Project Report

STUDY OF EFFECTS OF PYRIPROXYFEN ON THE DEVELOPMENT OF *DROSOPHILA MELANOGASTER* UNDER THE IMPACT OF VARIOUS FACTORS

Dissertation submitted to the University of Kerala in partial fulfillment of the requirements for the award of the degree of

Bachelor of Science
in
ZOOLOGY

(B.Sc Zoology, 2015- 18 batch)

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DEPARTMENT OF ZOOLOGY
TKM COLLEGE OF ARTS AND SCIENCE
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March- 2018

CERTIFICATE

This is to certify that the dissertation entitled **STUDY OF EFFECTS OF PYRIPROXYFEN, ON THE DEVELOPMENT OF *DROSOPHILA MELANOGASTER* UNDER THE IMPACT OF VARIOUS FACTORS** is an authentic record of the work done by .....with Reg. No..... under my supervision as partial fulfillment of the requirements for the Degree of *Bachelor of Science* in Zoology and this report has not been submitted earlier for the award of any degree or diploma or any other similar titles anywhere.

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EXAMINERS :

1

2

DECLARATION

I do hereby declare that this dissertation entitled entitled **STUDY OF EFFECTS OF PYRIPROXYFEN, ON THE DEVELOPMENT OF *DROSOPHILA MELANOGASTER* UNDER THE IMPACT OF VARIOUS FACTORS** is a bonafide report of the project work carried out by me, under the supervision and guidance of **Aseeb A K**, Asst. Professor, Department of Zoology,TKM College of Arts and Science, Kollam as partial fulfillment of the requirements for the award of the Degree of Bachelor of Science in Zoology.

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StudentS

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DEDICATED TO MY PARENTS & TEACHERS...

INTRODUCTION

1. INTRODUCTION

Drosophila melanogaster is a species of small flies, belonging to the genus *Drosophila* and family Drosophilidae. The entire genus of *Drosophila* contains more than 1500 species and is very diverse in appearance, behavior and breeding habit. They are found all around the world, with more species in the tropical regions. They can be found in deserts, tropical rain forest, cities, swamps and alpine zones. Most species breed in various kinds of decaying plant and fungal material, including fruit, bark, slime fluxes, flowers and mushrooms. *Drosophila melanogaster* is a fruit fly, a little insect about 3mm long, of the kind that accumulates around spoiled fruit. It is a common pest in homes, restaurants, and other occupied places. It is also one of the most valuable of organisms in biological research, particularly in genetics and developmental biology. *Drosophila melanogaster* has been used as a model organism for research for almost a century, and today, several thousand scientists are working on many different aspects of the fruit fly. *D. melanogaster* exhibits sexual dimorphism: females are about 2.5mm long; males are slightly smaller with darker backs. Males are easily distinguished from females based on colour differences, with a distinct black patch at the abdomen, less noticeable in recently emerged flies and the sex combs (a row of dark bristles on the tarsus of the first leg) Further more males have a cluster of spiky hairs (claspers) surrounding the reproducing parts used to attach to

the female during mating. *D. melanogaster* is closely associated with humans and are often referred to as domestic species.

1.1 LIFE HISTORY OF *DROSOPHILA MELANOGASTER*

Drosophila melanogaster display a holometabulous method of development, meaning that they have 4 distinct stages of their life cycle, each with a radically different body plan: egg, larva, pupa and finally adult. The eggs have one or more respiratory filaments near the anterior end; the tips of these extend above the surface and allow oxygen to reach the embryo. Larvae feed not on vegetable matter itself but on the yeasts and micro organisms present on the decaying breeding substrate. Development time varies widely between species and depends on the environmental factors such as temperature, breeding substrates and crowding.

Fruit flies begin their lives as an embryo in an egg. This stage lasts for about one day. During this time embryo develops into a larva. The larva is white, segmented and worm like. The larval stage is a feeding stage and consists of three subdivisions called instars.

The first instar larva hatches out of the egg, crawls into a food source and eats. The larva in each stage eats as much as possible. After a day, the first instar larva molts and becomes the second instar larva. Again the larva in this stage eats and eats. After a day in this stage, the larva molts again to

become the third instar larva. After 2 days of eating in this stage, the larva crawls out of the food source and molts again.

Following this molt, the larva stops moving and forms a pupa. *Drosophila* stays in the pupa for about five days. During this time, the metamorphosis or change from larva to adult is occurring. Adult structures like, wings, legs and eye develop.

When the adult emerge from pupa, they are fully formed. They become fertile after about 10 hours, copulate, the female lay eggs, and the cycle begins again. The whole life cycle takes about 10 -12 days.

The first, second and third instars of *Drosophila* can distinguished only by their size differences. Second instar larvae are twice the length of first instar. Third instar larvae is twice the length of second instar.

Adult *Drosophila* is small flies, typically pale yellow to reddish brown to black, with red eyes. The plumose arista, bristling of the head and thorax, and wing venation are characters used to diagnose the family. Most are small about 2-4 millimeters long.

1.2 INSECT HORMONES OF *DROSOPHILA MELANOGASTER*

Almost every aspect of an insect's life is regulated by hormones at one time or another. Molting and metamorphosis are the most obvious of the endocrine stimulated events in the insect life cycle, and the best studied. But

hormones also control such disparate physiological and developmental phenomena as metabolism, water balance, seasonal polymorphisms, caste determination, reproductive cycles and diapause as well as behaviors such as eclosion, pheromone production, migration and social dominance. Insect hormones have a pervasive role in the regulation of post embryonic development.

1.2.1 ECDYSONE

Ecdysone is a steroidal prohormone of the major insect molting hormone 20-hydroxy ecdysone, which is secreted from the prothoracic glands.

1.2.2 JUVENILE HORMONE

Juvenile Hormones are a group of acyclic sesquiterpenoids that regulate many aspects of insect physiology. . It has a wide range of functions in regulating development and physiological process such as metamorphosis, caste determination, ovarian maturation, diapause and migration in insects (Riddiford., 1994 1996, Wyatt & Davy., 1996, Goodman and Granger., 2005).

Juvenile hormones are secreted by a pair of endocrine glands behind the brain called the corpora allata

1.2.3 EFFECTS OF JUVENILE HORMONE IN INSECTS

A holometabolous insect molt several times during the larval stages and then undergoes metamorphosis, first into a pupa and then into an adult. These processes are largely regulated by JH and the steroid hormone ecdysone (Riddiford *et al.*, 2001). In the presence of JH, ecdysone causes a molt to a similar stage, but in the absence of JH, ecdysone causes a metamorphic molt (Riddiford., 1994). This effect of JH is defined as its "status quo" action (Williams ., 1952).

