OPTIMIZATION OF FRUIT FLY (*Drosophila melanogaster*) CULTURE MEDIA FOR HIGHER YIELD OF OFFSPRING

By

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ABSTRACT

OPTIMIZATION OF FRUIT FLY (*Drosophila melanogaster*) CULTURE MEDIA FOR HIGHER YIELD OF OFFSPRING

Tee Sui Yee

Drosophila melanogaster is one of the most widely used model organism in research on genetics and genome evolution. Mass culture of *D. melanogaster* is important to produce enough amounts of flies for research purposes. Various culture media have been formulated using simple and economic methods to produce large amounts of *Drosophila*. In this study, ten different culture media were formulated to culture inbred *D. melanogaster* and used as attractant to collect Kampar wild-type *Drosophila* species. Banana medium was used as the positive control medium and plain agar was used as the negative control medium. For inbred *D. melanogaster*, the number of pupal cases and hatched flies were calculated for two generations while only the number of pupal cases was calculated for wild-caught *Drosophila* species. The results were analyzed by using one-way ANOVA, Tukey's HSD multiple range test and paired sample t-test. One-way ANOVA showed that there were significant differences ($p \leq 0.05$) in the numbers of inbred offspring and also the numbers of wild-caught *Drosophila* species among different culture media. For inbred *D. melanogaster,* the banana and egg medium managed to breed the highest number of offspring for both generations. Meanwhile, the medium with a mixture of fruits and potato

managed to attract and propagate the highest number of wild-caught *Drosophila* species.

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Last but not least, I would like to show my appreciation to my friends and those who lent me a hand, supported and guided me in the process of completing this final year project.

DECLARATION

I hereby declare that the project report is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UTAR or other institutions.

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Tee Sui Yee

APPROVAL SHEET

This thesis entitled "**OPTIMIZATION OF FRUIT FLY (*Drosophila melanogaster*) CULTURE MEDIA FOR HIGHER YIELD OF OFFSPRING"** was prepared by TEE SUI YEE and submitted as partial fulfillment of the requirements for the degree of Bachelor of Science (Hons) in Biotechnology at Universiti Tunku Abdul Rahman.

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PERMISSION SHEET

It is hereby certified that **TEE SUI YEE** (ID No: **08ALB05459**) has completed this thesis/dissertation entitled "**OPTIMIZATION OF FRUIT FLY (*Drosophila melanogaster*) CULTURE MEDIA FOR HIGHER YIELD OF OFFSPRING**" under the supervision of Dr. Gideon Khoo from the Department of Science, Faculty of Science.

I hereby give permission to my supervisors to write and prepare a manuscript of these research findings for publishing in any form, if I did not prepare it within six (6) months time from this date, provided, that my name is included as one of the authors for this article. Arrangement of names will depend on my supervisors.

Yours truly,

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(TEE SUI YEE)

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LIST OF ABBREVATIONS

ANOVA	Analysis of Variance
HSD	Honestly Significant Difference
df	Degree of Freedom
H_0	Null Hypotheses
H_1	Alternative Hypothesis
cm	Centimeter
mL	Milliliter
g	Gram
℃	Degree Celsius

CHAPTER 1

INTRODUCTION

The genus *Drosophila* consists of various species of fruit flies which are widely used as model organisms in research on genetics and genome evolution. As model organisms, genus *Drosophila* has well-defined phylogeny and most of the *Drosophila* species have extensive literature on their genetics, development and ecology. Besides, the short generation time of *Drosophila* aided the studies on genetics especially studies of the laws of heredity (Markow & O'Grady, 2006). The first *Drosophila* research paper was published by Thomas Hunt Morgan in 1910, entitled "Sex limited inheritance in *Drosophila*" (Kenney & Borisy, 2009).

One of the most extensively used model organisms is *Drosophila melanogaster*, which is also known as the Cinderella of genetics (Ranganath, 1999). *D. melanogaster* is widely used as the model system in genomic research for nearly a century (Kenney & Borisy, 2009).This species of fruit flies not only possesses of well-defined genetics information, they also have short generation time which one generation only requires about two weeks. In addition, one pair of parent flies is able to produce several hundreds offspring which ease the process of genetics (Demerec & Kaufmann, 1996).

As similar to most of the insects, *Drosophila melanogaster* has four main stages for their life cycle, which are egg, larva, pupa and adult stages. Duration of life cycle for *D. melanogaster* is largely affected by temperature. For optimal results of culturing these flies, cultures are usually incubated at a constant temperature between 20 ℃ and 25 ℃ (Sandhyarani, 2010; Purves, Orians, Heller & Sadava, 1998).

Drosophila melanogaster can be raised easily wherever fermentation is in progress. Fermentation is one of the most important elements in breeding *Drosophila* although the roles of fermentation are still being unknown (Dirkson, 2009). In laboratories, *D. melanogaster* are usually not bred directly with overripe and fermented fruits because the fruit culture media will become too soft by the time new flies begin to hatch due to the process of fermentation. Therefore, *D. melanogaster* are bred in more solid culture media with yeast added manually to ferment those culture media. One of the most common ingredients added to fruit culture media in order to solidify the culture media is agar (Demerec & Kaufmann, 1996).

In addition, sugar content in *Drosophila* culture media must be sufficient too. This sugar content is not only vital for the growth of *Drosophila*, it is also required by yeast for fermentation. Hence, fruits with high sugar contents are usually chosen to be made into *Drosophila* culture media. The most common fruit used to culture *Drosophila* is banana, which is known for its carbohydrate content. Banana

culture medium is the current standard culture medium for *Drosophila* (Stocker & Gallant, 2007). This medium has been used since 1907 when Thomas Hunt Morgan performed his research on *Drosophila* (Hanschen, 1993). There have not been many improvements on the ingredients of this medium because other tropical fruits have not been tested as a suitable medium to complement banana medium.

There are two main objectives of carrying out this study. The first aim was to test the effect of different culture media on the hatching and growth of *Drosophila melanogaster*. Secondly, the effect of different culture media on collecting and hatching wild-caught *Drosophila* species were examined. The combination of objectives of this study is to investigate and develop a more suitable culture medium for culturing *D. melanogaster*.

In this study, five different types of single fruit culture media and another five different types of culture media with a mixture of fruits and/or other ingredients were formulated and prepared. Banana medium was prepared as standard medium (positive control), and plain agar was used as negative control medium. The different types of media comprised different nutritional values and sugar contents. Therefore, rate of regeneration of *D. melanogaster* in each medium was expected to be different compared to the others.

Inbred *Drosophila melanogaster* were used to investigate the effect of different culture media on their hatching and growth. Wild-type *D. melanogaster* from stock culture were subcultured into the ten different types of test culture media, positive and negative control media to produce F1 generation. The F1 generation of flies was then subcultured to fresh media to produce F2 generation. The numbers of pupal cases and imago of both F1 and F2 generations were calculated. The results were analyzed using one-way ANOVA test, Tukey's HSD multiple range test and paired sample t-test to determine which culture media promote the hatching and growth of *D. melanogaster*.

These ten culture media along with the positive and negative control media were used as attractants to collect Kampar wild-type *Drosophila* species from open environment. The number of pupal cases produced by wild-caught *Drosophila* in each medium was calculated and analyzed using one-way ANOVA test and Tukey's HSD multiple range test. The analysis helps in determining the more attractive culture media for wild-caught *Drosophila* species to lay and hatch their eggs.

The null hypothesis of this study stated that no significant difference in the numbers of pupal cases and imago would be observed among the different culture media for inbred *Drosophila melanogaster*. The null hypothesis also assumed that the numbers of pupal cases produced by wild-caught *Drosophila* species were equal in all types of culture media.

CHAPTER 2

LITERATURE REVIEW

2.1 The classification of *Drosophila melanogaster*

The fruit fly, *Drosophila melanogaster* is classified under Phylum Arthropoda. The characteristics of an arthropod are invertebrate, exoskeleton, a segmented body and jointed appendages. Exoskeleton is the external skeleton which composed of chitin in insects while appendages refer to the external body parts which protrude from an organism's body. Examples of appendages which can be observed on *Drosophila* are wings, antennae, and mouthparts (Lankester, 1904).

Under Phylum Arthropoda, *Drosophila melanogaster* is classified under class insecta and order diptera (Cranston & Gullan, 2002). As an insect, *D. melanogaster* has the characteristics of chitinous exoskeleton, segmented body (head, thorax and abdomen), three pairs of legs, compound eyes and two antennae. Compound eyes are eyes which composed of thousands of individual photoreceptor units, known as ommatidia (Chapman, 1998; Rash & Carde, 2003). Diptera is the branch group of true flies which each of the fly possesses only a pair of wings (Blagoderov, Lukashevich & Mostovski, 1758).

The superfamily of flies is known as Ephydroidea, consists of several families and the two largest families are Ephydridae and Drosophilidae. Drosophilidae is

further divided into two subfamilies, Steganinae and Drosophilinae. The Steganinae is a poorly understood subfamily and contains about 400 described species only. Unlike Steganinae, subfamily Drosophilinae is a bigger subfamily and more well-studied. *D. melanogaster* is categorized under family Drosophilidae and subfamily Drosophilinae (Throckmorton, 1975; Markow & O'Grady, 2006).

The subfamily Drosophilinae is divided into seven genera, of which *Drosophila* is the largest and most well-studied genus. There are more than 1500 described species under genus *Drosophila* and they are divided into ten different subgenera. Among the subgenera, subgenus *Drosophila* is the largest group followed by subgenus *Sophophora.* Both of these groups comprise approximately 90% of the diversity in the genus *Drosophila* (Okada, 1986).

The subgenus *Sophophora* was created by Sturtevant (1939) and *D. melanogaster* is categorized under this subgenus (O'Grady & Kidwell, 2002). The species *melanogaster* is the most extensively used *Drosophila* species in research on genetics and genome evolution. *D. melanogaster* is intensively studied and its genome is completely sequenced. It has been used for genomic research for nearly a century. "Cinderella of genetics" is a name given to *D. melanogaster* due to its contribution in this field. Although new species emerged for research along these years, *D. melanogaster* is still one of the most widely used species (National

Aeronautics and Space Administration [NASA], 2006; Ashburner, Golic & Hawley, 1989).

2.2 Distribution of *Drosophila*

The studies on *Drosophila* distribution by Patterson and Stone (1952) is one of the best studies on *Drosophila* distribution and the locality data in this genus. This study consists of the distribution of all *Drosophila* species (more than 600 species) known before the year of 1952. The wide range of *D. melanogaster* habitats and survival are mainly limited by low temperature and the lack of water. Therefore, *D. melanogaster* is found in almost every continent of the world except Antarctica but the distribution of *D. melanogaster* is changing with the worldwide climate changes (Markow & O'Grady, 2006; Miller, 2005).

2.3 The life cycle of *Drosophila melanogaster*

Drosophila melanogaster has four main stages in its life cycle, which are egg, larva, pupa and adult stages (Purves, Orians, Heller & Sadava, 1998). Duration of life cycle for *D. melanogaster* is largely affected by temperature. For optimal results of culturing these flies, cultures are usually incubated at a constant temperature of 20 ℃ to 25 ℃ (Sandhyarani, 2010).

The whole life cycle requires 15 days to complete if the cultures are kept at 20 ℃ but it requires only 10 days to complete the life cycle if the cultures are kept at 25 ℃. Culturing *D. melanogaster* at temperatures above 30 ℃ may lead to

sterilization or death of the flies. However, culturing the flies at temperature lower than 20 ℃ will decrease the viability of flies and prolong the life cycle (Demerec & Kaufmann, 1996).

Fertilization of eggs in *D. melanogaster* does not occur directly after mating with male flies. *D. melanogaster* females receive and store sperm in their sperm storage reservoirs, known as seminal receptacle and spermatheca during mating. The sperm of *D. melanogaster* are "giant sperm" which are about 1.76 mm long, 300 times longer than human sperm. Sperm are stored inside female flies' bodies up to two weeks and those stored in the seminal receptacle are used first before those stored in spematheca, based on a "last in-first out" basis. Fusion of sperm and egg occurs only when egg is about to be laid. The entire sperm enters the egg at the anterior pole and development of the embryo can be seen in the gut region (Gilbert, 1997; Pitnick, Markow & Spicer, 1999).

The egg of *Drosophila melanogaster* is about 0.5 mm long with rounded ventral surface and flatter dorsal side. The transparent and chitinous vitelline membrane of egg is coated by an opaque outer membrane called the chorion. A pair of filaments extends from the anterodorsal surface of the egg. This pair of filaments prevents the egg from sinking into soft food in which it may be laid (Tyler, 2003; Sandhyarani, 2010).

Within 24 hours after eggs are laid, larvae of same size as the eggs will hatch as first instar larvae. The larval stage is divided into three instar stages. Each larva will undergo two molting processes before pupation. The first instar and second instar stages take about 24 hours to complete each stage while the third instar stage requires 2.5 to 3 days before reaching pupal stage (Griffiths, Miller, Suzuki, Lewontin & Gelbart, 2000; Gilbert, 2000; Geiger,2002).

The sex of *D. melanogaster* can be determined at the larval stage by observing their gonads. Testes of male larvae are much larger in size compared to the ovaries of female larvae, and therefore experienced workers can determine the sex of a particular larva by observing through the transparent body wall of the larva, without any dissection (Kerkis, 1930).

