International Journal of Pharmaceutical Applications ISSN 0976-2639.
Vol 2, Issue 4, 2011, pp 225-230 http://www.bipublication.com

ANALYSIS OF *ACHYRANTHES ASPERA (ASH)* ON REPRODUCTIVE FITNESS OF *DROSOPHILA MELANOGASTER.*

B. R. Guru Prasad*[1], Pankaj Pathak[2]
[1]Department of Life Science, Kannada Bharthi College, Khusal Nagar, Coorg, India
[2]JSS Ayruvedic Medical College, Mysore.

ABSTRACT

Drosophila is a suitable model organism to test phytochemicals. The objective of our study was to explore the potential of *Achyranthes aspera* on reproductive fitness of *Drosophila melanogaster* using larval and adult feeding methods. The decrease in the fecundity, fertility, developmental time, ovarioles number, hatchability and viability was observed in adult feeding than larval feeding methods. According to the Chi-square the percentage of the hatchability is decreased in treated compare with control one and significant difference in viability in adult feeding method. Our findings shows the adult one which are exposed are more sensitive compare with larval stage.

Keywords: *Drosophila,* fecundity, fertility, hatchability, viability,
Running title: Effect of *Achyranthes aspera* on reproductive fitness

[1] INTRODUCTION

Today, the fruit fly *Drosophila* is an ideal organism to screen the few phyto-chemicals. This fly serves as a system for many investigations in developmental and cellular processes common to higher eukaryotes, including man. This fly is being extensively used for genetic studies since almost a century. Studies on *Drosophila* have enabled biologists to make significant contribution to the fields as diverse as basic medicine science, genetics, evolutionary biology and molecular biology. It could be maintained in large numbers having short life cycle, easy and inexpensive in culturing. Today several *Drosophila* species with mutant and transgenic stock is available at different laboratories through out the world. Presence of giant polytene chromosome and less number of the chromosomes are added advantages of the flies for genetic studies. Its genome has also been sequenced recently [1]. Vertebrates have about four homologous for every gene found in *Drosophila.* The species shares large numbers of homologous genes with mammals, 13,601 with humans. These have been analyzed to identify sequences related to those causing human diseases [2].

The evolutionary conservation of gene function between humans and *Drosophila,* make it an ideal model system for the study of the molecular mechanisms of human disease. In *Drosophila* and many other insects body size is positively correlated with mating success, longevity, fecundity and other fitness characters and best phenotypic heritable characters [3]. All these findings demonstrated the advantage of size in mating success and fitness. Various workers demonstrated the fitness studies in *Drosophila* such as fecundity, fertility [4] and longevity [5,6]. Generally, newer drugs include some herbal drugs have a more favorable adverse effect profile. Although a

number of drugs produce rare, but serious adverse effects, overdose toxicity is a matter of greater importance especially in patients who are in risk. *Drosophila* is best suitable organism to screen some drugs which are found in some of the plants which have medicinal value. The *D.melanogaster* belongs to *D.melanogaster* species group and found along with human habitation. Here authors had tried to evaluate the effect of *Achyranthas aspera* on reproductive fitness in *Drosophila melanogaster*.

MATERIALS AND METHODS

To study the effect of toxicity of *Achyranthas aspera* (ash) on reproductive fitness parameters of *D.melanogaster* flies were obtained from *Drosophila* stock center, Mysore were used. The pure culture of these flies was maintained under standard food medium [7, 8]. The *Achyranthas aspera* is a cosmopolitan herb found in and around wild localities of Mysore, Karnataka. The homogenized product of the root, stem and leaf of this plant (referred as Ash powder) is used as Aryurvedic drug in India.

To study the effect of these drugs in larval and adult feeding methods two sets of culture media is prepared 1. Control group which is considered as normal media wheat cream agar media (wheat cream agar medium was prepared by boiling 1000ml of distilled water along with 100g of jagery (sugar). When jagery dissolved in it, 100g of wheat powder (soji or rava) was added to the medium and then 10g of agar agar and 7.5ml of propionic acid (anti-fungal) were added gently). 2. Treated group where 10 drops of ash of *Achyranthas aspera* were thoroughly mixed in wheat cream agar medium. This media was distributed to glass vials of 8×2.5 cm size. The mouth of the vials was kept closed with cotton. One day later one or two drops of yeast solution were added to the food media. This medium was used after 24 hours. At every step heat vials were used for preparing medium to avoid the fungal infection and to prevent outbreak of pests and diseases. Similarly sterilized cotton was used to plug the vials. For larval feeding technique, *Drosophila* eggs of the same age ± 3 hours collected by procedure of [9] were placed in both culture group vials (control and experimental group) containing density of 25 eggs per vial. After 3 days newly hatched larvae were continuously fed on food medium supplemented with above groups. Virgin females and bachelor males emerged from the normal (control) and treated media were isolated under ether anesthesia within 3 hours of eclosion and maintained them separately for 5 days in normal media. After 5^{th} day both the virgin and bachelor male were transferred to fresh normal wheat cream agar media. The reproductive fitness parameters such as fecundity, fertility, developmental time ovarioles numbers and viability were analyzed from mated parents. For fecundity test mated females were transferred to vials containing normal food media and allowed to lay eggs for 24 hours. After 24 hours, the flies were individually transferred to fresh vial containing food media. The number of eggs laid (fecundity) during the following ten days was scored using stereomicroscope for both control and experimental groups. Twenty five replicates were maintained for each of the control and experiment studies. The egg hatchability was also measured by counting the number of the