1.3 INSECT GROWTH REGULATORS (IGRS)

The IGRs belong to a class of compounds which interfere with normal growth, development and reproduction of insects. These compounds are thought to be particularly attractive in pest control programmes as they are ecologically stable and environmentally safer substances, to which insects are unable to develop resistance. Juvenile Hormone Analogue is an example for insect growth regulators.

1.4 JUVENILE HORMONE ANALOGUES

Based on the studies of various physiological effects of JHs in insects, Williams (1967) suggested that this hormone or its analogues could be used as specific control agents to which pest species may be unable to develop resistance. This led to the discovery of juvenile hormone analogues (JHAs).

Advantages of JHAs are they are species specific, less or zero toxicity to other animals, their fast penetrance through the insect cuticle and they get degraded to non toxic compounds in a short time period.

1.5 PYRIPROXYFEN

Pyriproxyfen is a pyridine based pesticide which is found to be effective against a variety of arthropoda. It was introduced to the US in 1996 to protect cotton crops against whitefly. It has also found use protecting other crops and can also be used as a treatment for cat fleas.

1.6 LD 50 VALUE

LD stands for "Lethal Dose". LD_{50} is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals.

1.7 EFFECT OF PYRIPROXIFEN ON INSECT DEVELOPMENT

Pyriproxyfen is JH analogue preventing larvae from developing into adulthood and thus rendering them unable to reproduce. Nowadays Pyriproxyfen is effectively used as an Insect Growth Regulator (IGR) as a part of Integrated Pest Management system (IPM). Pyriproxyfen is reported to be effective in controlling Lepidopteran pests. But its efficacy towards diptera was also documented but to a lesser degree. It has a significant influence on larval instars of Drosophila melanogaster.

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1.8 EFFECT OF TEMPERATURE ON INSECT DEVELOPMENT

In this study, we consider the direct effects of temperature on chemically treated insect development. Existing studies suggest that direct effects of temperature are likely to be larger and more important than any other factor. Direct effects of temperature rise on insects may be greater in the Polar Regions than in temperate or tropical zones, reflecting the more severe environmental conditions, the tighter constraints and the prediction of much larger proportional temperature rises in these areas. The aim of this study is to explore the direct impacts of temperature elevation, as a principal driver of climate change, on the phenology, life cycles and distribution of insects .

1.9 EFFECT OF MOBILE RADIATION ON INSECT DEVELOPMENT

Mobile radiation has been reported to produce a number of biological effects on biomolecules, cells, and whole organisms, including changes in intracellular ionic concentrations, the synthesis rate of different biomolecules, cell proliferation rates, the reproductive capacity of animals, etc. During recent years, mobile phones, the most powerful RF transmitters in our everyday environment, have become widely and increasingly used by the public and to date there is no clear evidence about their possible biological effects. Our experiments were designed to test the effect of mobile radiation along with temperature on the development of chemically treated 3rd instar larvae of *Drosophila melanogaster*.

2 REVIEW OF LITERATURE

2.1 *DROSOPHILA MELANOGASTER* DEVELOPMENTAL STAGES

Laboratory egg to adult development time between the relatively optimal temperatures of 20–27°C varied by about 1 week from the shortest reported time of 9.7 days to longest development time of 17.1 days. Currently, little is known about the effect of yeast on larval diet suitability, development time, and survival rate although work in other *Drosophila* indicates that yeasts increase host suitability (Tatum 1939; Becher et al. 2012). The importance of yeast as a protein source is known for *D. melanogaster* (Good and Tatar 2001; Tu and Tatar 2003), and appears necessary for egg production in lab-reared *Drosophila* (A. Wallingford, unpublished data).

Chippindale *et al.* (1993) and Leroi *et al.* (1994 a,b) documented the apparent disappearance of the well-known life-history tradeoff between early fertility and late fertility/ survival described by Rose (1984). No general relationship exists between evolutionary rate and the time of genes expression. This may be true if the morphological conservation of developmental time points depends on a small number of developmentally important genes (Arthur 1997; Raff 1996).

2.2 ROLE OF JUVENILE HORMONE IN INSECT DEVELOPMENT

A holometabolous insect molt several times during the larval stages and then undergoes metamorphosis, first into a pupa and then into an adult. These

processes are largely regulated by JH and the steroid hormone ecdysone (Riddiford *et al.*, 2001). In the presence of JH, ecdysone causes a molt to a similar stage, but in the absence of JH, ecdysone causes a metamorphic molt (Riddiford., 1994). This effect of JH is defined as its "status quo" action (Williams., 1952).

Unlike lepidoptera and most other holometabolous insects, higher Diptera such as *Drosophila melanogaster* have lost most of their sensitivity to JH. Topical JH application cannot prevent the transition from larva to pupa in *D. melanogaster* (Ashburner.,1970; Postlethwait.,1974). Nevertheless application of high amount of JH or a JH mimic before or at the time of pupariation causes the formation of pupal–adult mosaic, a transparent abdomen covered with pupal cuticle with no or few short bristles and a relatively normal appearance of adult head and thorax (Ashburner.,1970; Postlethwait.,1974; Riddiford and Ashburner.,1991; Zhou & Riddiford., 2002).

Titers of the juvenile hormone III varied according to the stage of development. Highest levels were found in post feeding larvae and adults; only low levels were found in feeding last stadium larvae and in pupae. None of the other known JHs were detected (T.J. Sliter, B.J. Sedlak, F.C. Baker D.A. Schooley.,1987).

Using the hydroxyapatite assay, a JH III binding protein with a native molecular weight of nearly 400 kDa has been identified in the haemolymph of post feeding third instar larvae of *D. melanogaster*. The very high binding affinity and high specificity suggest that this JH III binding protein has a critical role in JH transport in the haemolymph (Lirim Shemshedini, Thomas, G. Wilson.,1988).

In all species, JH and their mimics, stimulate ovarian maturation and vitellogenin synthesis and that high doses of exogenous JHs or their mimics stimulate ovarian ecdysteroid synthesis at least in *Aedes aegypti, Aedes abropalpus,* and *D. melanogaster.* In *Musca domestica* and possibly *D. melanogaster*, 20-hydroxy ecdysone is present in the haemolymph at vitellogenic levels in newly emerged females and may persist in ovaryectomized adults. This may be the reason why topical application of JH or its mimics, to ovaryectomized isolated abdomens is effective in stimulating vitellogenin synthesis in the absence of the ovaries (Thomas J. Kely.,T.S. Adams, Margaret B. Schwartz, Mark T. Binnbaum, Elaine C. Rubenstein and Richard B. Imberski.,1987). The production of control levels of vitellogenin requires both JH and 20- hydroxy ecdysone (T.S. Adams & P.A. Filipi.,1987).