Before pupation, most of the larvae will creep from the culture medium and adhere to the side of culture bottle or any paper toweling that has been inserted into the culture medium. Larvae pupate within their last larval skin by gradually hardening and darkening the skin. Fully formed flies usually emerge 3.5 to 4.5 days after pupation through the anterior end of the pupal case (Griffiths et al., 2000). Newly emerged flies are relatively long and light in color and have curly wings. They will shorten and darken their bodies, and expand their wings during the first few hours (Demerec & Kaufmann, 1996; Woodrow Wilson Biology Institute, 1994).

The life span of *Drosophila melanogaster* varies depending on its surrounding environment. They can live for more than 100 days when the conditions for their survival are good. Nevertheless, the average life span for *D. melanogaster* is 26 days for females and 33 days for males. (Arnini, 2001)

2.4 Morphological characters of *Drosophila*

Morphology of *Drosophila* larvae is totally different from adult flies. Adult flies are divided into three major body segments: head, thorax and abdomen (Figure 2.1). Different morphology of each region is used to differentiate and identify different species of *Drosophila* (Demerec, 1994).

The head of *Drosophila* has a relatively complex structure and different morphology in different species which is useful in taxonomy classification. The shape of head and face, the number of relative size of oral setae and compound eye are the main regions for species identification (Markow & O'Grady, 2006).

The thorax segment is further divided into three segments named prothorax, mesothorax and metathorax. There is a region of humeral callus on the prothorax segment which bears with a pair of setae. This pair of setae is very useful in identifying the species. Mesothorax is the largest segment which contains the musculature that powers the wings. The important character for species identification in the mesothorax is the number and disposition of the acrostichals which lines the mosonotum (Markow & O'Grady, 2006; Demerec, 2008).

Each thorax segment consists of a pair of legs (Figure 2.1). The main leg segments on Drosophila are coxa, trochanter, femur, tibia and tarsus. The tarsus is further divided into five small tarsal segments. The coloration, chaetotaxy and length of leg segments are widely used to identify different species of *Drosophila*. Chaetotaxy, in this case refers to the arrangement of bristles on the leg of the flies. Besides, the existences of sex combs can be used to identify various species too as only certain species have sex combs (Demerec, 1994).

The abdomen of *Drosophila* is covered by chitin. The dorsal region of the abdomen segment is known as tergite while the ventral region is called sternite. The pigmentation patterns of tergites are not only important in taxonomy studies, they can also be used to differentiate between male and females flies. Furthermore, male and female flies can be easily differentiated by observing their sternite because female flies have six quadrilaterally-shaped sternites which make female flies bigger in size compared to male flies which possess only four sternites (Markow & O'Grady, 2006).

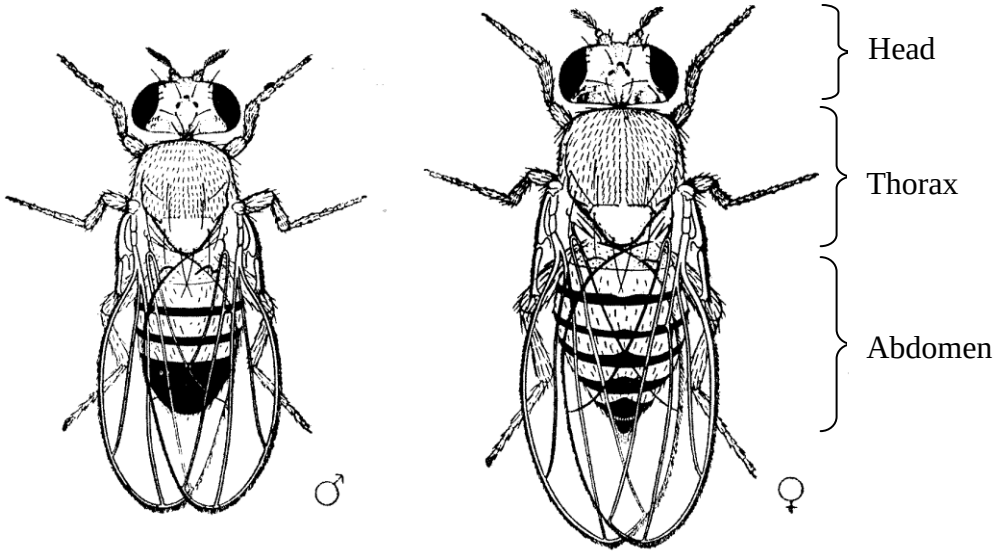

Figure 2.1: Imago of *Drosophila melanogaster*: male at left, female at right (Demerec & Kaufmann, 1996).

2.5 Breeding *Drosophila*

Drosophila melanogaster can be raised easily wherever fermentation is in progress. In our daily life, fruit flies can be easily observed on overripe fruits, especially those that had begun to ferment. Similar to most other fruit flies, *D. melanogaster* can be bred using these fermented fruits. Fermentation is one of the most important elements in breeding *Drosophila* although the roles of fermentation are still unknown (Dirkson, 2009; Demerec & Kaufmann, 1996).

For research purposes, *D. melanogaster* are not bred with overripe and fermented fruits because the fruit culture media would have become too soft by the time new flies begin to hatch due to the process of fermentation. Therefore, *D. melanogaster* are bred in harder culture media in which yeast are added manually to ferment the culture media. One of the most common ingredients added to fruit

culture media in order to harden the culture media is agar (Demerec & Kaufmann, 1996).

Sugar content in *Drosophila* culture media must be sufficient. Sugar content in culture media is not only vital for the growth of *Drosophila* but is also required by yeast for fermentation. Hence, fruits with high sugar contents are usually chosen to be made into *Drosophila* culture media. The most common fruit used to culture *Drosophila* is banana. Banana culture medium is also known as standard culture medium for culturing *Drosophila* (Stocker & Gallant, 2007).

Yet, there are numerous recipes in preparing culture media for *D. melanogaster* and different laboratories or research centers have their own formulated culture media for these little fruit flies. The Bloomington *Drosophila* stock center at Indiana University is one of the most popular *Drosophila* research centers. Seven different types of culture media were used in this research center and each of the medium has its own strengths in culturing the flies. The standard medium used by this research center is constituted by soy flour, cornmeal, light malt extract, light corn syrup, propionic acid and agar (Bloomington *Drosophila* Stock Center, 2007).

There are also culture media with reduced agar content and without agar content. Cornmeal and oatmeal content are used to substitute agar to harden the culture media. However, this type of culture medium may have difficulties in pouring the

medium into culture bottles before the cultivation of flies. These culture media with a minimum of agar are useful when the agar supplies are depleted (Spencer, 1943).

Contamination of culture media with mold will lower the survival rate and retard the growth of *Drosophila melanogaster* as heterotrophic fungi will consume a large portion of the nutrient in culture media. Low fermentation processes due to lack of nutrients for yeast further retards the growth of *D. melanogaster*. Therefore, growth of fungi needs to be inhibited in *D. melanogaster* culture media. There are various types of mold inhibitors available commercially nowadays. For instance, Tegosept M (methyl-*p*-hydroxybenzoate), propionic acid and *p*-hydroxy-benzoic acid methyl ester are the most commonly used mold inhibitors (Niesel, Bewley, Miller & Armstrong, 1980). Autoclaving is another method of avoiding the growth of fungi by ensuring that the culture media in sterile condition (Bloomington *Drosophila* Stock Center, 2007; Demerec & Kaufmann, 1996).

CHAPTER 3

MATERIALS AND METHODS

3.1 Media preparation

The experiments were divided into two major parts. The first part was carried out with the inbred wild-type *Drosophila melanogaster* while and second part was tested on the Kampar wild-type *Drosophila* species. Ten different types of culture media were formulated: five different types of single fruit media and five other types of culture media. Banana (*Musa acuminate* Colla) medium was used as standard medium and plain agar was used as negative control medium.

The agars used to prepare the culture media were strip-type agars from KCL Sdn. Bhd., Malaysia. 8.3 g of these agars were fully dissolved in 450 mL distilled water at 100 ℃. The liquefied agar was used as negative control medium or mix with other ingredients for other formulated culture media. For the preparation of standard banana medium and five types of single fruit culture media, 100 g of fruits were blended and mixed well with 100 mL of liquefied agar. The five types of fruits used in the preparation of single fruit culture media were guava (*Psidium guajava*), pineapple (*Ananas comosus*), starfruit (*Avverhoa carambola*), dragonfruit (*Hylocereus undatus*) and papaya (*Carica papaya*).

Another five types of culture media were formulated and they were labeled as Method 1 (M1), Method 2 (M2), Method 3 (M3), Method 4 (M4) and Method 5 (M5). M1 medium had a formula of 150 mL of liquefied agar, 150 g of blended banana and a class A egg. Meanwhile, M2 medium was the mixture of 15 g of granulated white sugar (Prai, Malaysia), 25 g of milk powder (Every Day, Philippines), 100 g of potato (*Solanum tuberosum*) and 150 mL of liquefied agar.

M3 medium was prepared by mixing 200 g of blended banana, 25 g of granulated white sugar and 150 g of oatmeal (Pristine, Australia). Ingredients for the preparation of M4 medium were 150 mL of distilled water, 150 mL of liquefied agar, 25 g of soyabean powder (Hei Hwang, Malaysia), 50 g of cornmeal (Cap Bintang, Malaysia) and two teaspoons of golden syrup (Nona, Malaysia). Soyabean powder and cornmeal were dissolved in the distilled water before mixing with liquefied agar to avoid the clumping of those powders. For M5 medium, 40 g of banana, 50 g of apple (*Malus*), 75 g of grapes (*Vitis*), 100 g of potato and 10 g of brown sugar (Prai, Malaysia) were mixed well in 150 mL of liquefied agar.

In the first part of this experiment, the test for each medium was carried out in triplicate. Each type of culture medium was poured into three glass tubes with the dimension of 2.5 cm × 7.5 cm to about 1.5 cm height. Cotton wool was wrapped with gauze into a ball-shape and used as a stopper for each glass tube (Figure 3.1). The media were sterilized by autoclaving at 121 ℃ for fifteen minutes. Sterile

media were then incubated at 37 ℃ overnight. The purpose of incubation was to remove the condensation which formed inside the glass tubes after autoclaving.

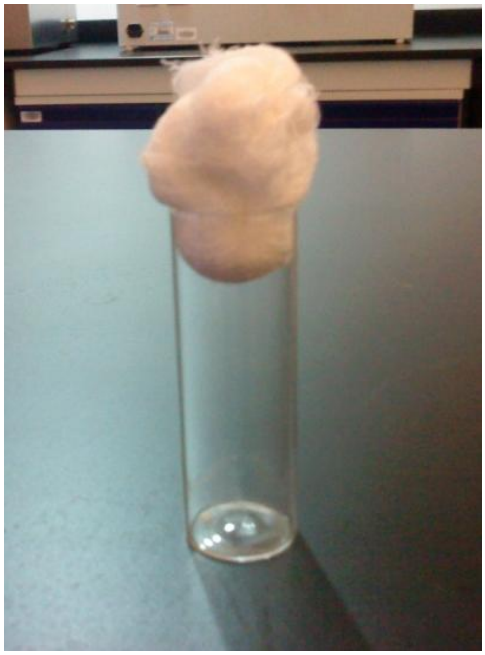

Figure 3.1: Glass tube for culturing *Drosophila melanogaster*.

In the second part of the experiment, the culture media were prepared in transparent plastic containers with the dimension 16 cm × 10 cm × 6.5 cm and the experiment was carried out in duplicate. The culture media were poured into the containers to about 2.5 cm and used as attractants to collect Kampar wild-type *Drosophila* species in open environment. These media were not autoclaved. Three openings with about 3 cm × 6 cm were cut on the cover of containers (Figure 3.2).

Figure 3.2: Container used to collect and culture Kampar wild-type *Drosophila.*

3.2 Cultivation of inbred wild-type *Drosophila melanogaster*

Wild-type *D. melanogaster* flies were shaken out from their original culture bottles (Figure 3.3) into a reagent bottle (Figure 3.4). Five drops of ether were dropped on the cotton wool which was stuffed into the cover of the reagent bottle. The reagent bottle was capped immediately after flies were transferred into the reagent bottle. Ether was used to anesthetize flies so that flies can be easily being examined. Flies were left in etherized bottle for 30 seconds until all the flies were anesthetized. The flies were then transferred from the reagent bottle onto an index paper (Figure 3.5) to avoid over etherization of flies which may cause the death of flies.

Figure 3.3: Culture bottle for inbred wild-type *Drosophila melanogaster*.

Figure 3.4: Ether and reagent bottle with the cover stuffed with cotton wool.

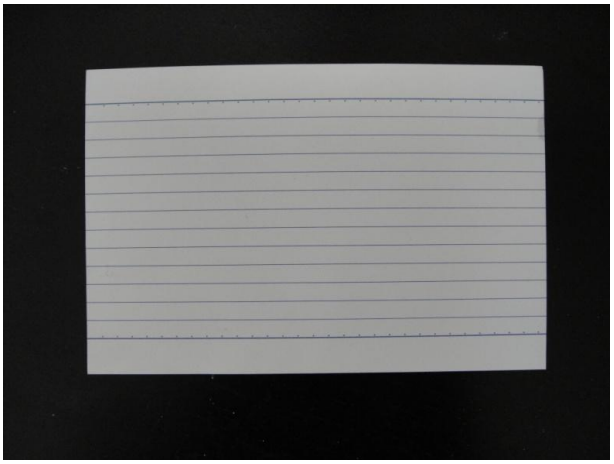

Figure 3.5: Index paper.

