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EFFECT OF AZADIRACHTIN ON THE PHENOTYPE OF DIFFERENT DEVELOPMENTAL STAGES OF *DROSOPHILA MELANOGASTER*

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ABSTRACT:

In the present study we have studied the effect of azadirachtin on insect model *Drosophila melanogaster. Drosophila melanogaster* is a small, common fly and exhibit complete metamorphism. *Drosophila melanogaster* captured from rotten food, ripped banana and kitchen. They were cultured in controlled culture media and also kept separate normal culture one. After the development of each stages various parameter have been taken place and statically compared the controlled and normal flies, and eventually we have observed decrease in the size of flies which were fed on controlled culture media.

KEY WORDS: *Drosophila melanogaster*, Azadirachtin.

INTRODUCTION:

A drug includes all chemicals other than food that affect living processes. If this effect helps the body than drug is acting as a medicine but if a drug causes a harmful effect on the body the drug is acting as a poison. To investigate about the drug effect on the human body the drug is not directly applied to the human body but various tests is done on various other organism, so far several studies have mainly been done on rat or mouse animal model and rarely with other animals like monkey, cat, dog, rabbit etc.

Regarding the various reason like more proliferation and low price scientist have focused on insect models. *Drosophila melanogaster* is a small, common fly found near unripe and rotted fruit. Drosophila is very valuable in terms of four pairs of chromosomes for genetic researchers, due to its small size, ease of culture and short generation time. There are several reason to be taken *Drosophila* as an experimental animal because they can be easily handled, anesthetized and manipulated individuals with very unsophisticated equipment. *Drosophila* are sexually dimorphic (males and females are different), making it is quite easy to differentiate the sexes. It is easy to obtain virgin males and females, as virgins are physically distinctive from mature adults. Flies have a short generation time (10-12 days) and do well at room temperature. The care and culture requires little equipment, is low in cost and uses little space even for large cultures.

Drosophila melanogaster exhibits complete metamorphism, meaning the life cycle includes an egg, larval (worm-like) form, pupa and finally emergence (enclosure) as a flying adult. This is the same as the well-known metamorphosis of butterflies and many other insects. The larval stage has three instars, or molts. After the third instars, larvae will begin to migrate up the culture vial in order to pupate.

Azadirachtin, a botanical pesticide derived from the neem tree, *Azadirachta indica* is one of the most promising natural compounds (Winkaler *et al*., 2007), where it is less harmful to the environment than the synthetic pesticides (Sundaram, 1996).

Azadirachtin is structurally similar to that of insect hormone known as ecdysones. The insect hormone ecdysone plays an important role during growth of insect when passing from larva to adult. As Azadirachtin is similar to ecdysone it controls the process of metamorphosis by affecting on corpus cardiacum (an organ which secrete hormone in insects) means controls the secretion of hormone.

MATERIALS & METHODS

Collection of *Drosophila* flies: - Fruit fly has cosmopolitan distribution, and flies were easily collected on ripe banana or fruit. Ripe banana was put in a Petridis in one place then, the fruit flies were attracted and feed on ripe banana and the flies were transferred to a conical flask in the lab, we cover to the opening of the flask with a Maslin cloth the flies were easily collected with a brush.

Preparation of *Drosophila* culture media

Material required

Potato, sugar, Propionic acid, yeast.

Potato	-	100g
Agar	-	1.09 g
Water	-	100 ml
Dextrose	-	1 gm
Propionic acid	-	0.8 ml
Yeast	-	0.5 gm

Boil the potato and clean it then weight up to 100 g. then add 1.09 g agar, 100 ml of H_2O added in it and I gm dextrose. Homogenize the mixture and make a fine paste. After formation of paste autoclave it at 15 IP for 10-15 min.

Cool the media up to 60^0C. Then add 0.8 ml propionic acid put the culture media up to 40^0C add 0.59 gm yeast in it by dissolving it in distilled water then sterilize it in autoclave at 15 IP.

Preparation of culture vials: - After sterilization of culture vials, transferred the culture, medium in ordered manner. First label the four cultured vials as A,B,C,D and then the vials with culture media. Arrange the vials at different azadirachtin concentrations.

Vials	A	-	75 µl
	B	-	35 µl
	C	-	18 µl
	D	-	Control

Transfer of *Drosophila* flies into culture vials:-

Drosophila flies were collected in collection bottle, the transfer 10-15 fruit flies into culture vials (half male and half female) and close the mouth of vials. Different concentration of drug was added to the culture as mentioned in the experimental set-up. These flies laid eggs in culture, hatch and completed their life cycle within 10-15 days. After completion of life cycle, various stages of the flies were collected and proceed for the measurement. The life cycle of Drosophila melanogaster were studied in these culture vials.