Male last instar larvae and adult females of *Locusta migratoria* respond positively to the application of the juvenile hormone analogues methoperene and Pyriproxyfen, showing increase in vitellogenin gene

expression (Dhadialla *et al.*,1987). Also in *Blatella germanica* females, vitellogenin gene expression is induced by topical application of JH (Comas *et al.*,1999). In Diptera it has been shown that the JH as well as ecdysone, are responsible for the induction of yolk protein gene expression (Hagedorn., 1994; Bownet.,1986, 1984).

Injection of *dl* juvenile hormone or C_{17} methyl ester into *Sarcophaga bullata* larvae prevents puparium formation or arrests development at about the 3rd day of pupal–adult development. Topical application to the abdomens of young pupae results in the secretion of a second pupal cuticle. This is the first reported morphogenetic effect of juvenile hormone on a fly (V.S. Srivastava and Lawrence I. Gilbert.,1968).

Application of Juvenoid on the Mediterranean fruit fly, *Ceratitis capitata* (Diptera, Tephritidae) within 24 hr before or after puparium formation caused disturbance in imaginal disc differentiation. Treated insects developed either into pupa-adult intermediates failing to emerge or into defective adults whose fecundity was severely decreased (D.S. Daoud & F. Sehnal.,1974).

2.3 APPLICATION OF JUVENILE HORMONE ANALOGUE (JHA)

Already 500 analogues with JH activity has been discovered (Slama *et al.*,1974; Romanuk.,1981). Among the well known JHAs are, Epofenonane (Handgartner *et al.*,1976), Methoprene (Henrick *et al.*,1976). Hydroprene

(Henrick *et al.*,1976) Kinoprene (Henrick *et al.*, 1976) and Phenoxyphenoxy carbamate (Peleg.,1982). The first JHA of commercial success were Methoprene and Hydroprene (C.A. Henrick, G.B staal and J.B. Siddal.,1973). Methoprene is active against dipteran insects and fleas and hydroprenes is active against cockroach. These compounds however, were too unstable under field conditions to be used for agriculture. Dorn *et al.* (1981) reported on the photostable JH analogue, fenoxycrab. Fenoxycrab was effective not only on household pest but also on agricultural pest such as leaf rollers, the codling moth and *psylla pyricola*.

Several JHAs were later synthesized and evaluated them for the control of household and agricultural pests, finally discovering pyriproxyfen as one of the most potent JHAs known to date (Masachika Hirano *et al.*,1998).

A few JHAs interfere with the activity of others hormones. Methoprene inhibits the secretion of prothoracicotropic hormone. This results in an inhibition in the secretion of ecdysone in *Mamestra brassicae* (Hiruma *et al.*, 1978). But in the last instar larvae of *M. brarrricae* and *Spodoptera mauritia* JHA stimulate the prothoracic glands to release β ecdysone (Hiruma *et al.*,1978, Balamani & Nair.,1989a).

JHAs affect the physiology of morphogenesis, reproduction and embryogenesis. Morphogenetic effect is more prominent during larval pupal transformation. Most common morphogenetic effect of JHA treatment is the

production of extra larval, nymphal or pupal form (Sehnal.,1971; Novak.,1974; Santha & Nair.,1987). The formation of extra larval instar depends on stage and age of the larvae at the time of treatment. Effects of JH analogue on the morphology of *Aedes scutellaris malayensis collers* (Diptera: Culicidae) was studied. It caused many morphogenetic aberrations on its larvae and pupae (Yodbestra, S. *et al*., 1985).

Embryogenesis is inhibited if JHAs are applied to eggs. Studies on *Bernisia tabaci* – a sweet potato white fly have revealed a juvenile hormone analogue – Pyriproxyfen is found to suppress embryogenesis and adult formation (Ishaaya I & Horowitz A.R., 1992). Various types of effects ranging from ovicidal effects to delayed effects during post embryonic life have been reported (Riddiford., 1971). Studies on *Pyrrhocoris apterus* - a linden bug have revealed that JHA is effective in preventing the postembryonic metamorphosis of pyrrhocoridae (Linn M. Riddiford and Carroll M. Williams.,1967).

2.4 EFFECT OF TEMPERATURE ON INSECT DEVELOPMENT

Climate change is occurring (Houghton et al. 1996, 2001). During the past 100 years global-average surface temperatures have increased by approximately 0.6 °C (the largest increase of any century during the past 1000 years), with the 1990s the warmest decade and 1998 the warmest year since instrumental records began (Houghton et al. 2001). The Third IPCC report

predicts that global-average surface temperature will increase further by 1.4±5.8 °C by 2100 with atmospheric carbon dioxide (CO2) concentrations expected to rise to between 540 and 970 p.p.m. over the same period. It is predicted that the British Isles will experience temperature and CO2 elevations of this magnitude (CCIRG 1991, 1996; Hulme & Jenkins 1998; Houghton et al. 2001). Rates of development of herbivore insects vary within season, depending on temperature and host plant condition. Example: the butterfly Aglais urticae on Urtica dioica (Bryant et al. 1997) and autoecious aphids such as Drepanosiphon platanoidis on Acer pseudoplatanus and Aphis rumicis on Rumex sp. (Dixon 1998). Temperature may induce changes in life-cycle duration (rate of development), voltinism, population density, size, genetic composition, extent of host plant exploitation as well as local and geographical distribution linked to colonization and extinction. These effects are likely to be greatest in above-ground herbivores, exposed to the full variability of micro- and macroclimate, while soil-dwelling species experience thermal regimes that are buffered by the denser soil environment. Many species are limited in their distribution by summer heat availability rather than the lethal effect of extreme temperatures. Examples include the Arctic aphid Acyrthosiphon svalbardicum on Dryas octopetala (Strathdee et al. 1993), the spittle bug Neophilaenus lineatus along an altitudinal temperature gradient in Scotland (Whittaker & Tribe 1996), the psyllids, Cacopsylla palmeni, C. brunneipennis and C. palmeni on Salix lapponum in

Norway (Hill & Hodkinson 1995), the British Strophingia sp. on Calluna sp. and Erica sp. (Miles et al. 1997; Hodkinson et al. 1999) and many British butter¯ies (Dennis 1993).

2.5 EFFECT OF MOBILE RADIATION ON INSECT DEVELOPMENT

In earlier experiments, performed to study the effects of magnetic fields (Ma and Chu, 1993; Ramirez et al., 1983) or RF fields (Pay et al., 1978), on the reproduction of the same insect, the procedures comprised counting of laid eggs, as a basic part. In addition in one of those works (Ma and Chu, 1993) flies were taken from the general stock population. Eggs from older flies have a considerable percentage of mortality. In another of those works (Ramirez et al., 1983), only the female flies were exposed, which were 4 days old and already mated when placed in the field. In the last of the above experiments (Pay et al., 1978), they studied the oviposition of individual pairs of adult flies that were developed from pupae that were exposed for only 10 min in a very intense microwave field (able to produce large temperature increases) 100 hr posthatching. Therefore, the exposure took place many hours before the beginning of oogenesis, which in Drosophila starts during the last stages of pupation (King, 1970).