Anesthetized flies were examined under a stereo microscope (Leica Zoom 2000, Model no. Z45V, Germany) to distinguish male and female flies by observing their physical appearances. Male flies had rounded abdomen tips while female flies had elongated abdomen tips and were slightly bigger in size compared to male flies. Sterile glass tubes with media were placed horizontally and three pairs of flies were transferred into each glass tube.

Figure 3.6: Adult flies of *Drosophila melanogaster* under stereo microscope: female (left), male (right).

The culture media were then incubated in an air-conditioned room at 22 ℃. The condition of the culture media and flies were observed every day. The three pairs of parental flies were removed from the culture media on Day 7 of culture. Parental flies were shaken out from glass tubes into a reagent bottle for prolonged etherization to euthanize them. Dead flies were then transferred into a "morgue" bottle filled with ethanol-mineral oil preservative.

The first generation pupa and flies hatched from the eggs of these three pairs of parental flies were named as F1 generation. Number of pupal cases in each glass tube was counted on Day 7 of culture. Numbers of pupal cases were easily counted by dotting each observed pupal cases with marker pen on the outer wall of the glass tubes because pupal cases were immobile and most of the times adhere on the inner wall of glass tubes above the medium.

Number of flies in each culture medium was counted on the 7th day since the first fly hatched. Due to the mobility of flies, all the flies were anesthetized before the number of flies can be counted. All the flies from each glass tube were transferred out from culture glass tube into a reagent bottle for etherization. Anesthetized flies were spread on an index paper for counting purpose. Flies were then euthanized, transferred back to their original culture media for further observations or subcultured to fresh media.

First generation flies were subcultured into fresh media after the number F1 pupal cases and hatched flies were counted. The purpose of subculturing is to examine the differences between the numbers of F1 generation offspring and F2 generation offspring in each culture medium. F1 flies were shaken out from their culture glass tubes into reagent bottle for etherization. Flies were examined under stereo microscope and three pairs of F1 flies were subcultured into fresh culture media. Numbers of F2 pupal cases were counted on Day 7 of subculture and numbers of F2 flies were counted on the 7th day since first fly hatched.

3.3 Collecting and culturing wild-caught *Drosophila* species

All media containers with openings on the top of the cover were placed at Block C cafeteria, Universiti Tunku Abdul Rahman (UTAR) Kampar, Perak campus for a week long. During the period, Kampar wild-type *Drosophila* flies were attracted by the culture media and laid eggs on their preferable media. After a week of exposure to the open environment, the media containers were collected. Flies trapped inside the containers were released and the openings of the containers were sealed. Ten pin-sized holes were made on the cover for each container to enable exchange of gases. Media were then incubated in the laboratory at 22 ℃ for another week until the eggs hatched into larvae and undergo pupation. Hatched flies were released and only the number of pupal cases in each container was counted.

3.4 Statistical analysis

The SPSS software version 11.5 was used to analyze the results obtained in this experiment. Three different analyses were performed to analyze the results of inbred wild-type *Drosophila melanogaster*: Analysis of Variance (ANOVA), Tukey's HSD multiple range tests and paired sample tests. Two tests were performed to analyze the results of Kampar wild-type *Drosophila* species: Analysis of Variance (ANOVA) and Tukey's HSD multiple range tests.

ANOVA test was used to compare the means of the results from all types of media. The Tukey's HSD multiple range test were performed after ANOVA test to determine which of the medium had significantly different result compared to other media. Three significance levels: 0.05, 0.01 and 0.001 were used to evaluate the differences among the parameters in this experiment. The hypothesis used in these two tests was shown below:

H_0: There is no significant difference in the number of offspring among the culture media.

H_1: There is significant difference in the number of offspring among the culture media.

There were two types of paired sample tests performed to evaluate the results obtained in cultivation of inbred wild-type *D. melanogaster*: t-test and correlation test. The paired sample t-test was performed to determine the significant

difference in the population mean between two sets of results. The two hypotheses used in t-test were shown below:

Hypothesis 1:

H_0: There is no significant difference between the numbers of pupal cases and hatched flies in all the culture media.

H_1: There is significant difference between the numbers of pupal cases and hatched flies in all the culture media.

Hypothesis 2:

H_0: There is no significant difference between the numbers of first generation offspring and the second generation offspring in all the culture media.

H_1: There is significant difference between the numbers of first generation offspring and the second generation offspring in all the culture media.

The paired sample correlation test was performed to measure the relative strength of a linear relationship between two sets of results. The two hypotheses used in paired sample correlation test were shown below:

Hypothesis 1:

H_0: There is no linear relationship exists between the numbers of pupal cases and hatched flies in all the culture media.

H_1: There is a linear relationship exists between the numbers of pupal cases and hatched flies in all the culture media.

Hypothesis 2:

H_0: There is no linear relationship exists between the numbers of first generation offspring and the second generation offspring in all the culture media.

H_1: There is a linear relationship exists between the numbers of first generation offspring and the second generation offspring in all the culture media.

CHAPTER 4

RESULTS

4.1 Population density of first and second generations of inbred *Drosophila melanogaster* in different culture media

Figure 4.1 shows the number of pupal cases for the first generation offspring in different culture media. There were no pupal cases observed in the negative control medium, pineapple medium and M3 medium. The three pairs of parent flies cultured in guava medium produced only an average of 10 pupal cases after a week of cultivation. Standard banana medium showed a moderate result where an average of 45 pupal cases was observed. M1 medium showed the production of an average of 151 pupal cases, the highest number of pupal cases produced among all other culture media. M2 medium ranked the second higher as an average of 135 pupal cases was observed produced in the culture medium.

Figure 4.2 shows the number of first generation flies hatched in the different culture media. The trend is almost similar as in Figure 4.1 where the pointers for negative control, pineapple and M3 media were fall on the x-axis. M1 culture medium remained to have the highest number of offspring, an average of 140 flies was hatched in M1 culture medium. However, M2 medium showed a drastically drop in Figure 4.2 compared to Figure 4.1. An average of 135 pupal cases was observed in M2 medium but only an average of 37 flies was hatched from these pupal cases.

Figure 4.3 shows the average number of second generation pupal cases produced in different culture media. First generation *Drosophila* flies were directly affected the results of second generation offspring as first generation flies were needed to be subcultured into fresh culture media in order to produce the second generation of offspring. Therefore, negative control medium, pineapple medium and M3 medium did not produce any offspring since no F1 generation flies could be subcultured to produce F2 offspring. Low number of flies hatched in guava medium (an average of 2 flies) also produced no offspring after subculturing. Moreover, due to the sticky starfruit medium, most of the F1 flies hatched were stuck on the medium and unable to be subcultured into fresh medium, causing no F2 generation offspring to be produced.

Besides those cultures without new *D. melanogaster* production, other culture media showed higher numbers of offspring for the F2 generation (Figure 4.3-4.4) compared to the F1 generation (Figure 4.1-4.2). Number of F2 pupal cases produced in M1 medium was the highest, an average of 277 pupal cases was observed, which was much higher than the number of pupal cases observed in the F1 generation (an average of 151). M2 medium ranked the second highest with an average of 178 pupal cases and followed by M4 medium for which an average of 170 pupal cases was observed.

Figure 4.4 shows the numbers of second generation flies hatched in different culture media. M1 medium showed the highest number of hatched flies with an

average of 167 flies, followed by M4 medium which an average of 135 flies was observed. M2 medium showed a unique trend in both first and second generations where numbers of pupal cases observed were very high (an average of 135 pupal cases in F1 generation and an average of 178 pupal cases in F2 generation) but numbers of hatched flies were much lower compared to the pupa numbers (an average of 37 hatched flies in F1 generation and an average of 31 hatched flies in F2 generation).

4.2 Comparison of population density among all types of culture media for the first and second generations of *Drosophila melanogaster*

One-way ANOVA analyses were carried out for the comparison on the number of pupal cases and hatched flies in both first and second generations. Table 4.5 shows the results of these ANOVA tests. From Table 4.5, all four sets of results from ANOVA test show the significant differences in the numbers of pupal cases or hatched flies among all the culture media in both first and second generations at the significance level of 0.000.

Tukey's HSD multiple range tests were performed following the one-way ANOVA tests and the results were shown in Table 4.2 - 4.5. Table 4.2 shows the comparison on the numbers of pupal cases for the first generation. M1 and M2 media which had the highest number of pupal cases showed significant differences with M4 medium at the significance level of 0.05 and all other culture media at the significance level of 0.01.

From Table 4.2, culture media which showed no significant difference with negative control medium include guava, pineapple, starfruit and M3 media. Meanwhile, starfruit, dragonfruit, papaya and M4 media showed no significant difference with standard banana medium. This suggested that *Drosophila melanogaster* cultured in M1, M2 and M5 media are able to produce much higher numbers of pupa compared to standard banana medium.

The Tukey's HSD multiple range test on the number of first generation hatched flies was shown in Table 4.3. The highest number of hatched flies was observed in M1 medium and this result showed significant differences with all other culture media at the significance level of 0.001. This suggested that *Drosophila melanogaster* cultured in M1 medium are able to produce much higher number of flies compared to the cultures using all other culture media tested in this study.

Besides guava, pineapple, starfruit and M3 media, the addition of dragonfruit culture medium showed no significant difference with negative control medium in the first generation hatched flies. Based on the data in Figure 4.2 and Table 4.3, the numbers of first generation hatched flies in M1 and M5 media were much larger compared to standard banana medium and showed significant differences in Tukey's tests at the significance level of 0.05 and 0.001 respectively.

Table 4.4 shows the result of Tukey's HSD multiple range test on the numbers of second generation pupal cases among the different culture media. The high

number of pupal cases observed in M1 medium (an average of 277) makes this medium had significant differences with all other culture media in Tukey's test at the significance level of 0.001.

As for the first generation pupa, second generation pupa observed in guava, pineapple, starfruit and M3 media showed no significant difference with negative control medium (Table 4.4). However, in second generation, number of pupal cases observed in dragonfruit, M1, M2 and M4 media were much higher compared to the standard medium. These four culture media showed significant differences with standard medium in Tukey's test at 1% significance level for dragonfruit medium and 0.1% significance level for M1, M2 and M4 media.

The result of Tukey's HSD multiple range test on the numbers of second generation hatched flies among all different culture media was shown in Table 4.5. M1 and M4 media which had the highest numbers of hatched flies showed significant differences with all other culture media at significance level of 0.1%. This indicates that flies cultured in M1 and M4 media were able to produce much larger numbers of offspring compared to the flies cultured in standard medium.

Guava, pineapple, starfruit and M3 media showed no significant difference with negative control medium in the number of pupal cases and hatched flies in both first and second generations. This suggested that these media are not suitable in breeding *D. melanogaster*. Conversely, flies cultured in M1 medium were able to

produce a much higher number of offspring compared to standard medium and most of the other culture media. This suggested that M1 medium is very suitable for culturing *D. melanogaster*.

4.3 Comparison of population density using paired sample t-tests

Table 4.6 shows the comparison of population mean between the number of pupal cases and the number of hatched flies in both first and second generations. Meanwhile, Table 4.7 shows the comparison of population mean between the first generation offspring and the second generation offspring. The t-value for negative control medium, pineapple medium and M3 medium could not be calculated as there were no results generated in this media.

At the significance level of 0.001, the F1 offspring in dragonfruit medium and the F2 offspring in M2 medium showed significant differences between the number of pupal cases and hatched flies (Table 4.6). At then significance level of 0.05, the F2 offspring in papaya medium and the F1 offspring in M5 medium showed significant differences between the number of pupal cases and hatched flies (Table 4.6). These significant differences indicate the numbers of hatched flies were much lower compared to the numbers of pupal cases observed.

From Table 4.7, the number of pupal cases produced in F1 generation showed a significant difference with the number of pupal cases produced in F2 generation ($p \leq 0.001$). At the significance level of 0.05, the number of pupal cases observed for F1 generation and F2 generation in papaya medium showed a significant

difference. Besides, the numbers of offspring observed in M4 medium also showed significant differences between the first and second generations ($p \leq 0.05$). These significant differences indicate that the reproductions of *D. melanogaster* in the F2 generation were much higher compared to the reproduction of the F1 generation.

4.4 Analysis on Kampar wild-type *Drosophila*

Figure 4.5 shows the numbers of pupal cases produced by wild-caught *Drosophila* flies in different culture media. An average of 666 pupal cases was observed in M5 medium, which was the highest among all other culture media. M1 medium was the second highest where an average of 565 pupal cases was observed produced in this medium. Pineapple medium was following the M1 medium and an average of 424 pupal cases was observed in this medium. However, most of the flies were stuck and drown in the sticky and soft pineapple medium after hatching from their pupal cases.

There was an average of 26 pupal cases observed in negative control medium but no hatched fly was observed. Besides, there were also a low number of pupal cases observed in M3 and M4 media, only an average of 88 and 124 pupal cases respectively. Other culture media including standard medium showed moderate results in attracting and culturing these wild-type flies, with the average of pupal cases observed in each medium was within the range of 223 to 306.