Observation of various stages: - Male and female copulate after 2-3 hours of copulation, female lay fertilized eggs. After 24 hrs of fertilization eggs hatches into first instars larva. First instar larvae change into second instars larvae, after 3-days. After the fifth day second instar larvae changes into third instar larvae and in sixth day third instars larvae changes into pupa. The sizes of larvae changes in all stages were found to be shrinkage than in normal condition in case of azadirachtin fed larvae.

Collection of adult flies: - The pupa changes in adult fly. After the development of adults, flies were collected for the observation and measurement of various parameters. First etherized them and collected in Petridis.

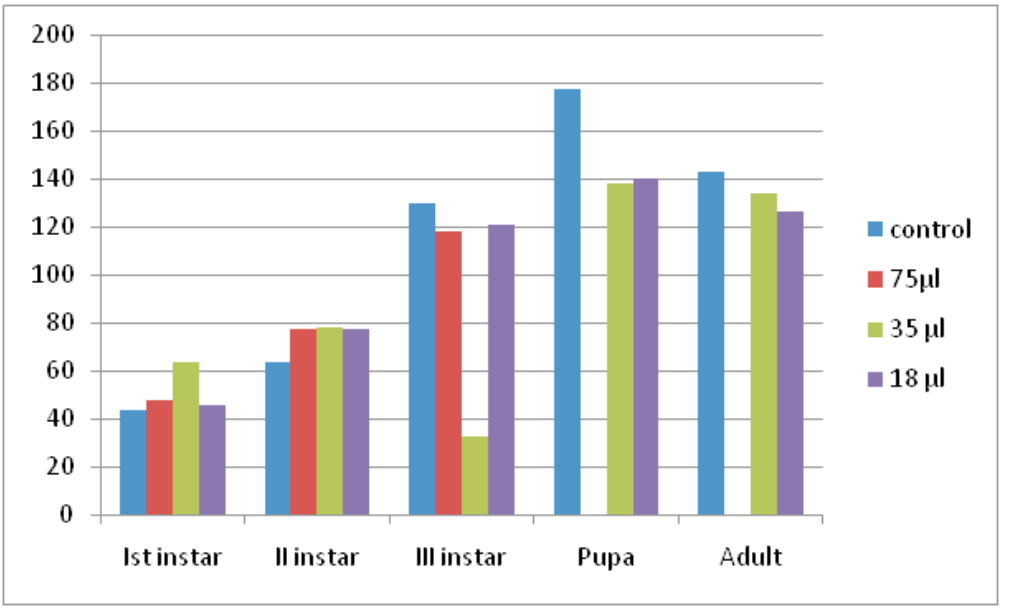
Measurement of various parameters: - The measurement of different developmental stages of the flies was done in under the microscope. These observations made under 10 x magnifications by using occulometer having scale given in micrometer (um).

OBSERVATIONS AND RESULT

In our present work we observed the effect of azadirachtin on the molting of the flies and we found the inhibition of the molting with the increased dosages of azadirachtin, at higher doses (1ml, 0.5ml, 0.01ml) we observed the total mortality which shows the highly toxicity of azadirachtin. But at (18µl, 35µl, 75µl) respectively we noticed the development of 3rd instar larvae into pupa and adult incase of azadirachtin (18µl and 35µl) but at (75µl) no 3rd instar larvae developed into pupa thus the pupae development was inhibited or we may say the moulting was inhibited.

Sr. No.	Devpt.-stage / Concentration	Ist Instar (µm)	IInd Instar (µm)	IIIst Instar (µm)	Pupa (µm)	Adult (µm)
1	Control	42.83±5.15	63.5±6.80	130.16±15.38	177.83±4.99	143.16±9.6
2	75µl	48±4.73^{NS} (10.77)	77.66±4.45** (18.23)	118.5±3.44^{NS} (9.83)		
3	35µl	63.83±4.30** (32.89)	78.5±15.01* (19.10)	32.5±4.27^{NS} (1.76)	138.5±13.18** (28.39)	134.16±10.12* (13.16)
4	18µl	46±7.48^{NS} (6.89)	77.66±5.31** (6.20)	120.83±6.64^{NS} (7.72)	140.5±6.09** (26.56)	126.5±6.44* (7.90)

Values are mean ± SD of six observations, * p<0.05>, ** p<0.01 & NS- Not Significant. Values are in paranthesis indicate the percent change in size over the control.