3 OBJECTIVES OF THE PRESENT STUDY

The present study was undertaken with the aim of understanding the changes in the developmental stages of *Drosophila melanogaster,* a dipteran pest, after the treatment with a juvenile hormone analogue pyriproxyfen under the impact of different parameters like temperature, mobile radiation etc. Pyriproxyfen is reported to be operative in controlling lepidopteran pest. But its efficacy towards diptera was also documented to a lesser degree. Similarly studies are currently going on concerning the effect of different parameters on insect development. But at present, there are no well-defined documents available for the study of the effect of pyriproxifen under various situations such as high temperature and presence of mobile radiation. To study the influence on diptera, a model organism called *Drosophila melanogaster* was selected because of its short life cycle, ease in rearing and availability of genome sequence. Our atmosphere is now polluted with various types of radiations and so many other solid, gaseous and liquid contaminants, and we are also facing severe issues of global warming. In the scenario of increasing atmospheric pollution and temperature, study of the effect of these things on drosophila development is somewhat pertinent. Most of the pesticides, particularly insect growth regulators, are generally resorted to without testing its effects under different environmental circumstances. If its effects have substantial increase or decrease under these conditions, then, there may be changes in its prescribed amount. Pyriproxifen like IGR compounds have a

particular dosage value. If there is significant differences in its dosage value due to the variations in the environmental conditions, pesticide may become either nonfunctional or hazardous. Furthermore, as the role of insect is vital in the conservation of biodiversity, we should try to safeguard them from such environmental threats, if it has significant effects on its developmental stages. We should consider both of these situations and find an effective solution. In this context, the present study is more significant and apposite, and it is a pioneer step.

4 MATERIALS AND METHODS

4.1 INSECT

Drosophila is a genus of small flies, belonging to the family Drosophilidae and order Diptera

4.2 EXPERIMENTAL SETUP

Wooden chambers, thermometers, heat generating apparatus, mobile phone, culture bottle

4.3 COLLECTION OF EGGS AND LARVAE OF *DROSOPHILA MELANOGASTER*

Drosophila stock culture brought from Calicut University cultural lab is maintained in the Zoology laboratory of the college at room temperature.

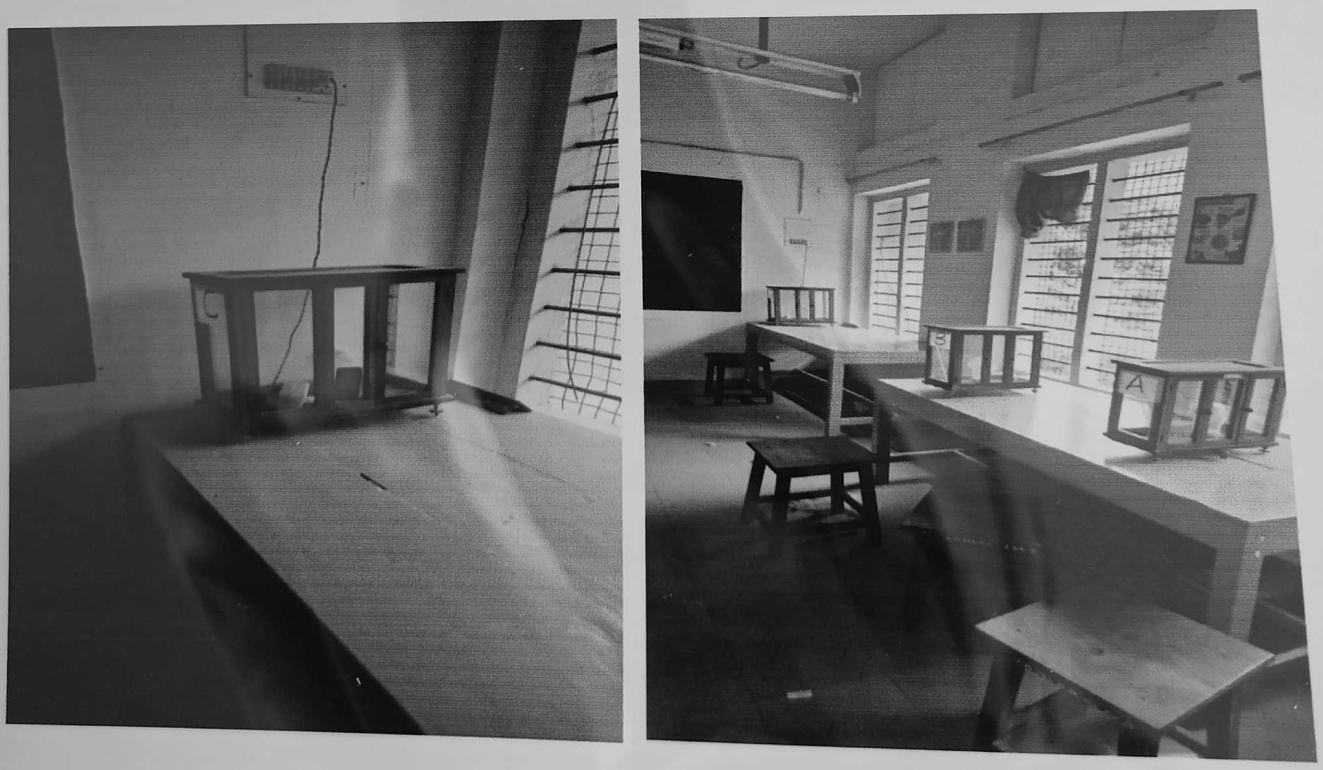
4.4 PREPARATION OF CULTURE MEDIA

Drosophila culture media was prepared according to the standard protocol followed by Mysore University. The composition of standard media, used in the present work, is given below.

Rawa	:	100 gms
Jaggery	:	100 gms
Bakers yeast	:	15 gms
Agar agar	:	10 gms

Plate – 1 *Drosophila Melanogaster* culture medium preparation

Plate – 2 Experimental setup for finding the effect of various factors on the development of 3rd Instar larvae *of Drosophila Melanogaster*



Water : 1 litre

The above medium was boiled in a vessel for 30 minutes and the temperature of the medium was brought to room temperature and 7.5 ml propionic acid was added as a mould inhibitor. The medium prepared was poured into sterile culture bottles. While preparing culture bottles, a uniform quantity of medium by weight was added in all trials (50 ml). Several grains (but not more) of yeast were also scattered to the media surface before adding flies. The bottles were well covered with sterilized cotton clothes.

4.5 EXPERIMENTAL SETUP FOR UN-TREATED LARVAE

4.5.1 EXPERIMENTAL SETUP FOR THE STUDY OF THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF 3RD INSTAR LARVAE.

Two equal sized wooden chambers covered with glasses were selected for the experiment and labeled as control, A and test, B. A heat producing apparatus was arranged in the test chamber B, and thermometers were also placed in all chambers for measuring the temperature. Culture bottles, each with 50 ml culture medium, were prepared, and placed one each in the chambers. Equal numbers of 3rd instar larvae were then transferred to both of these culture bottles. The bottles were continuously observed and the results were noted.