One-way ANOVA test was carried out to compare the population means and the result was shown in Table 4.7. At the significance level of 0.05, there are significant differences in the numbers of pupal cases observed among all the culture media. Therefore, Tukey's HSD multiple range test was performed to determine which culture media showed significant differences in the number of pupal cases compared to other media. From Table 4.8, most of the population mean of Kampar wild-type *Drosophila* in various media showed no significant differences except for the population mean in negative control medium was significantly different from the population means in M1 medium and M5 medium. Besides, population mean of Kampar wild-type flies in M5 medium is significantly different from M3 and M4 mediums too.

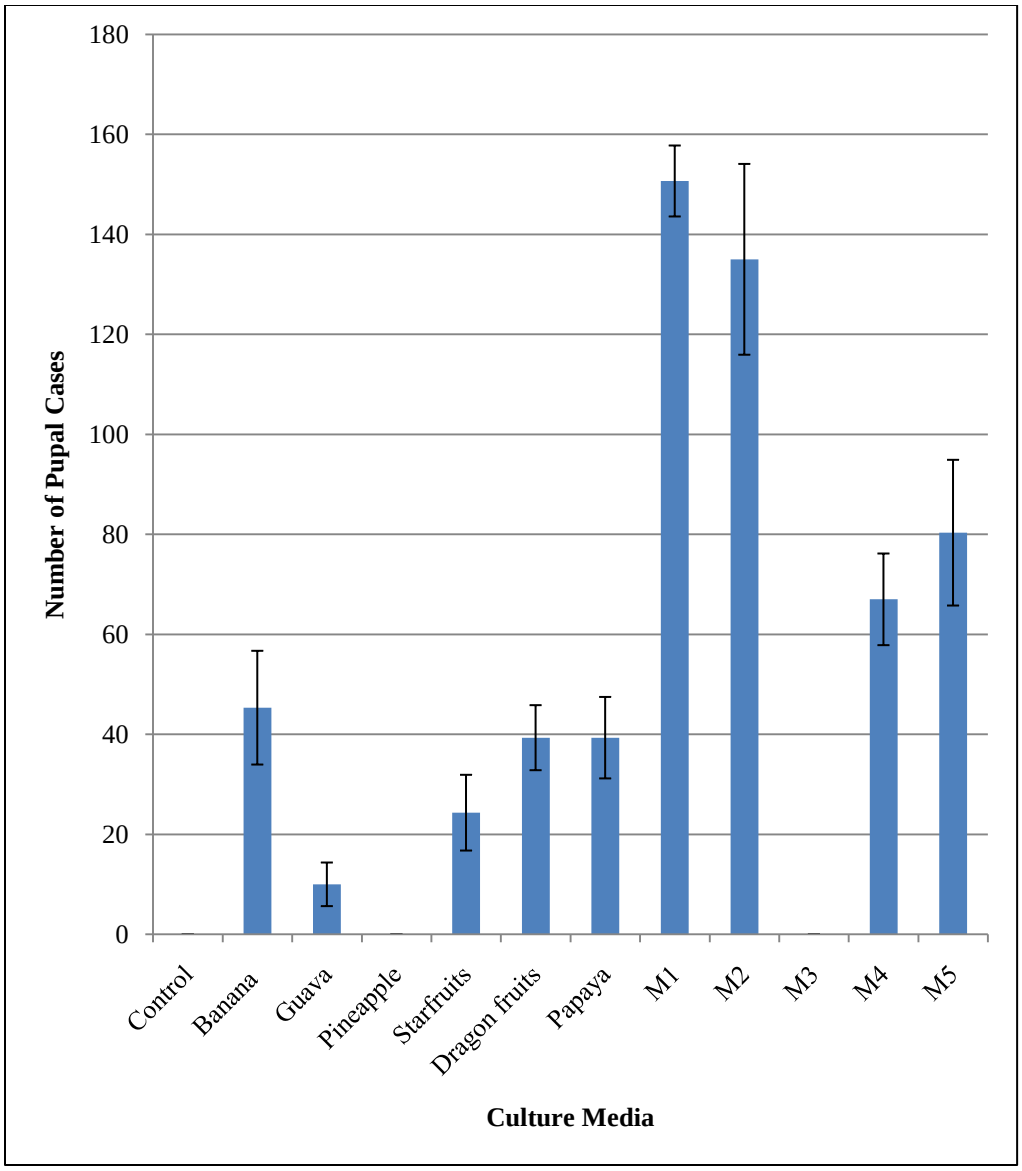

Figure 4.1: Number of pupal cases for the first generation of *D. melanogaster* in different culture media.

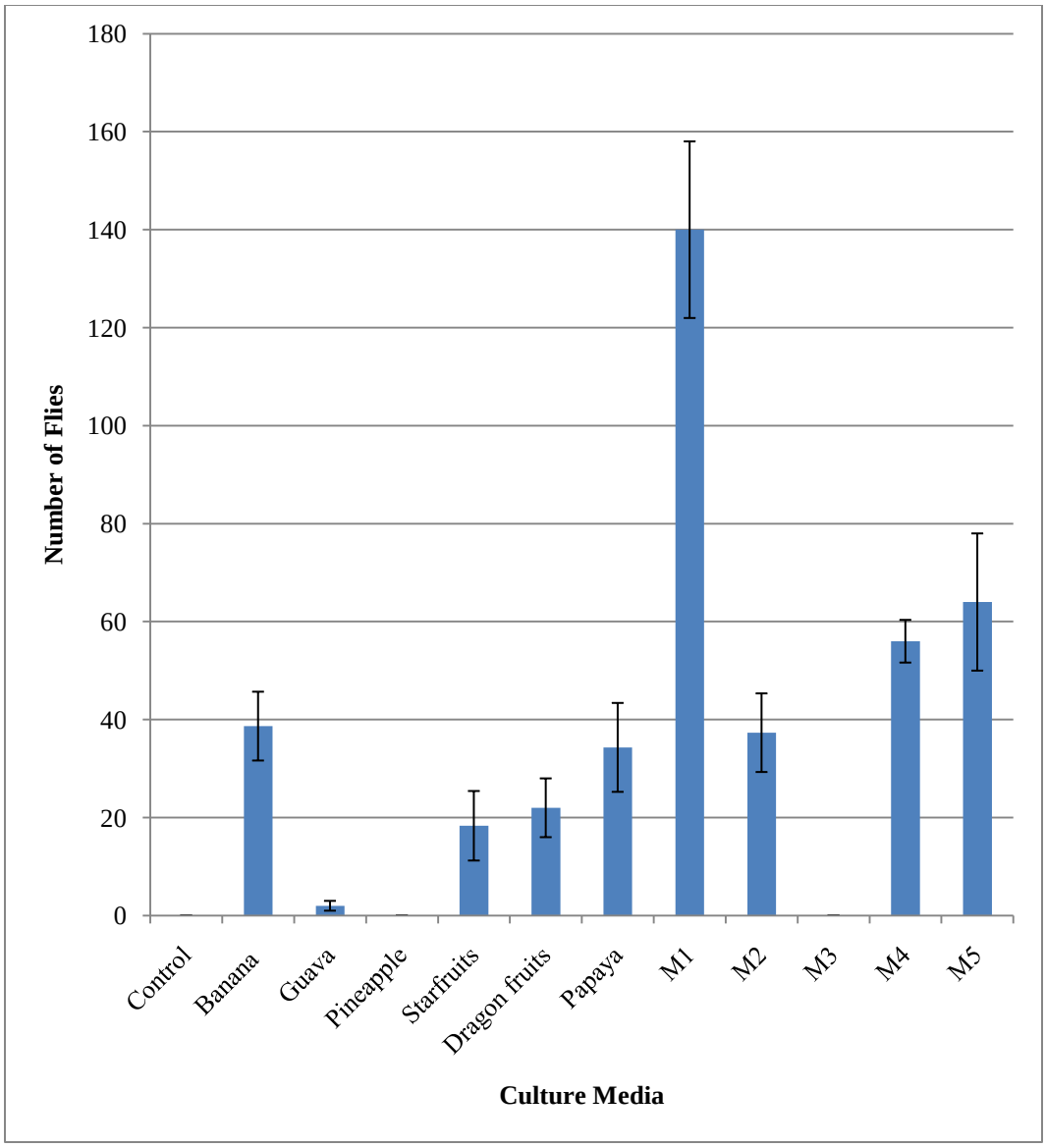

Figure 4.2: Number of hatched flies for the first generation of *D. melanogaster* in different culture media.

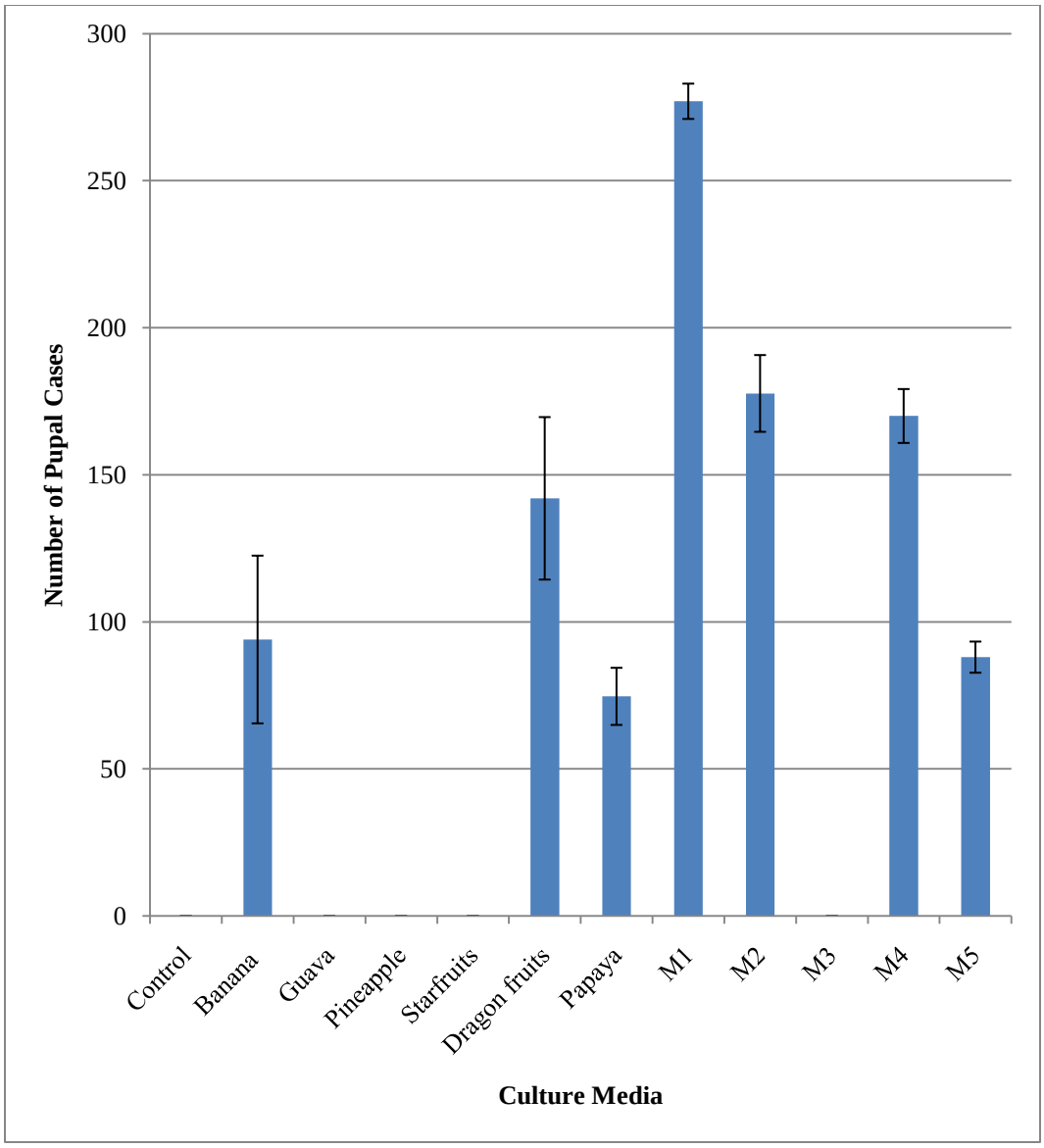

Figure 4.3: Number of pupal cases for the second generation of *D. melanogaster* in different culture media.