eggs hatched after 48 hours from the pair mated. The fertility was measured by counting the number of the progeny produced by a single mated female. For testing fertility, each mated female was kept in an individual food vial for a period of one day and then transferred to a fresh food vial every day. Ten successive changes were made and the total number of flies that emerged from each vial was counted. Twenty five replicates were maintained for each of the experimental and control under study. To study the viability (survival value) the number of flies emerged out of each vial are recorded every day until the last day of emergence. Data were pooled and the mean number of flies per female was calculated. Counting of ovarioles number was done by selecting the twenty five virgin females separately and maintained in both control and experimental cultures and aged 4 days. These virgin females were dissected for left ovaries in saline and bundles of ovarioles were separated by fine needle and counted under a stereomicroscope. In adult feeding technique, virgin females and bachelor males emerged from the normal media were isolated under ether anesthesia within 3 hours of eclosion and maintained them separately in normal media for 3 days and then transferred separately to control and experimental group treated media fed for 2 days (48 hours). Thus they were aged for 5 days. The parameters such as fecundity, fertility, developmental time, ovarioles number and viability and were analyzed according to larval feeding method. Statistical methods such One way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT)and Chi square test was applied for all parameters using SPSS 10.5 software.

RESULTS

Effect of *Achyranthas aspera* (ash) on *D. melanogaster* in control and treated culture in both the larval and adult feeding methods is shown in table 1. In the larval feeding method such as fecundity, fertility, developmental time and ovarioles number was non-significant difference between control and experimental groups according to one way analysis of variance (ANOVA) (fecundity=1.675; fertility= 2.379; developmental time = 1.256; ovarioles number = 1.121). On the contrary, in adult feeding method (table 1), all parameters such as fecundity, fertility, developmental time and ovarioles number of both culture groups showed high significant difference. This difference is shown in mean values and F-values of control group versus experimental group (fecundity (109.20 v/s 52.15; F value=15.203), fertility (110.90 v/s 56.40; F value= 13.665), developmental time (11.65 v/s 15.63; F value= 6.571), ovarioles number (14.04 v/s 17.04; F value = 15.75). ANOVA depicts the significant difference between two culture groups $P<0.05$) in all the above parameters. Table 2 incorporates the results of effect of *A. aspera* on egg hatchability and viability of both control and experimental groups. In control groups of the both larval and adult feeding methods hatching is 96% and 95% respectively. Hatching was reduced to 95% to 75.5% in adult feeding method and chi-square shows significant between control group and experimental groups. This trend is absent in larval feeding method.

Data obtained in table 2 also shows the effect of *Achyranthas aspera* (ash) on viability in various concentrations in both larval and adult feeding methods. Viability in control group is highest compared to experimental group. In larval feeding method viability was 90.5% and in adult feeding it was 85.4%. Viability decreased in experimental groups in both the feeding techniques. However, lowest percent of viabilities in adult feeding was 69.3% and 71% in larval feeding method. The difference in viability between control and experimental were statistically significant in both feeding method according to the chi square.

DISCUSSION

Sexual reproduction is very important phenomena for continuity of life. Sexually reproducing animals are endowed with special features, first to produce fertile offspring and second to adapt to a particular environment. The reproduction is preceded by a series of courtship acts where in males and females show unique rituals to attract each other, mate and produce the offspring. The courtship and mating although are genetic, are also influenced by various factors acoustic, visual, chemical, and tactile signals that culminate in copulation [10,11,] Such signals are also species specific and carry information about species, gender or receptivity [12]This behavior not only is influenced by the mutual stimuli and response generated by courting individuals but also by the environmental factors [8]. In the present study an effort is made to study the effect of *Achyranthas aspera* (ash) on reproductive fitness parameters