4.5.2 EXPERIMENTAL SETUP FOR THE STUDY OF EFFECT OF MOBILE RADIATION ON THE DEVELOPMENT OF 3RD INSTAR LARVAE.

Two equal sized wooden chambers covered with glasses were selected for the experiment and labeled as A and B. A fully charged mobile is placed in the test chamber B from 10 AM to 4 PM, with a view to producing mobile radiation throughout the experimental time. Culture bottles, each with 50 ml culture medium, were prepared, and placed one each in the chambers. Equal numbers of 3rd instar larvae were then transferred to both of these culture bottles. The bottles were continuously observed, and the results were noted.

4.5.3 EXPERIMENTAL SETUP FOR THE STUDY OF COLLECTIVE EFFECT OF TEMPERATURE AND MOBILE RADIATION ON THE DEVELOPMENT OF 3RD INSTAR LARVAE.

Two equal sized wooden chambers covered with glasses were selected for the experiment and labeled as A and B. A heat producing apparatus and a fully charged mobile (from 10 AM to 4 PM) was placed in the test chamber B with the aim of producing high temperature and mobile radiation simultaneously throughout the experimental time. Culture bottles, each with 50 ml culture medium, were prepared, and placed one each in the chambers. Equal numbers of 3rd instar larvae were then transferred to both of these culture bottles. The bottles were continuously observed, and the results were noted.

4.6 CHEMICAL AND TREATMENT

4.6.1 PYRIPROXYFEN

Pyriproxyfen is a pyridine based pesticide. It is a JH analogue which prevents larvae from developing into adulthood, and thus rendering them unable to reproduce.

4.6.2 TREATMENT TO TEST THE TOXICITY OF PYRIPROXYFEN.

To calculate the lethal dose, different concentrations of Pyriprixyfen (0.02 µg/µl to 0.08 µg/µl) were prepared and added to the bottle containing 50 ml sterile culture medium. A control bottle was also prepared, using equivalent volume of acetone. The 3rd instar larvae were then transferred to these treated bottle.

Dilution of Pyriproxyfen for Experiment

Pyiproxifen stock- 11.23 %

11.23 % = 11.23 g/100 ml

= 112.3 µg/ µl

Preparation of different dilution of Pyriproxyfen for 50 ml culture bottle

1. Preparation of 0.02 µg/ µl

Stock solution - 112.3 µg/ µl

Take 8.9 µl Pyriproxyfen stock and add 91.1 µl acetone into it and the final solution is added into the 50 ml culture bottle.

2. Preparation of 0.03 µg/ µl

Take 13.35 µl Pyriproxyfen stock and add 86.65 µl acetone into it and the final solution is added into the 50 ml culture bottle.

3. Preparation of 0.04 µg/ µl

Take 17.8µl Pyriproxyfen stock and add 82.2 µl acetone into it and the final solution is added into the 50 ml culture bottle.

4. Preparation of 0.05 µg/ µl

Take 22.25 µl Pyriproxyfen stock and add 77.5 µl acetone into it and the final solution is added into the 50 ml culture bottle.

5. Preparation of 0.06 µg/ µl

Take 26.7 µl Pyriproxyfen stock and add 73.3 µl acetone into it and the final solution is added into the 50 ml culture bottle.

6. Preparation of 0.08 µg/ µl

Take 35.6 µl Pyriproxyfen stock and add 64.4 µl acetone into it and the final solution is added into the 50 ml culture bottle.

Analysis

After a definite time interval, the bottles were checked to count the number of larvae that have been pupated, and compared them with the control larvae. By using these data LD 50 concentration was identified.

4.7 EXPERIMENTAL SETUP FOR TREATED LARVAE

4.7.1 EXPERIMENTAL SETUP FOR THE STUDY OF THE EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF 3RD INSTAR LARVAE TREATED WITH PYRIPROXIFEN.

Three equal sized wooden chambers covered with glasses were selected for the experiment, and labeled as A, B and C. A heat producing apparatus was arranged in the test chamber C, and thermometers were also placed in all chambers for measuring the temperature. Sub lethal concentration of pyriproxyfen was selected for the treatment and introduced into the bottles present in the chambers B and C. An equivalent volume of acetone was introduced in to the bottle of chamber A. The 3rd instar larvae were then transferred to all of these culture bottles. By using heating apparatus, Bottle C was exposed to high temperature. The bottles were continuously observed and the results were noted.

4.7.2 EXPERIMENTAL SETUP FOR THE STUDY OF EFFECT OF MOBILE RADIATION ON THE DEVELOPMENT OF 3RD INSTAR LARVAE TREATED WITH PYRIPROXIFEN.

Three equal sized wooden chambers covered with glasses were selected for the experiment and labeled as A, B and C. A fully charged mobile (from 10 AM to 4 PM) is placed in the test chamber C with the aim of generating mobile radiation. Sub lethal concentration of pyriproxyfen was selected for the treatment, and introduced into the bottles present in the chambers B and C. An equivalent volume of acetone was introduced into the bottle of chamber A. The 3rd instar larvae were then transferred to all of these culture bottles. The bottles were continuously observed and the results were noted.

4.7.3 EXPERIMENTAL SETUP FOR THE STUDY OF CUMULATIVE EFFECT OF TEMPERATURE AND MOBILE RADIATION ON THE DEVELOPMENT OF 3RD INSTAR LARVAE TREATED WITH PYRIPROXIFEN.

Three equal sized wooden chambers covered with glasses were selected for the experiment and labeled as A, B and C. A heat producing apparatus and mobile phone (from 10 AM to 4 PM) for generating mobile radiation were arranged in the test chamber C, and thermometers were also placed in all chambers for measuring the temperature. Sub lethal concentration of pyriproxyfen was selected for the treatment and introduced into the bottles present in the chambers B and C. An equivalent volume of acetone was introduced in to the bottle of chamber A. The 3rd instar larvae were then transferred to all of these culture bottles. In the presence of heating apparatus and Mobile phone, bottle C was simultaneously exposed to both high temperature and mobile radiation. The bottles were continuously observed and the results were noted.