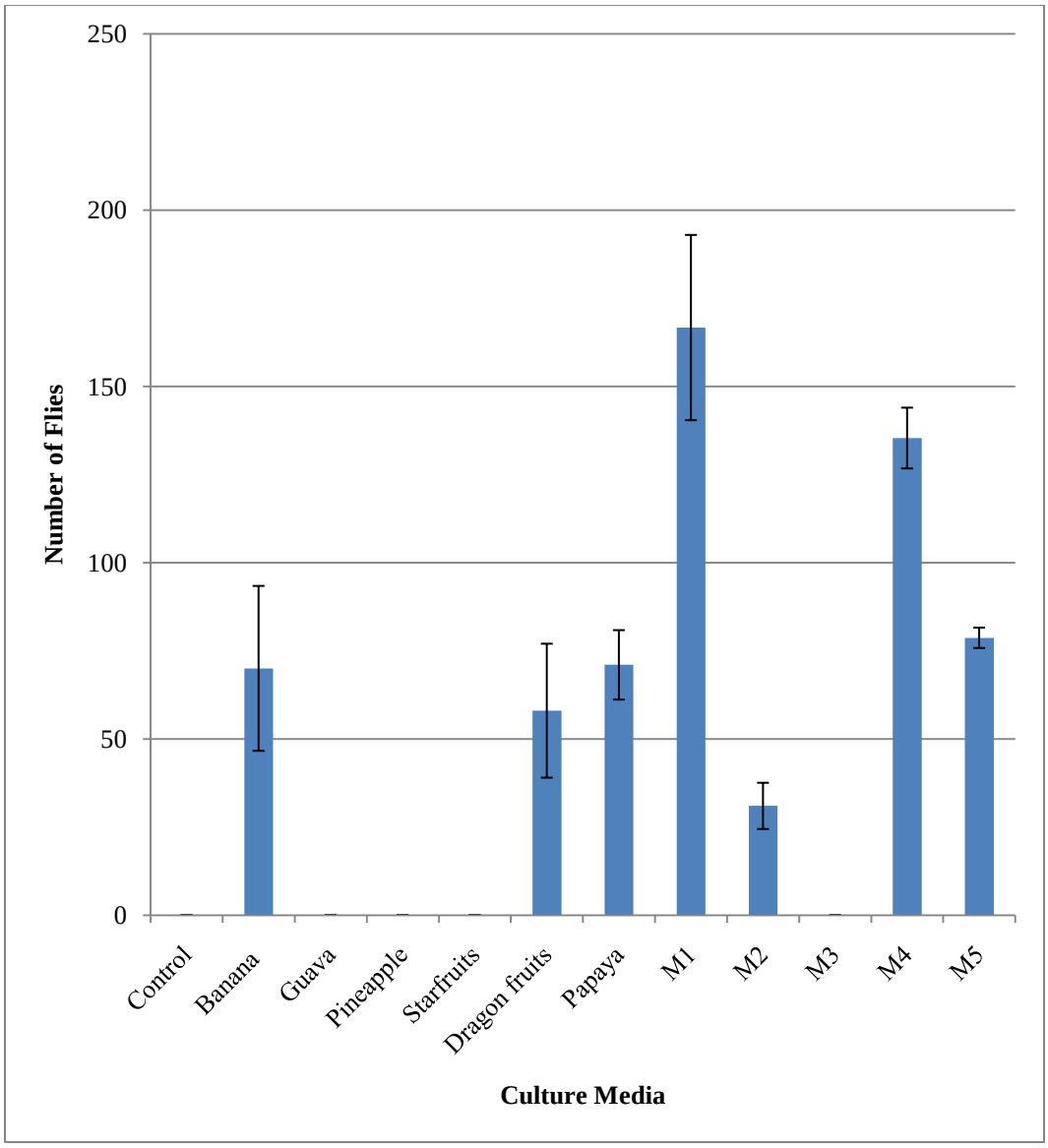

Figure 4.4: Number of hatched flies for the second generation of *D. melanogaster* in different culture media.

Table 4.1: One-way ANOVA table for the comparison of the mean number of pupal cases and hatched flies in the first and second generations.

Generation (pupae/flies)		Sum of Squares	df	Mean Square	F	Sig.
Number of pupal cases in first generation	Between Groups	85709.222	11	7791.747	91.220	0.000***
	Within Groups	2050.000	24	85.417		
	Total	87759.222	35			
Number of flies in first generation	Between Groups	52597.889	11	4781.626	69.692	0.000***
	Within Groups	1646.667	24	68.611		
	Total	54244.556	35			
Number of pupal cases in second generation	Between Groups	276737.889	11	25157.990	151.808	0.000***
	Within Groups	3977.333	24	165.722		
	Total	280715.222	35			
Number of flies in second generation	Between Groups	106413.556	11	9673.960	63.749	0.000***
	Within Groups	3642.000	24	151.750		
	Total	110055.556	35			

*significant at $p \leq 0.05$ **significant at $p \leq 0.01$ ***significant at $p \leq 0.001$

Table 4.2: Multiple range test (Tukey's HSD) for the comparison of the number of pupal cases in the first *D. melanogaster* generation among the different culture media.

	Negative	**Standard**	**Guava**	**Pineapple**	**Starfruit**	**Dragonfruit**	**Papaya**	**Method1**	**Method2**	**Method3**	**Method4**	**Method5**
Negative	-	0.000***	0.967	1.000	0.110	0.001***	0.001***	0.000***	0.000***	1.000	0.000***	0.000***
Standard		-	0.004**	0.000***	0.248	0.999	0.999	0.000***	0.000***	0.000***	0.213	0.005**
Guava			-	0.967	0.749	0.027*	0.027*	0.000***	0.000***	0.967	0.000***	0.000***
Pineapple				-	0.110	0.001***	0.001***	0.000***	0.000***	1.000	0.000***	0.000***
Starfruit					-	0.697	0.697	0.000***	0.000***	0.110	0.000***	0.000***
Dragonfruit						-	1.000	0.000***	0.000***	0.001***	0.044*	0.001***
Papaya							-	0.000***	0.000***	0.001***	0.044*	0.001***
Method1								-	0.642	0.000***	0.000***	0.000***
Method2									-	0.000***	0.000***	0.000***
Method3										-	0.000***	0.000***
Method4											-	0.820
Method5												-

*significant at p≤0.05 **significant at p≤0.01 ***significant at p≤0.001

Table 4.3: Multiple range test (Tukey's HSD) for the comparison of the number of hatched flies in the first *D. melanogaster* generation among the different culture media.

	Negative	**Standard**	**Guava**	**Pineapple**	**Starfruit**	**Dragonfruit**	**Papaya**	**Method1**	**Method2**	**Method3**	**Method4**	**Method5**
Negative	-	0.000***	1.000	1.000	0.279	0.104	0.002**	0.000***	0.001***	1.000	0.000***	0.000***
Standard		-	0.001***	0.000***	0.167	0.405	1.000	0.000***	1.000	0.000***	0.351	0.037*
Guava			-	1.000	0.433	0.183	0.003**	0.000***	0.001***	1.000	0.000***	0.000***
Pineapple				-	0.279	0.104	0.002**	0.000***	0.001***	1.000	0.000***	0.000***
Starfruit					-	1.000	0.462	0.000***	0.237	0.279	0.000***	0.000***
Dragonfruit						-	0.791	0.000***	0.522	0.104	0.002**	0.000***
Papaya							-	0.000***	1.000	0.002**	0.115	0.008**
Method1								-	0.000***	0.000***	0.000***	0.000***
Method2									-	0.001***	0.257	0.024*
Method3										-	0.000***	0.000***
Method4											-	0.985
Method5												-

*significant at p≤0.05 **significant at p≤0.01 ***significant at p≤0.001

Table 4.4: Multiple range test (Tukey's HSD) for the comparison of the number of pupal cases in the second *D. melanogaster* generation among the different culture media.

	Negative	**Standard**	**Guava**	**Pineapple**	**Starfruit**	**Dragonfruit**	**Papaya**	**Method1**	**Method2**	**Method3**	**Method4**	**Method5**
Negative	-	0.000***	1.000	1.000	1.000	0.000***	0.000***	0.000***	0.000***	1.000	0.000***	0.000***
Standard		-	0.000***	0.000***	0.000***	0.006**	0.782	0.000***	0.000***	0.000***	0.000***	1.000
Guava			-	1.000	1.000	0.000***	0.000***	0.000***	0.000***	1.000	0.000***	0.000***
Pineapple				-	1.000	0.000***	0.000***	0.000***	0.000***	1.000	0.000***	0.000***
Starfruit					-	0.000***	0.000***	0.000***	0.000***	1.000	0.000***	0.000***
Dragonfruit						-	0.000***	0.000***	0.078	0.000***	0.301	0.001**
Papaya							-	0.000***	0.000***	0.000***	0.000***	0.976
Method1								-	0.000***	0.000***	0.000***	0.000***
Method2									-	0.000***	1.000	0.000***
Method3										-	0.000***	0.000***
Method4											-	0.000***
Method5												-

*significant at p≤0.05 **significant at p≤0.01 ***significant at p≤0.001

Table 4.5: Multiple range test (Tukey's HSD) for the comparison of the number of hatched flies in the second *D. melanogaster* generation among the different culture media.

	Negative	**Standard**	**Guava**	**Pineapple**	**Starfruit**	**Dragonfruit**	**Papaya**	**Method1**	**Method2**	**Method3**	**Method4**	**Method5**
Negative	-	0.000***	1.000	1.000	1.000	0.000***	0.000***	0.000***	0.145	1.000	0.000***	0.000***
Standard		-	0.000***	0.000***	0.000***	0.985	1.000	0.000***	0.027*	0.000***	0.000***	0.999
Guava			-	1.000	1.000	0.000***	0.000***	0.000***	0.145	1.000	0.000***	0.000***
Pineapple				-	1.000	0.000***	0.000***	0.000***	0.145	1.000	0.000***	0.000***
Starfruit					-	0.000***	0.000***	0.000***	0.145	1.000	0.000***	0.000***
Dragonfruit						-	0.972	0.000***	0.291	0.000***	0.000***	0.656
Papaya							-	0.000***	0.022*	0.000***	0.000***	1.000
Method1								-	0.000***	0.000***	0.136	0.000***
Method2									-	0.145	0.000***	0.004**
Method3										-	0.000***	0.000***
Method4											-	0.000***
Method5												-

*significant at $p \leq 0.05$ **significant at $p \leq 0.01$ ***significant at $p \leq 0.001$

Table 4.6: Paired sample t-tests of the number of pupal cases versus the number of hatched flies for the first and second generations in different culture media. (df=2)

Culture media	Number of pupal cases versus hatched flies	t	Significance level of 2-tailed test
Negative	Generation 1	-	-
	Generation 2	-	-
Standard	Generation 1	2.500	0.130
	Generation 2	3.170	0.087
Guava	Generation 1	4.000	0.057
	Generation 2	-	-
Pineapple	Generation 1	-	-
	Generation 2	-	-
Starfruit	Generation 1	6.000	0.027*
	Generation 2	-	-
Dragonfruit	Generation 1	52.000	0.000***
	Generation 2	3.461	0.074
Papaya	Generation 1	8.660	0.013*
	Generation 2	11.000	0.008**
Method 1	Generation 1	1.684	0.234
	Generation 2	9.255	0.011*
Method 2	Generation 1	6.781	0.021*
	Generation 2	39.044	0.001***
Method 3	Generation 1	-	-
	Generation 2	-	-
Method 4	Generation 1	2.144	0.165
	Generation 2	3.873	0.061
Method 5	Generation 1	18.520	0.003**
	Generation 2	3.150	0.088

*significant at $p \leq 0.05$ **significant at $p \leq 0.01$ ***significant at $p \leq 0.001$

Table 4.7: Paired sample t-tests of the first generation offspring versus the second generation offspring for the number of pupal cases and number of flies in different culture media. (df = 2)

Culture medium	Generation 1 Versus Generation 2	t	Significance level of 2-tailed test
Negative	Number of pupal cases	-	-
	Number of flies	-	-
Banana	Number of pupal cases	-3.879	0.061
	Number of flies	-3.121	0.089
Guava	Number of pupal cases	3.974	0.058
	Number of flies	3.464	0.074
Pineapple	Number of pupal cases	-	-
	Number of flies	-	-
Starfruit	Number of pupal cases	5.566	0.031*
	Number of flies	4.476	0.046*
Dragonfruit	Number of pupal cases	-7.198	0.019*
	Number of flies	-3.427	0.076
Papaya	Number of pupal cases	-4.635	0.044*
	Number of flies	-4.521	0.046*
Method1	Number of pupal cases	-38.482	0.001***
	Number of flies	-3.439	0.075
Method2	Number of pupal cases	-11.099	0.008**
	Number of flies	0.883	0.470
Method 3	Number of pupal cases	-	-
	Number of flies	-	-
Method4	Number of pupal cases	-12.875	0.006**
	Number of flies	-10.990	0.008**
Method5	Number of pupal cases	-0.681	0.566
	Number of flies	-1.777	0.218

*significant at $p \leq 0.05$ **significant at $p \leq 0.01$ ***significant at $p \leq 0.001$

