8	100	1:1	0.60	8.0	84	31.2
	2.0	6.1	80	5:3	0.56	7.0	61	31.0
	3.0	6.6	70	3:4	0.53	6.5	26	29.0
	4.0	7.0	50	3:2	0.48	5.5	0	26.2
	5.0	7.7	30	2:1	0.42	5.0	0	23.7

Table 3.1- Growth and Development of *Drosophila* lines of 50% Yeast Diet under UV Induced Stress

Yeast Conc. In Media (%)	Radiation Period (min)	Average Weight of Larvae before Radiation (mg)	Average Grids Covered (cm/min)	Average Grids Covered at 0 hrs.	Average Grids Covered at 24 hrs.	Average Decrease in Activity after 24 hrs. (cm/min)	Average Duration of Pupation (hrs.)
50%	1.0	1.70	6.7	4.8	2.9	3.8	72.0
	2.0	1.70	8.2	5.2	3.6	4.6	60.0
	3.0	1.70	7.8	4.7	2.5	5.3	55.2
	4.0	1.70	6.8	4.1	1.2	5.6	50.4
	5.0	1.70	7.6	4.4	1.2	6.4	43.2

Table 3.2- Growth and Development of *Drosophila* lines of 50% Yeast Diet under UV Induced Stress

Yeast Conc. In Media (%)	Radiation Period (min)	Average Duration of Eclosion (days)	Percentage of Pupa Eclosed (%)	Male-Female Ratio (M:F)	Average Weight of Fly (mg)	Negative Geotaxis (cm/5s)	No. of Eggs Produced	Average Longevity (days)
50%	1.0	4.8	100	3:2	0.92	10.0	182	30.2
	2.0	5.5	80	1:1	0.87	9.5	116	29.5
	3.0	6.0	50	3:2	0.82	8.5	0	26.8
	4.0	6.3	40	3:1	0.74	8.0	0	24.3
	5.0	7.0	10	1:0	0.68	7.0	0	21.0

Table 4.1- Growth and Development of *Drosophila* lines of 100% Yeast Diet under UV Induced Stress

Yeast Conc. In Media (%)	Radiation Period (min)	Average Weight of Larvae before Radiation (mg)	Average Grids Covered (cm/min)	Average Grids Covered at 0 hrs.	Average Grids Covered at 24 hrs.	Average Decrease in Activity after 24 hrs. (cm/min)	Average Duration of Pupation (hrs.)
100%	1.0	1.50	5.3	3.3	1.9	3.4	50.4
	2.0	1.50	5.2	3.3	1.6	3.6	48.0
	3.0	1.50	5.4	2.7	1.6	3.8	43.2
	4.0	1.50	5.7	2.1	1.5	4.2	38.4
	5.0	1.50	5.4	2.1	1.0	4.4	31.2

Table 4.2- Growth and Development of *Drosophila* lines of 100% Yeast Diet under UV Induced Stress

Yeast Conc. In Media (%)	Radiation Period (min)	Average Duration of Eclosion (days)	Percentage of Pupa Eclosed (%)	Male-Female Ratio (M:F)	Average Weight of Fly (mg)	Negative Geotaxis (cm/5s)	No. of Eggs Produced	Average Longevity (days)
100%	1.0	4.7	90	2:1	0.78	8.0	116	29.2
	2.0	5.0	90	5:4	0.73	7.0	82	28.6
	3.0	5.7	60	2:1	0.62	6.5	37	25.8
	4.0	5.8	40	3:1	0.57	6.0	0	22.3
	5.0	6.0	20	2:0	0.51	5.0	0	19.5