5 RESULTS & DISCUSSION

5.1 EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF 3RD INSTAR LARVAE.

Third instar larvae of *D.melanogaster* were exposed to high temperature (35 to 40°C). Duration for the larval developmental stages from 3rd instar larvae, number of larvae pupated and the total number of insect emerged etc. were counted from the test bottle and compared to that of the control bottle. Percentage pupated in the test was calculated by taking the number pupated in control as 100%. The results are shown in table-1. At high temperature, duration of the conversion from larvae to pupae was reduced by one day. In both control and test, the number of pupae formed was almost same. There was no significant difference. But the number of insect emerged was reduced to almost half in the test compared to control. From the above information we can clearly say that temperature has an active role in the development of *Drosophila melanogaster.*

5.2 EFFECT OF MOBILE RADIATION ON THE DEVELOPMENT OF 3RD INSTAR LARVAE.

3rd instar larvae of *D.melanogaster* were exposed to mobile radiation almost 6 hours a day (from 10 AM to 4 PM). Duration for larval developmental stages from 3rd instar larvae, number of larvae pupated and the total number of insect emerged etc. were counted from the test bottle and compared to that of

control. Percentage pupated in the test was calculated by taking the number pupated in control as 100%. The results are shown in table-2.

When the 3rd Instar larvae of Drosophila melanogaster were exposed to mobile radiation, there was no change in larvae to pupae and pupae to larvae conversion as shown in table 2. Exposed larvae percentage survival was almost 70 %. There was no significant difference in the number of pupae, comparing both control and test. There seems to be no remarkable variations in the exposure of mobile radiation. But, the number of insect emerged was reduced a little. From the above information, we could not predict evidently the efficacy of mobile radiation on insect development.

5.3 COMBINED EFFECT OF TEMPERATURE AND MOBILE RADIATION ON THE DEVELOPMENT OF 3RD INSTAR LARVAE.

3rd instar larvae of *D.melanogaster* were exposed to both high temperature (35-40 °C) and mobile radiation (from 10 AM to 4 PM) synchronously. Duration for larval developmental stages from 3rd instar larvae, number of larvae pupated and the total number of insect emerged etc. were counted from the test bottle and compared to that of control. Percentage pupated in the test was calculated by taking the number pupated in control as 100%. The results are shown in table-3

In the presence of mobile radiation and high temperature, duration for larvae to pupae conversion was reduced by one day. There was no significant

TABLE 1 - EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF 3RD INSTAR LARVAE OF *DROSOPHILA MELANOGASTER*

	Duration (Days)		Total number of larvae	Number of larvae pupated	Number of insects emerged
	Larvae to pupae	Pupae to adult			
Room temperature (control)	1	5	20	19	11
High temperature (35- 40 °C - Test)	1	4	20	17	6

TABLE 2 - EFFECT OF MOBILE RADIATION ON THE DEVELOPMENT OF 3RD INSTAR LARVAE OF *DROSOPHILA MELANOGASTER*

	Duration (Days)		Total number of larvae	Number of larvae pupated	Number of insects emerged
	Larvae to pupae	Pupae to adult			
Control	1	5	20	18	10
Exposed to mobile radiation	1	5	20	19	7

TABLE 3 – COMBINED EFFECT OF TEMPERATURE & MOBILE RADIATION ON THE DEVELOPMENT OF 3RD INSTAR LARVAE OF *DROSOPHILA MELANOGASTER*

	Duration (Days)		Total number of larvae	Number of larvae pupated	Number of insects emerged
	Larvae to pupae	Pupae to adult			
control	1	5	20	18	12
High temperature + mob Radiation	1	4	20	17	6

difference in the number of pupae, comparing both control and test. But the number of insect emerged was reduced to half in the test compared to control. Comparing table 1, 2 and 3, we can indubitably say that the presence of high temperature and not the mobile radiation which is the reason for delay in 3rd Instar larvae molting into pupae. These two factors have no substantial effect in pupation. Number of pupation is almost same in all cases. Combined effects of temperature and mobile radiation decrease insect emergence.

5.4 TOXICITY OF PYRIPROXYFEN TO 3RD INSTAR LARVAE OF *DROSOPHILA MELANOGASTER.*

3rd instar larvae of *D.melanogaster* were exposed to different concentrations (.01 µg/µl,.02 µg/µl,.o4 µg/µl,.06 µg/µl,.08 µg/µl) of Pyriproxyfen, to test the toxicity. Number of larvae pupated after the treatment was counted and compared to that of control. Percentage pupated in the treatment was calculated by taking the number pupated in control as 100%. With increase in the concentration of Pyriproxyfen, the percentage of 3rd Instar larvae survived up to pupation decreased as shown in Table 4. Form the data we can clearly state that the effects of Pyriproxyfen on third instar larvae of Drosophila melanogaster were dependent on concentrations, and pupation decreased from 75% (0.08 µg/µl) to 0% (0.02 µg/µl) of control. From the Dose responsive curve as shown in the figure 1, the LD 50 value of Pyripoxifen For third instar larvae of Drosophila melanogaster was determined to be 0.035 µg/µl. Beyond

this sub lethal concentration, more than 50 % of the death of total larvae usually occurred.

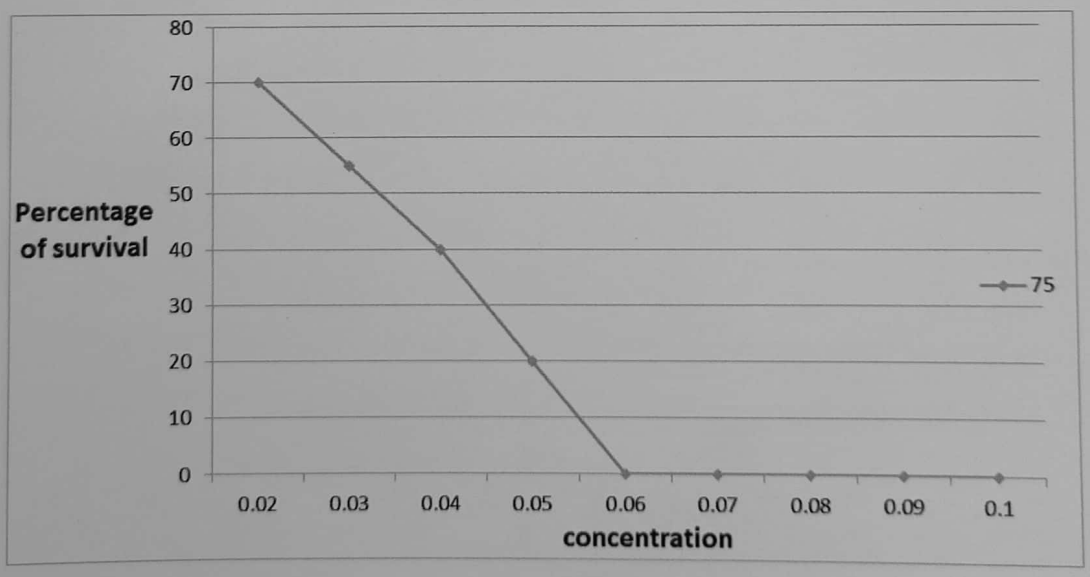
5.5 EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF 3RD INSTAR LARVAE TREATED WITH PYRIPROXIFEN.