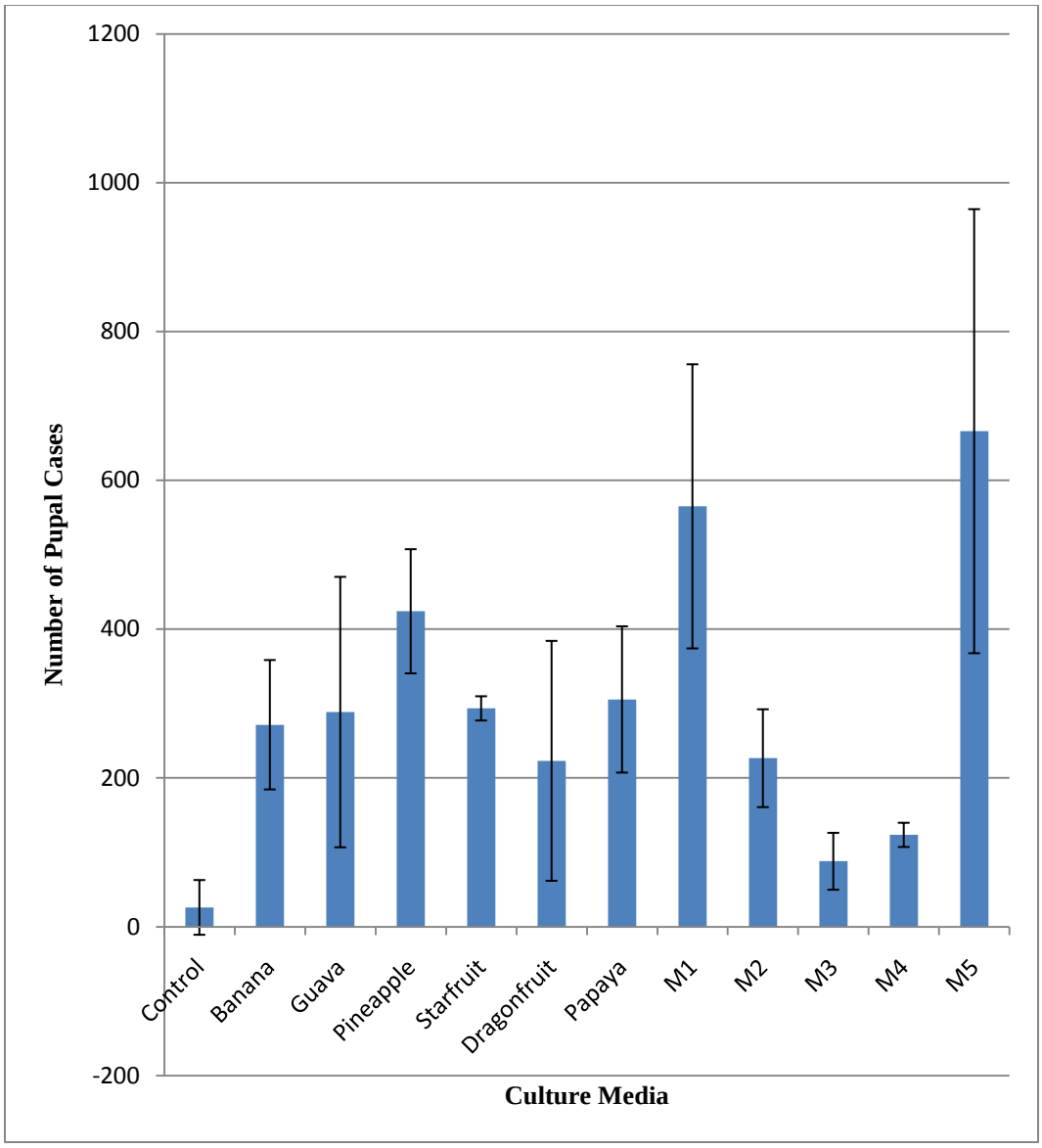

Figure 4.5: Number of pupal cases for the wild-caught *Drosophila* species in different culture media.

Table 4.8: One-way ANOVA of the number of pupal cases from Kampar wild-type *Drosophila* species.

	Sum of squares	df	Mean square	F	Sig.
Between groups	764521.500	11	69501.955	3.855	0.014*
Within groups	216359.000	12	18029.917		
Total	980880.500	23			

*significant at p≤0.05 **significant at p≤0.01 ***significant at p≤0.001

Table 4.9: Multiple range test (Tukey's HSD) for the comparison of the number of pupal cases for Kampar wild-type *Drosophila* among different culture media.

	Negative	**Standard**	**Guava**	**Pineapple**	**Starfruit**	**Dragonfruit**	**Papaya**	**Method1**	**Method2**	**Method3**	**Method4**	**Method5**
Negative	-	0.778	0.712	0.229	0.692	0.925	0.642	0.047*	0.927	1.000	1.000	0.014*
Standard		-	1.000	0.985	1.000	1.000	1.000	0.584	1.000	0.950	0.988	0.237
Guava			-	0.994	1.000	1.000	1.000	0.655	1.000	0.917	0.975	0.282
Pineapple				-	0.996	0.916	0.998	0.992	0.923	0.416	0.555	0.791
Starfruit					-	1.000	1.000	0.675	1.000	0.905	0.969	0.296
Dragonfruit						-	1.000	0.395	1.000	0.994	1.000	0.141
Papaya							-	0.724	1.000	0.872	0.953	0.333
Method1								-	0.407	0.096	0.143	1.000
Method2									-	0.993	0.999	0.146
Method3										-	1.000	0.029*
Method4											-	0.045*
Method5												-

*significant at $p \leq 0.05$ **significant at $p \leq 0.01$ ***significant at $p \leq 0.001$

CHAPTER 5

DISCUSSION

5.1 Effect of different culture media on inbred *Drosophila melanogaster*

In this study, inbred wild-type *Drosophila melanogaster* were used for population studies to test the effect of different culture media on their populations. Ten different recipes of culture media were formulated and tested in this study. This is to investigate the suitability of each culture medium in culturing and breeding *D. melanogaster* and develop a suitable culture medium to mass produce *D. melanogaster* with minimum production cost. *D. melanogaster* is bred commercially as it is widely used in genetic studies (Werren & Beukeboom, 1998).

The reproduction of *D. melanogaster* and the length of their life cycle are dependent mainly on temperature, substrate (culture medium), predation pressure and heterogeneity environment (Wayne, Soundararajan & Harshman, 2006; Burnell, Reaper & Doherty, 1991). Some other factors such as pH of culture media might also affect the reproduction of *D. melanogaster* (Bridges & Darby, 1933). This study was focused on the effect of different culture media to the reproduction of *D. melanogaster*. Predation pressure and heterogeneity environment were absent in this study and the temperature during the flies cultivation was fixed at 22 ℃.

Banana medium was used as standard medium (positive control) in this study. The stock culture of *Drosophila melanogaster* used to produce F1 generation flies in this study was also cultured in banana medium. Therefore, there was no significant difference in the number of pupal cases ($p=0.061$) and number of flies ($p=0.089$) between first generation and second generation offspring (Table 4.7). This is because the flies did not require long adaptation time to the fresh culture medium before they can reproduce (Mitrovski & Hoffman, 2001).

The efficiency of standard banana medium in breeding *D. melanogaster* was moderate compared to the other ten types of culture media in this study. Besides negative control medium, flies cultivated in pineapple medium and M3 medium were unable to survive and produced no offspring (Figure 4.1). The F1 hatched flies in guava medium and starfruit medium became stuck on the sticky media before they were subcultured and thus did not produce any second generation offspring (Figure 4.2). The results in these media showed significantly differences ($p=0.000$) to the results of standard medium.

For the first generation offspring, the number of pupal cases observed in standard medium was significantly lower than in M1 and M2 media ($p\leq0.001$) and M5 medium ($p\leq0.01$). Meanwhile, the number of flies observed was significantly lower than in M1 medium ($p\leq0.001$) and M5 medium ($p\leq0.05$). For second generation offspring, the number of pupal cases observed in standard medium was significantly lower than in dragonfruit medium ($p\leq0.01$), M1, M2 and M4 media

($p \leq 0.001$). The number of F2 flies observed in standard medium was significantly lower than in M1 medium and M4 medium ($p \leq 0.001$) (Table 4.2 - 4.5). The nutritional values of culture media have great effects towards the survivability, growth and the development of *D. melanogaster* (Sang, 1956; Sang & Robert, 1961). Therefore, different culture media gave significantly different results in the number of offspring produced.

The numbers of offspring produced in second generation were much higher compared to the numbers of offspring produced in first generation in most of the culture media. Flies from the stock culture were subcultured into ten different types of culture media. These ten different culture media were new environments for the flies from stock culture as they were originally cultured in standard banana medium. Adaptation to the new environment might delay the reproduction of *Drosophila melanogaster* (Mitrovski & Hoffman, 2001). Therefore, low numbers of first generation offspring were encountered. Higher numbers of second generation offspring showed that the flies had adapted to their new environment.

From Table 4.7, dragonfruit, papaya, M1, M2 and M4 media showed significant differences in the numbers offspring between two generations. Number of pupal cases observed in M1 medium for the first and second generations was the most significant difference ($p=0.001$). In M1 medium, an average of 151 pupal cases was observed in first generation but an average of 277 pupal cases was observed in second generation. Meanwhile, the difference observed in M2 medium and M4

medium was significant at significance level of 0.01 and the difference observed in dragonfruit and papaya media were significant at significance level of 0.05.

In this study, the numbers of hatched *D. melanogaster* in M1 medium (medium of banana with egg) were the highest for both first and second generations compared to the flies hatched in the other types of culture media. The addition egg in M1 culture medium leads to the high protein content in this medium. From the research by Sang (1962), protein content is able to increase the efficiency in reproducing *D. melanogaster*. Therefore, the high numbers of pupal cases and hatched flies in M1 medium might be due to the high protein content in this medium. Besides, the pupal cases formed in M1 culture medium were larger in size compared to the pupal cases observed in other culture media. This might also due to the high protein content in M1 medium (Uysal, Aydogan & Algur, 2002).

Drosophila melanogaster cultured in M2 medium (medium of sugar, milk and potato) produced high number of pupal cases but relatively low number of hatched flies. This trend was observed in both first and second generations. There was a significant difference ($p \leq 0.05$) between the number of pupal cases and number of hatched flies for the first generation while there is a significant difference ($p \leq 0.001$) for second generation (Table 4.6). Low number of hatched flies might be due to the effect of nutrients in M2 medium which delayed the hatching of flies.

D. melanogaster cultured in M4 medium showed no significant difference with the result in standard medium in the comparison of the first generation offspring. However, the number of second generation offspring produced in M4 medium showed significant differences to the result produced in standard medium. It might be due to the adaptation period of flies during the first culture (67 pupal cases and 56 flies). They were able to produce a higher number of offspring in second culture (170 pupal cases and 135.33 flies) after they became adapted to their new environment (Mitrovski & Hoffman, 2001).

M4 medium, composed mainly of soy flour and cornmeal, was the modified recipe from the standard *Drosophila* medium used by Bloomington *Drosophila* Stock Center at Indiana University. This recipe was originally from the malt medium formulated by Lakovaara (1969). This medium is firm enough to resist liquefaction caused by larval activity and thus it is useful in mass culture the larvae of *Drosophila* (Bloomington *Drosophila* Stock Center, 2007).

The M5 medium, composed of mixture of banana, apple, grapes and potato, was a recipe formulated by Miller (2005), which was named the "power mix" medium. The numbers of offspring produced in this medium showed no significant difference between F1 generation and F2 generation (Table 4.7). From this study, it is suggested that this complex medium with mixture of various ingredients is not suitable in breeding *D. melanogaster* because the complexity of medium preparation steps did not show higher production of *Drosophila* offspring. The

medium might lack of one or more of the five major nutrients (casein, cholesterol, choline, RNA and fructose) required in culturing *D. melanogaster* larvae (Sang & King, 1961), thus producing relatively low number of offspring.

From Figure 4.1 and 4.2, *D. melanogaster* cultured in negative control medium, pineapple medium and M3 medium (medium of banana, sugar and oatmeal) died before the production of any offspring. As nutritional values of culture media are essential for growth, reproduction and the development, *D. melanogaster* was not able to survive in the negative control medium which consisted of only agar (Sang, 1956; Sang & Robert, 1961).

For pineapple medium, the failure to cultivate *D. melanogaster* was due to the liquefied culture medium. High acidic properties of pineapple might have hydrolyzed the agar and prevented the agar medium from solidifying. (Sayed, 1983) Flies were not able to survive in this culture medium as they were easily stuck in the liquid medium. Increasing the agar content is necessary to harden the pineapple culture medium before it could be used to test its suitability in culturing the *D. melanogaster* in the future.

On the other hand, M3 medium failed to culture flies in this study. This might be because the culture medium was too dry to cultivate *D. melanogaster*. From the research study by Catchpoole (2005), desiccation stress causes death of flies as flies lose their body moisture too fast. The surviving flies in desiccated condition

might produce some offspring but these hatched larvae could hardly survive in such conditions as well. Thus, moisture content in M3 medium must be increased to make it a more suitable environment for *D. melanogaster*.

From Figures 4.3 and 4.4, there were no production of offspring in guava medium and starfruit medium in second generation although low production of offspring were observed in the first generation (Figure 4.1 and 4.2). These two culture media became sticky and liquefied at the end of the experiment and thus the newly hatched F1 flies were stuck to the media. The stuck flies were unable to transfer out from their culture bottles to subculture to fresh culture media. The reasons which caused the sticky and liquefied culture media were the autoclaving process and the fermentation by yeast. During the sterilization process, high temperatures altered and lowered the pH values of the culture media (Skirvin et. al., 1986). From the research by Smith (1932), high temperature tends to destruct sugar into acidic products and increases the acidity of culture media.

Additional acidic products produced during fermentation further increased the acidity of the culture media (Remize, Roustan, Sablayrolles, Barre & Dequin, 1998). Culture media were softened and liquefied due to the hydrolysis of agar by acid. In future studies, the agar content must be increased as high agar content greatly reduces the alteration of pH during autoclaving process (Skirvin et. al., 1986). High agar content also enables the culture media to withstand the acidic products produced during fermentation and remain in solid state.