In *Drosophila*, The fecundity remains one of the less known quantitative traits. Along with fecundity, the fertility, developmental time and ovarioles number, hatchability and viability also estimated. Estimation of fecundity and fertility is important in routine toxicology testing of various chemicals including pytochemicals. This gives an insight into the extent of effect on ovarioles and physiological factors, which is expressed in the terms of egg, offspring production and viability. Table 1 reveals that mean fecundity, fertility (eggs/female), developmental time (days) and ovarioles number (per female) in both cultures was not so significant in larval feeding method. This is completely reverse in case of adult feeding method. According to ANOVA have shown that increased fecundity in larval feeding and decreased in adult feeding were significant in treated group. This indicates that the mode of administration is also important factor, which one should consider while assessing the effect of any chemical on any biological system. In the present study fecundity is not so affected in larval compare to adult feeding method. This is due to larva has undifferentiated ovary. Perhaps the absence of *Achyranthas aspera* (ash) enhances the capacity of egg production when they develop adults. When the larva develops into adults, the effect of drug would vanish hence lay more eggs. In contrast to this in adult feeding, the decrease in fecundity may be accounted for the fact that the flies are under the influence of *Achyranthas aspera* (ash), hence they might not have been able to lay eggs rather than producing less egg. This finding agrees with the observation of [13] where they noticed oviposition rhythm in

D.melanogaster and [14] has demonstrated that certain aziridine analogous have discernible effect on fecundity in *Drosophila*. Table 2 also shows mean fertility per female in adult diet with *Achyranthas aspera* (ash) was seriously impaired and they were less fertile than control, compare to larval diet. ($P<0.05$ according to ANOVA). Several workers have made studies on the effect of different chemicals on fertility in *D.melanogaster* [15,16, 17]. The present study of the author agrees with them. The Developmental time taken in the larval feeding technique was less compare to adult feeding technique in experimental culture group. In case of ovarioles numbers suggest that there is influence of the *Achyranthas aspera* (ash) on the development of ovarioles. Ovarioles is the functional unit of *Drosophila egg*. The developed and maturation of ovarioles is during adult so this is affected in adult feeding, but not in larval feeding technique, Where there is no secondary sexual characters (ovarioles) are not developed in larva.

Egg hatchability in control and treated one in larval feeding is given in table 2. The data shows was non significant ($P<0.05$, λ^2 test). It is possible that the chemicals have not affect the hatching of *D.melanogaster* in all treated versus control, by larval and adult feeding methods. Viability is one of the adoptive traits of any population and determines the rate of increases or decrease of population in an environment. Any change in viability reflects the somatic effect induced by them [18] provided the analysis is made in a uniform environment. Environmental factors which would affect viability mainly include such as temperature, food, space and population density [19]. Viability of *D.melanogaster* in both the feeding methods is shown in table 2 in controls viability was very high compare to treated one. On the other hand in adult feeding method viability was seriously reduced compare to the larval feeding, (table 2). There is the significant difference in viability in adult feeding ($P<0.05$, λ^2 test).

CONCLUSION

Thus it can be concluded from the above discussion that *A. aspera* ash have affected the reproductive fitness in adult not larva stage of *D.melanogaster* in both adult and larval feeding methods. Ash which was prepared using the *A. aspera* has effect on the fitness parameters of *Drosophila*

ACKNOWLEGDEMENT

Authors are very grateful to the Principal of Kannada Bharthi College for providing facility and helping for this experiment output.

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Tables:

	Parmeters / concentrations	Fecundity Eggs/female	Fertility Adults/female	Developmental time	Ovarioles Number
Larval feeding	control	119.10 ± 6.3	115.6 ± 5.37	10.02 ± 0.07	14.00 ± 0.10
	Experimental	111.13 ± 5.2	112.00 ± 0.92	11.03 ± 0.14	12.00 ± 0.62
	F value	1.575	2.379	1.256	1.121
Adult feeding	control	109.20 ± 4.2	111.90 ± 5.20	11.65 ± 0.08	14.04 ± 0.16
	Experimental	62.15 ± 5.15	66.40 ± 0.81	14.63 ± 0.15	15.04 ± 0.71
	F value	15.203*	13.665*	6.571*	15.75 **

Table 1. Effect of *Achyranthas aspera* on *D.melanogaster* (values are represent mean and their standard errors)

*Mean difference is significant at 0.05 levels according to ANOVA

	Parmeters / concentrations	Total eggs laid	Hatching (%)	λ^2 for hatching	Viability	$\lambda^2$for Viability
Larval feeding	control	1138	1100 (96%)		1120 (90.5%)	
	Experimental	1145	1018 (90.1%)	1.351	873 (71.0%)	18.521
Adult feeding	control	1077	1121(95%)		1114 (85.4%)	
	Experimental	435	353(75.5%)	0.166	236 (69.3%)	5.021 *

Table **2** Effect of *Achyranthas aspera* in *D.melanogaster* on hatchability and viability.

*significant by λ^2 test at 5% level, when compared to control