Out of three chambers labeled as A, B and C, Culture medium present in the chamber C was exposed to high temperature (35-40°C). All the chambers except A were treated with Pyriproxifen. Duration for larval developmental stages from 3rd instar larvae, number of larvae pupated and the total number of insect emerged etc. were counted from the test bottle and compared to that of control. Percentage pupated in the test was calculated by taking the number pupated in control as 100%. The results are shown in table-5. Pyriproxifen treated larvae shows a delay in its molting to pupae at room temperature compared to its control. But, at high temperature, it turned out to be normal as control. There was no delay for pupation, and it seems to be the abolishing effect of high temperature over the outcome of pyriproxifen. A reverse reaction was happened in the total duration for pupal period. Pupal period normally lasts for 5 days. But, at high temperature, duration for pupae to adult conversion was reduced by one day, which was evidently specified in table-1. But as shown in table-5, the presence of pyriproxifen upturned this effect and it became normal as control. There was a substantial reduction in the number of pupae of A compared to B, and it shows that pyriproxifen has a direct role for reducing the pupation rate of *Drosophila melanogaster*. Number of

TABLE 4 - TOXICITY OF PYRIPROXYFEN TO THE LARVAE OF *D.MELANOGASTER*

Different concentrations of Pyriproxyfen	Number of LARVAE		Percentage survived in the Test
	Test	Control	
0.08 µg/µl	0	20	0 %
0.06 µg/µl	0	20	0 %
0.05 µg/µl	4	20	20 %
0.04 µg/µl	8	20	40 %
0.03 µg/µl	11	20	55 %
0.02 µg/µl	15	20	75 %

FIG 1. DOSE RESPONSIVE CURVE FOR LD 50 CALCULATION



From the Dose responsive curve that is shown above (Fig.1), the LD 50 value was determined to be 0.035 µg/µl

pupae in B and C is almost same, which indicates that temperature has no definite role in larvae to pupae conversion. But the number of insect emerged was reduced to almost half in the B compared to A and in the C compared to B. It denotes that both pyriproxifen and high temperature will reduce the insect emergence. From the above information, we can clearly say that both pyriproxifen and high temperature have a dynamic role in the development of *Drosophila melanogaster*.

5.6 EFFECT OF MOBILE RADIATION ON THE DEVELOPMENT OF 3RD INSTAR LARVAE TREATED WITH PYRIPROXIFEN.

Out of three chambers labeled as A, B and C, Culture medium present in the chamber c exposed to mobile radiation from 10 Am to 4 PM. All the chambers except A were treated with Pyriproxien. Duration for larval developmental stages from 3rd instar larvae, number of larvae pupated and the total number of insect emerged etc. were counted from the test bottle and compared to that of control. Percentage pupated in the test was calculated by taking the number pupated in control as 100%. The results are shown in table-6. As per the table, Pyriproxifen treated larvae exhibits a delay in larvae to pupae conversion at room temperature compared to its control. It remained as same when it is exposed to mobile radiation. Similar to the previous experiments, this one also expressed no change in pupal period in all the bottles. There was a substantial drop in the number of pupae of A compared to B which shows that pyriproxifen has a direct role for reducing the pupation

TABLE 5 - EFFECT OF TEMPERATURE ON THE DEVELOPMENT OF 3RD INSTAR LARVAE TREATED WITH PYRIPROXIFEN.

Experiment	Duration (Days)		Number		
	Larvae to pupae	Pupae to adult	Total Larvae	pupated	Emerged insect
A- (control)	1	5	20	19	12
B-(Treated with Pyriproxifen	1.5	5	20	12	7
C-(Pyriproxifen + high temperature)	1	5	20	11	3

TABLE 6 - EFFECT OF MOBILE RADIATION ON THE DEVELOPMENT OF 3RD INSTAR LARVAE TREATED WITH PYRIPROXIFEN.

Experiment	Duration (Days)		Number		
	Larvae to pupae	Pupae to adult	Total Larvae	pupated	Emerged insect
A- (control)	1	5	20	17	11
B- (Pyriproxifen)	1.5	5	20	11	6
C- (Pyriproxifen + mobile radiation)	1.5	5	20	10	5

TABLE 7 - COMBINED EFFECT OF MOBILE RADIATION AND TEMPERATURE ON THE DEVELOPMENT OF 3RD INSTAR LARVAE TREATED WITH PYRIPROXIFEN.

Experiment	Duration (Days)		Number		
	Larvae to pupae	Pupae to adult	Total Larvae	pupated	Emerged insect
A- (control)	1	5	20	18	12
B-(Treated with Pyriproxifen)	1.5	5	20	11	6
C-Pyriproxifen+ High temperature + mobile radiation	1	5	20	5	1

rate of *Drosophila melanogaster*. Number of pupae in B and C is almost same which designates that exposure to mobile radiation will not generally affect drosophila for its larvae to pupae conversion. The number of insect emerged was reduced to almost half in the B compared to A. But there was only a minor difference in C compared to B and it hints that only pyriproxifen has a direct role in the insect emergence. The effect of mobile radiation is not much significant.

5.7 COMBINED EFFECT OF MOBILE RADIATION AND TEMPERATURE ON THE DEVELOPMENT OF 3RD INSTAR LARVAE TREATED WITH PYRIPROXIFEN.

Out of three chambers labeled as A, B and C, Culture medium present in the chamber C was exposed to both factors, that is, high temperature and mobile radiation (from10 AM to 4 PM). All the chambers except A were treated with Pyriproxien. Duration for larval developmental stages from 3rd instar larvae, number of larvae pupated and the total number of insect emerged etc. were counted from the test bottle and compared to that of control. Percentage pupated in the test was calculated by taking the number pupated in control as 100%. The results are shown in table-7

As per this result, Pyriproxifen treated larvae exhibits a delay for larvae to pupae conversion at room temperature comparing bottle B with control. But, Larvae exhibits normal rate of pupal conversion in bottle C in which treated larvae was exposed to high temperature and mobile radiation.

There was no delay for pupation. Analyzing the data in the tables from 1 to 7, we can evidently predict that it is the invalidating effect of high temperature over the effect of pyriproxifen. But in this case, mobile radiation has almost neutral role. There was no change in the pupal period in all cases. A substantial reduction in the number of pupae of A compared to B shows that pyriproxifen reduces the pupation rate of *Drosophila melanogaster*. Number of pupae in B and C are almost same. It shows that the high temperature and mobile radiation have no definite role in larvae to pupae conversion. But the number of insect emerged was reduced to almost half in the B compared to A. Only one insect that emerged in C as shown in table 7, specifies that, the collective effect of mobile radiation and high temperature highly reduces the insect emergence.