5.2 Effect of different culture media on the population of wild-caught *Drosophila* species

Figure 4.5 shows the mean number of pupal cases hatched from wild-caught *Drosophila* species. Culture media were used to attract Kampar wild-type *Drosophila* species and to culture the collected eggs. Therefore, the high number of pupal cases observed in one culture medium indicated that it attracted more flies to lay their eggs on the medium and also the suitability of the medium to culture the larvae produced by these wild-caught *Drosophila* species.

From the Figure 4.5, the highest number of pupal cases was observed in M5 medium (mean=666), followed by M1 medium (mean=565) and pineapple medium (mean=424). The M5 medium managed to attract and breed the highest number of Kampar wild-type *Drosophila*. Different *Drosophila* species have different nutritional requirements for their growth and reproduction (Royes & Robertson, 2005). M5 medium was found to be unsuitable for breeding inbred wild-type *D. melanogaster* but it was useful for attracting and culturing Kampar wild-type *Drosophila* species.

M1 medium which showed the best result in breeding inbred wild-type *D. melanogaster* in this study was also suitable in collecting and propagating Kampar wild-type *Drosophila* species as a high number of pupal cases was observed in the medium. From this result, it is suggested that the addition protein content in M1 medium aids in the process of hatching the eggs of Kampar wild-type *Drosophila* species.

On the other hand, pineapple medium managed to attract and breed a high number of Kampar wild-type *Drosophila* although this medium was failed in producing the next generation of inbred *D. melanogaster* in laboratory. The medium used as flies' attractant was not sterilized by autoclaving. Thus, the composition of pineapple medium was not altered by the treatment with high temperature during sterilization and was able to solidify (Smith, 1932). However, the medium became sticky and liquefied at the end of the cultivation due to fermentation and most of the hatched flies were stuck to the medium.

Growth of mold and bacteria were observed on all the culture media after being exposed to the open environment to collect Kampar wild-type *Drosophila*. This is because no sterilization process was performed for these media and no mold and bacteria inhibitors were added to the culture media. Contamination of culture media with mold will lower the survival rate and retard the growth of *Drosophila melanogaster* as heterotrophic fungi will consume a large portion of the nutrient in culture media. Low fermentation processes due to lack of nutrients for yeast further retards the growth of *D. melanogaster* (Indiana University, 2011).

The contamination of culture media by bacteria greatly reduced the reproduction of *Drosophila* species. This is because female flies might not lay eggs on surfaces with bacterial material. If they lay their eggs on the surfaces with bacteria, the eggs might not develop or the hatched larvae would be trapped in the bacterial slime (Markow & O'Grady, 2006). Slime is the extracellular material excreted by

bacteria as a protection against environmental changes such as desiccation. Bacterial slime is mostly composed of exopolysaccharides, glycoproteins and glycolipids (Holley, 2009).

For future studies, it is recommended to add mold and bacteria inhibitors into culture media in order to inhibit the growth of these contaminants on the culture media. There are various types of mold inhibitors available commercially nowadays. For instance, Tegosept M (methyl-*p*-hydroxybenzoate), propionic acid and *p*-hydroxy-benzoic acid methyl ester are the most commonly used mold inhibitors (Niesel, Bewley, Miller & Armstrong, 1980). Meanwhile, antibiotics such as penicillin and streptomycin can be used to inhibit the growth of bacteria (Meyer, 1966). Decreased in the pH of culture media may also help in inhibiting the growth of bacteria (Tortora, Funke & Case, 2010).

CHAPTER 6

CONCLUSION

This study tested the effect of different culture media on the reproduction of inbred *Drosophila melanogaster* and also the effect of different culture media for collecting and culturing wild-caught *Drosophila* species. Ten different culture media were tested along with banana medium (positive control) and plain agar (negative control).

From the results obtained, M1 medium (medium of banana with egg) was the most suitable culture medium for breeding *D. melanogaster*. The highest number of first and second generations offspring were observed hatched in this medium. From the Tukey's HSD test, the number of first generation pupal cases observed in M1 medium was significantly higher than the results for all other culture media. In the second generation, the number of offspring observed in M1 medium was significantly higher than the results for all other culture media except M5 medium. Conversely, pineapple, starfruit, guava and M3 media failed to breed *D. melanogaster* in this study. Parent flies cultured in these media failed to survive and did not produce any offspring.

For the study on wild-caught *Drosophila* species, M5 medium (the mixture of banana, apple, grapes and potato) gave the best result in collecting and

propagating this *Drosophila* species compared to the other media. Contamination of all unsterilized culture media by mold and bacteria was observed. The growth of contaminants affected the results of wild-caught *Drosophila* and the growth of larvae. Therefore, for future studies, antifungal and antibacterial agents are required to inhibit the growth of fungi and bacteria in unsterilized culture media.

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APPENDIX A

Raw data of the numbers of first generation pupal cases and adult flies.

Culture media	Number of pupal cases in first generation		Number of flies in first generation	
	Triplicate	Mean ± Standard deviation	Triplicate	Mean ± Standard deviation
Negative control	0 0 0	0.000 ± 0.000	0 0 0	0.00 ± 0.00
Standard	58 42 36	45.33 ± 11.37	46 38 32	38.67 ± 7.02
Guava	8 15 7	10.00 ± 4.36	2 3 1	2.00 ± 1.00
Pineapple	0 0 0	0.00 ± 0.00	0 0 0	0.00 ± 0.00
Starfruit	33 19 21	24.33 ± 7.57	26 12 17	18.33 ± 7.10
Dragonfruit	46 33 39	39.33 ± 6.51	28 16 22	22.00 ± 6.00
Papaya	43 45 30	39.33 ± 8.15	38 41 24	34.33 ± 9.07
Method1	157 152 143	150.67 ± 7.10	155 145 120	140.00 ± 18.03
Method2	155 117 133	135.00 ± 19.08	29 38 45	37.33 ± 8.02
Method3	0 0 0	0.00 ± 0.00	0 0 0	0.00 ± 0.00
Method4	77 59 65	67.00 ± 9.17	59 58 51	56.00 ± 4.36
Method5	82 94 65	80.33 ± 14.57	64 78 50	64.00 ± 14.00

Raw data of the number of second generation pupal cases and adult flies.

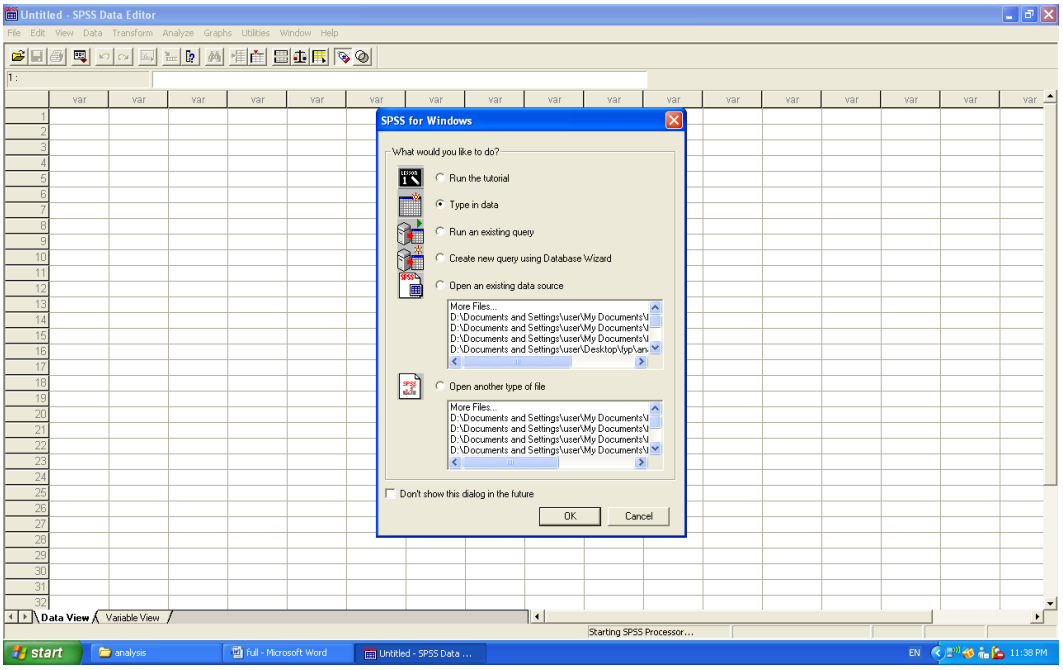
Culture media	Number of pupal cases in second generation		Number of flies in second generation	
	Triplicate	Mean ± Standard deviation	Triplicate	Mean ± Standard deviation
Negative control	0 0 0	0.00 ± 0.00	0 0 0	0.00 ± 0.00
Standard	123 66 93	94.00 ± 28.51	97 56 57	70.00 ± 23.39
Guava	0 0 0	0.00 ± 0.00	0 0 0	0.00 ± 0.00
Pineapple	0 0 0	0.00 ± 0.00	0 0 0	0.00 ± 0.00
Starfruit	0 0 0	0.00 ± 0.00	0 0 0	0.00 ± 0.00
Dragonfruit	145 113 168	142.00 ± 27.62	74 63 37	58.00 ± 19.00
Papaya	64 83 77	74.67 ± 9.71	60 79 74	71.00 ± 9.85
Method1	277 283 271	277.00 ± 6.00	176 187 137	166.67 ± 26.27
Method2	190 164 179	177.67 ± 13.05	37 24 32	31.00 ± 6.56
Method3	0 0 0	0.00 ± 0.00	0 0 0	0.00 ± 0.00
Method4	172 178 160	170.00 ± 9.165	126 137 143	135.33 ± 8.62
Method5	90 82 92	88.00 ± 5.29	82 77 77	78.67 ± 2.89

Raw data of the number of pupal cases for wild-caught *Drosophila* species.

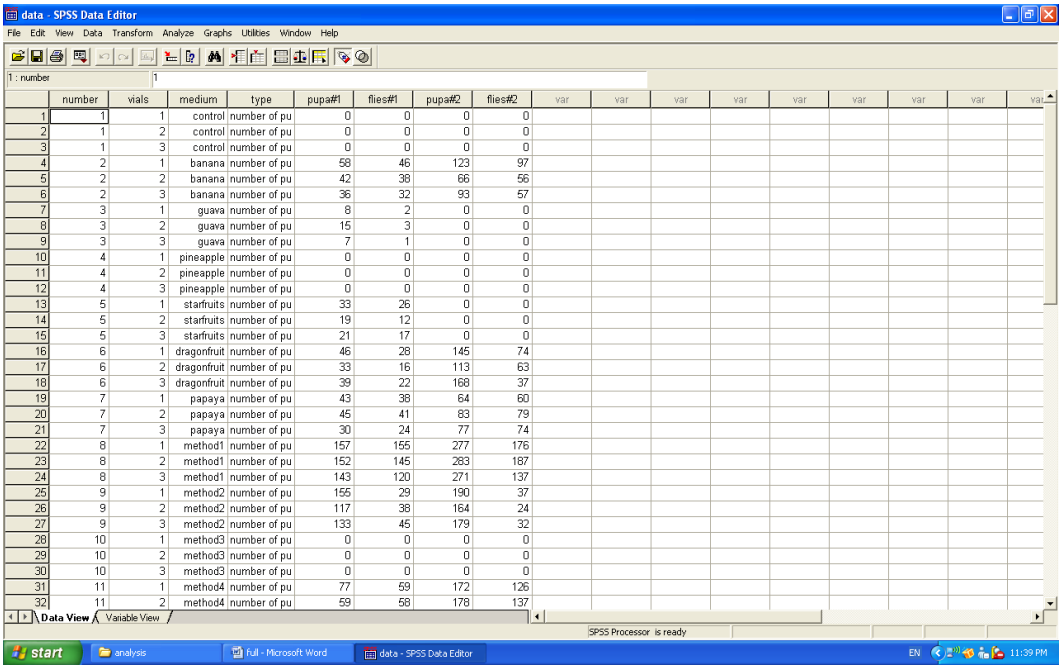
Culture media	Duplicate	Mean ± Standard deviation
Negative control	0 52	26.00 ± 36.77
Standard	333 210	271.50 ± 86.97
Guava	417 160	288.5 ± 181.73
Pineapple	365 483	424.00 ± 83.44
Starfruit	282 305	293.50 ± 16.26
Dragonfruit	109 337	223.00 ± 161.22
Papaya	375 236	305.50 ± 98.29
Method 1	700 430	565.00 ± 190.92
Method 2	180 273	226.50 ± 65.76
Method 3	61 115	88.00 ± 38.18
Method 4	135 112	123.50 ± 16.26
Method 5	455 877	666.00 ± 298.51

APPENDIX B

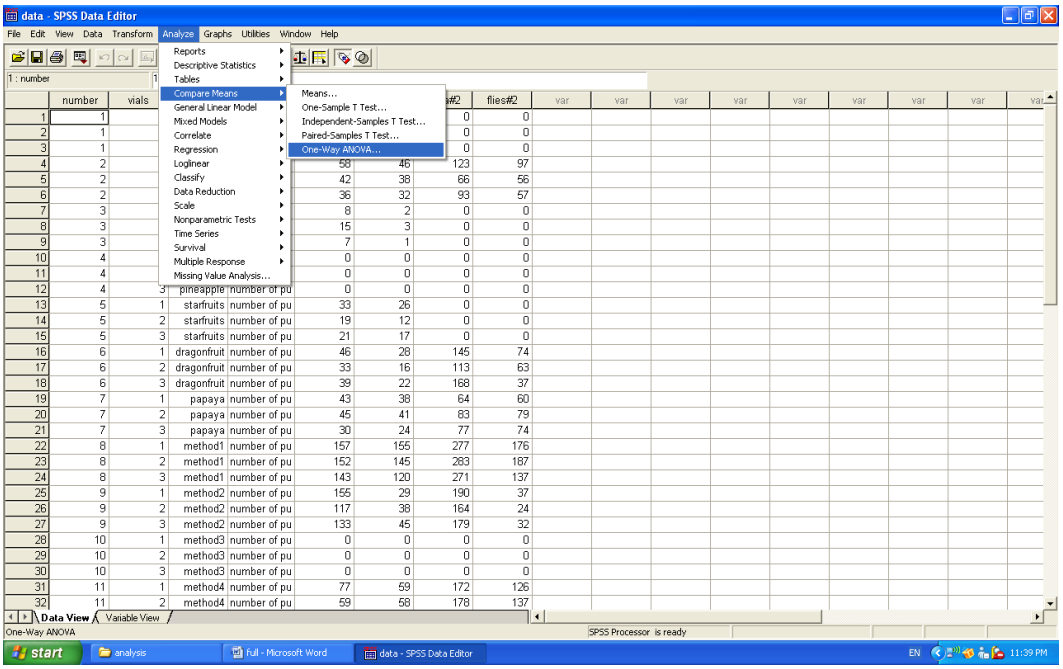
Steps involved in analyzing data with SPSS software (version 11.5).