Graph- Effect of azadirachtin on the size of different developmental stages *of Drosophila melenogaster.*

DISCUSSION AND CONCLUSION

Paul B. Tanzubil and Alan R. M. Caffery (1990) studied the treatment of larvae of the African armyworm (*Spodoptera exempta*) with azadirachtin and aqueous neem seed extracts produced a range of adverse effects that were dose dependent. High doses of up to 10 µg per larva of azadirachtin resulted in 100% larval mortality, but this effect was delayed and prolonged. At lower doses of azadirachtin, however, inhibition and disruption of molting was observed and larval-pupal intermediates or abnormal pupae were commonly found. Similar results were obtained with the aqueous extracts of neem seeds. The few pupae obtained from larvae treated with lower doses of the extracts (0.01 and 0.1 µg per larva) either failed to develop further or developed into adults that died during eclosion, or had frizzled, curled wings.

Low doses of azadirachtin merely prolonged the inter-moult stage, apparently due to a delayed

ecdysteroid peak. Medium and high doses suppressed adult ecdysis, and the larvae became permanent larvae, the longevity of which increased with rising doses. Although medium doses prevent ecdysis, apolysis and secretion of adult cuticle were taking place. The ecdysteroid peak was further delayed in these larvae and was somewhat lower than in controls. Permanent larvae induced by high azadirachtin doses showed neither ecdysis nor apolysis. Larvae also showed an ecdysteroid peak, which was considerably delayed and distinctly lower than in the controls. Thus, treatment with different azadirachtin doses allowed some dissection of the molting cycle into different steps, in which the hormonal regulation could be studied independently.

Menakshi Bhat et al (2011) in their study using invivo diabetic murine model, *AZadiracta indica* and *Bougainvillea spectabilis* chloroform, methanolic and aqueous extracts were investigated for the biochemical parameters important for controlling diabetes. It was found that *A. indica* chloroform extract and *B. spectabilis* aqueous, methanolic extracts showed a good oral glucose tolerance and significantly reduced the intestinal glucosidase activity. Interestingly, *A. indica* chloroform and B. spectabilis aqueous extracts showed significant increase in glucose-6-phosphate dehydrogenase activity and hepatic, skeletal muscle glycogen content after 21 days of treatment. In immunohistochemical analysis, they observed a regeneration of insulin-producing cells and corresponding increase in the plasma insulin and c-peptide levels with the treatment of *A. indica* chloroform and *B. spectabilis* aqueous, methanolic extracts.

A. J. Mordue et al (2008) studied the biological effect of azadirachtin on fifth instars nymphs of *Locusta migratoria migratorioides.* Azadirachtin injection at the beginning of the instar resulted in a dose-dependent range of developmental aberrations. Low concentrations (c. 1.7/µg/g body weight) resulted in adults with curled wing tips and reduced longevity; higher concentrations (c. 2.9µg/g) resulted in death during the imaginal molt; doses of c. 6.5µg/g cause death immediately prior to the molt; and doses of c. 7.3µg/g induce a greatly extended instar. Such doses were related to a proportionately slower growth rate of the insect and a significantly reduced food intake, as assessed by wet weight and faeces production. Doses of 80µg/g resulted in death within 24 h. Experiments *in vivo* and *in vitro* demonstrate a significant reduction with azadirachtin treatment in the rate of passage of food through the gut, and in gut motility. The significance of that direct effect on gut motility was discussed in relation to the mode of action of azadirachtin on growth and molting.

In our study *Drosophila melanogasters* were fed on culture containing different concentration of azadirachtin as at (0.01ml, 0.5ml, 0.01ml) we found the total death of the feeding drosophila that is the total mortality was observed the mortality hence may be attributed to the pesticidal effect of azadiractin and also concluded that azadirachtin responsible for decreasing the size of *Drosophila melanogaster.*

REFERENCES

1.A.J. Mourdue, P.K. Cottee, K.A. Evans,(2008). Studied the biological effect of azadirachtin on fifth instar nymphs of Locusta migratoria migratorioides.DOI: 10:1111-1365.

2.Menakshi Bhat, Sandeep Kumar K. Kothiwele, Amruta R. Tirmale, Shoba Y. Bhargava, and Bimba N. Joshi.,(2011). Study using invivo diabetic murine model, AZadiracta indica and Bougainvillea spectabilis chloroform, methanolic and aqueous extracts investigated for the biochemical parameters important for controlling diabetes. Doi,.10:1093- 033.

3.Paul B.Tanzubil & Alan R. M. Caffery, (1990). Studied the treatment of larvae of the African armyworm (Spodoptera exempta) with azadirachtin and aqueous neem seed

4.Sundaram, K. M. S., (1996). Azadirachtin, biopesticide: a review of studies conducted on its analytic

chemistry, environmental behavior and biological effects. J. Environm. Science Health B, 3: 913-948.
5.Winkaler, E. U. Santos, T. R. M.; Joaquim G. Machado-Neto, J. G. and Martinez, C. B. R., (2007).Acute lethal and sublethal effects of neem leaf extract on the neotropical fresh water fish Prochilodus lineatus.Comp. Biochem. Physiol. C 145: 236–244.

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