Percentage insect emergence graph (Fig.2) evidently shows far-reaching changes in the percentage emergence of insect while adding both of the factors simultaneously. In the control, the emergence of insect was 12 out of 18. Considering the control emegence as hundred percentage, only 8.3 percentage (1 out of 12) insect emergences were exhibited by the cumulative effect of both mobile radiation and temperature. There were not many changes in the pupation percentage graph (Figure 3) except in the combined effects of high temperature and mobile radiation on 3rd instar larvae treated with pyriproxifen which shows a significant decrease in the number of pupation.

FIG 2 PERCENTAGE INSECT EMERGENCE (CONTROL IS TAKEN AS 100 PERCENTAGE)

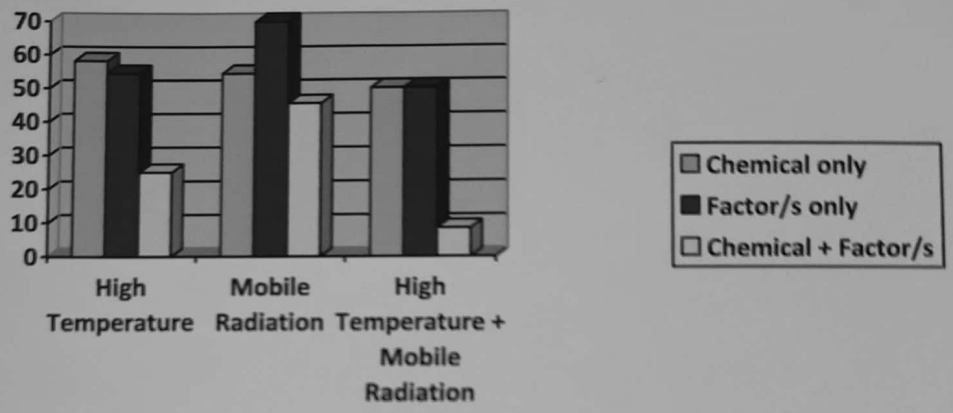
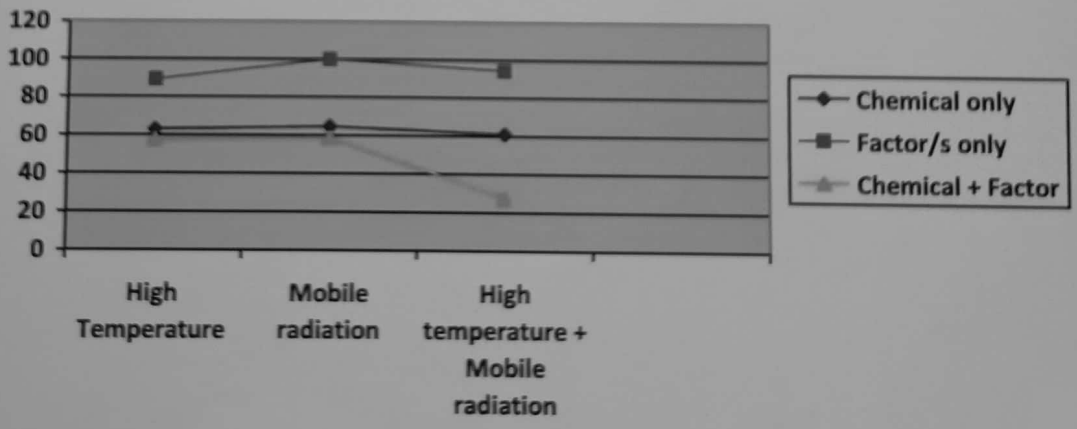


FIG 3 PUPATION PERCENTAGE (CONTROL IS TAKEN AS 100 PERCENTAGE)



During the past 100 years, global-average surface temperatures have increased by approximately 0.6 °C. The Third IPCC report predicts that global-average surface temperature will increase further by 1.4±5.8 °C by 2100. At the present time, our atmosphere is polluted with various types of radiations especially mobile radiation. In the circumstances of increasing atmospheric pollution and temperature, study of the effect of these things on drosophila development is somewhat pivotal.

Most of the pesticides, particularly, insect growth regulators are generally using without testing its effects under different environmental circumstances. In our study, we proved that, pyriproxifen, an insect growth regulator, used as a pesticide to control insects, has substantial rise in its activity under the state of affairs of high temperature and mobile radiation. If it is so, there may be changes in the sub lethal dose of pesticide not only pyriproxifen, but also other pesticides which are used all over the world. Generally pesticides are not highly specific and they may have effect on wide range of organism including non-targeting and beneficial organisms. So, we should be very carefull about the usage of such chemicals. The present study gives a warning that if the sub lethal doses of various chemicals changed from normal value, either decrease or increase due to global warming and or over use of mobile phone, and that may cause wide range of problems in the living world.

In most of the cases LD 50 were determined long before by using conventional methods, without properly considering the influence of environmental factors on it. So, we have to check the present toxicity of the pesticides immediately in order to safeguard the organisms and conserve biodiversity globally.

7 CONCLUSION

Pyriproxifen like IGR compounds has a precise dosage value. Pesticide may become either nonfunctional or hazardous if there is substantial differences in its dosage value in consequence of the disparities in the environmental situations. Furthermore, as the role of insect is vital in the conservation of biodiversity, we should try to protect them from such environmental threats, if it has significant effects on its developmental stages. As part of this study, we considered both of these situations.

The number of insect emerged was reduced to almost half in the test compared to control both in treated and untreated condition in the experiment conducted for finding the effects of temperature on the development of 3rd instar larvae of *Drosophila melanogaster*. From that, we can undoubtedly say that temperature has a dynamic role in the development of *Drosophila melanogaster*. There was no significant change in larvae to pupae and pupae to larvae transformation when the 3rd Instar larvae of Drosophila melanogaster were exposed to mobile radiation. But the number of insect emerged was reduced a little. The result was almost same in the case of treated larvae experiment. Insect emergence was highly reduced as a result of the joint effects of mobile radiation and high temperature. This reduction was extreme in the treated Drosophila. Percentage insect emergence graph (Fig.2) clearly shows

drastic changes in the percentage emergence of insect while adding both factors simultaneously.

In this study, we proved that, pyriproxifen an insect growth regulator, used as a pesticide to control insects, has substantial increase in its activity under the conditions of high temperature and mobile radiation. The present study gives a warning that it may cause wide range of problems in the living world if the sub lethal doses of various chemicals changed from normal value, either decrease or increase due to global warming and/or over use of mobile phones. In most of the cases, LD 50 were determined long before by using conventional methods, without properly considering the influence of environmental factors on it. So, in order to safeguard the organisms and conserve biodiversity globally, we have to check the present toxicity of the pesticides immediately. Generally, pesticides are not highly specific and they may have effects on wide range of organisms including non-targeting and beneficial organisms. It will be in an inconsequential amount normally. But it may negatively affect non-targeting or beneficial organisms if the dosage value is changed drastically.

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