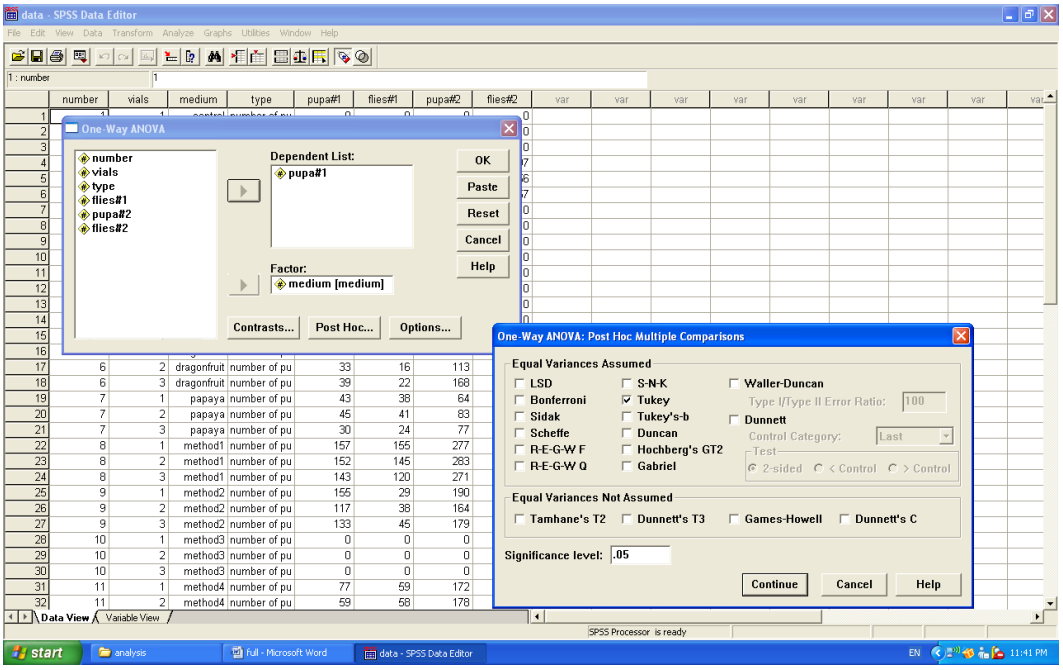
Step 1: Select “Type in data” and click “ok” to key in the data for analysis.



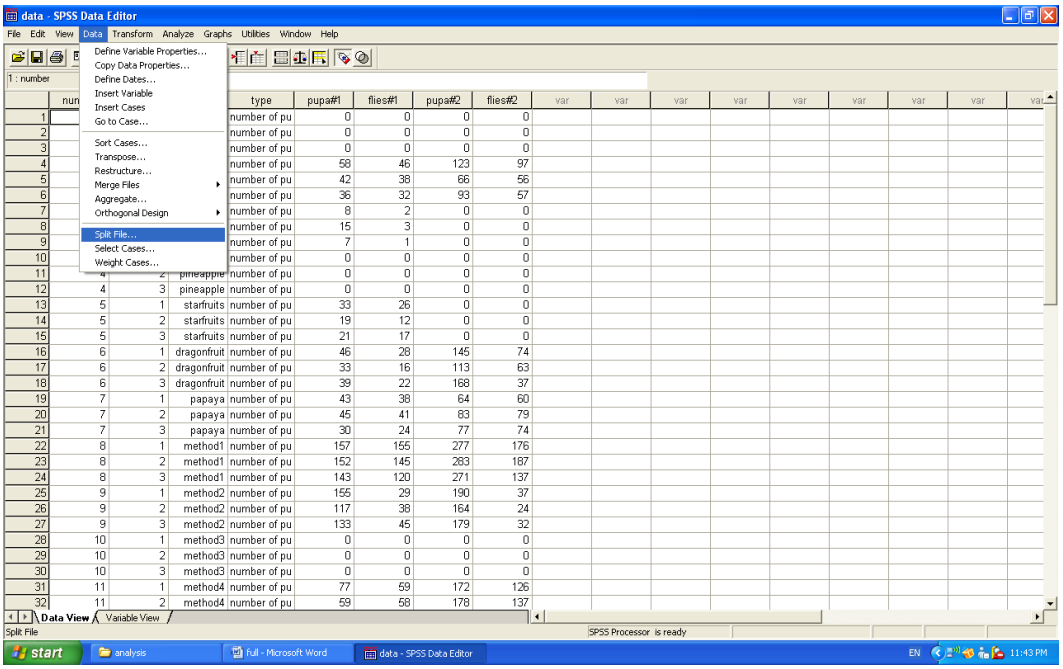
Step 2: Key in raw data.



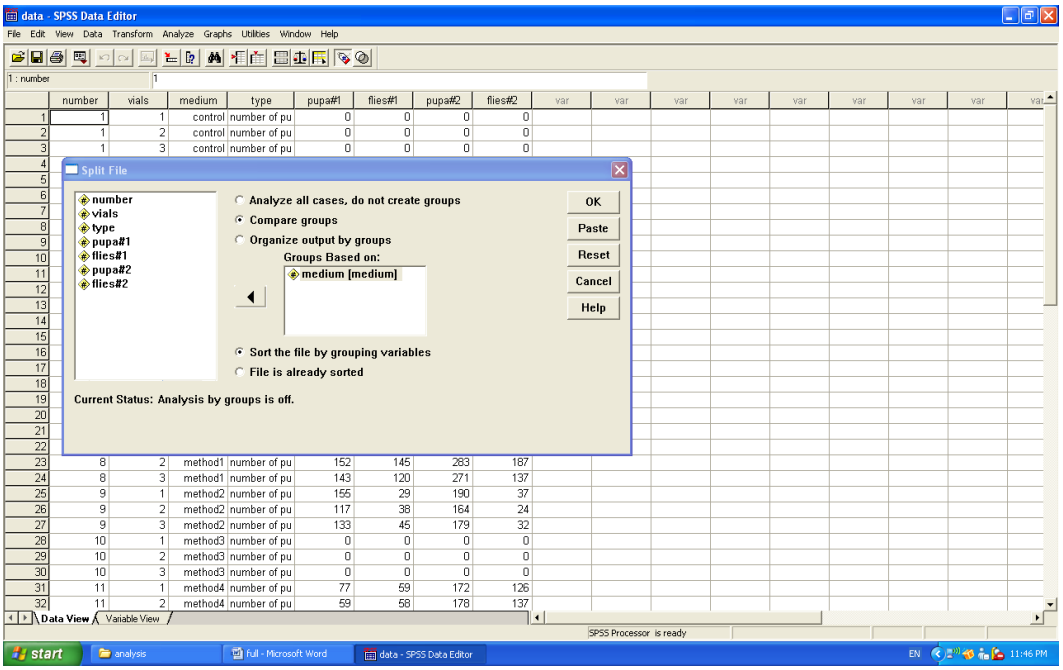
Step 3: From menu, select "Analyze", "Compare Means" then "One-way ANOVA".



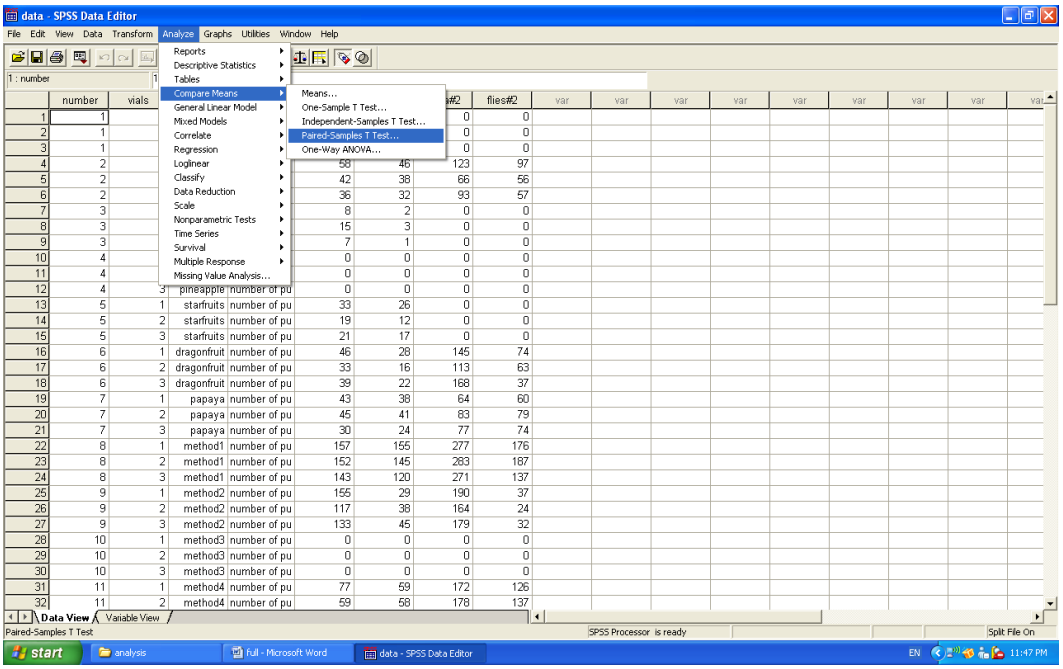
Step 4: Insert variables into Dependent List and Factor then click on "Post Hoc" to select "Tukey" for multiple comparisons.



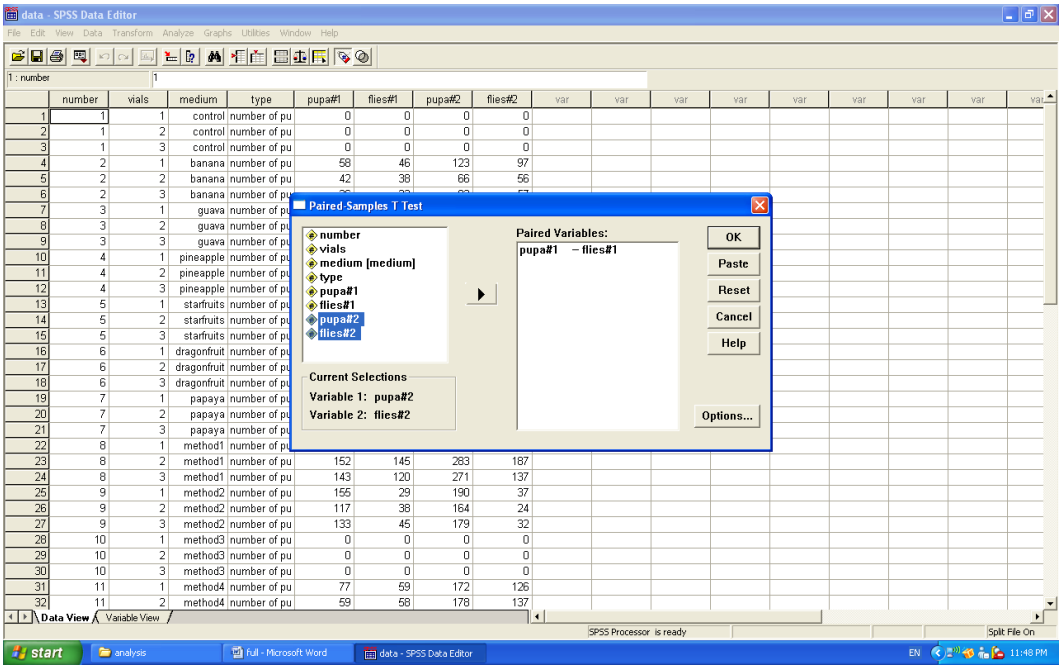
Step 5: From menu, select “Data” then “Split File”



Step 6: Click on “Compare group” then insert “medium” into the “Groups Based on” column.



Step 7: From menu, select “Analyze”, “Compare Means” then “Paired Sample T Test”.



Step 8: Insert variables in pairs into Paired Variables.