Storage Time Effect of Defibrinated and Irradiated Bovine Blood Feeding on *Glossina pallidipes* and *Glossina fuscipes fuscipes* under Laboratory Conditions for the Purpose of Sterile Insect Techniques

Temesgen Fikre Sira

MSc Thesis Submitted to the Department of Applied Biology, School of Applied Natural Science

Office of Graduate Studies

Adama Science and Technology University

February 2024

Adama, Ethiopia

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Advisor: Dr. Daniel Getahun

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Declaration

I hereby declare that this Master's Thesis entitled "Storage Time Effect of Defibrinated and Irradiated Bovine Blood Feed on *G. pallidipes* and *G. fuscipes fuscipes* under Laboratory Conditions for the Purpose of Sterile Insect Techniques" is my original work. That is, it has not been submitted for the award of any academic degree, diploma or certificate in any other university. All sources of materials that are used for this thesis have been duly acknowledged through citation.

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Approval of Advisor

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LIST OF ABBREVIATIONS AND ACRONOMS

AAT	African Animal Trypanosomosis
AHI	Animal Health Institute
HAT	Human African Trypanosomosis
ANOVA	Analysis of Variance
AW-IPM	Area-Wide Integrated Pest Management
CFU	Colony Factor Unit
DM	Daily Mortality
DMRT	Duncan Multiple Range Test
El-Pino	Medfly Rearing Facility in Guatemala
FNOS	Follicle Next in Ovulation Sequence
IBT	Irradiated Bioassay Test
KTFRC	Kality Tsetse Fly Research Center
NIBT	Non-irradiated Bioassay test
PPIF	Productivity of Pupae per initial female
RAD	Radiation-Absorbed Dose
RH	Relative Humidity
SAT	Sequential Aerosol Technique
SCVL	Sebeta Central Veterinary Laboratory
SIT	Sterile Insect Technique
SD	Standard Deviation

ABSTRACT

The use of sterile insect techniques in tsetse fly control and eradication campaign depends on mass production of good quality sterile males in laboratory. The mass production of the flies in turn requires a constant supply of blood diet with good quality because both males and females including the larvae within the uterus of pregnant female flies depend on the blood for their survival and reproduction. The aim of this study was to investigate storage time and irradiation effect on reproductive and mortality/ survival rate of G. pallidipes and G. fuscipes fuscipes under laboratory condition. A total of 540 females and males flies randomly sampled from each G. pallidipes and G. fuscipes fuscipes species to investigate both storage and irradiation effect respectively. Flies were fed on different batches of blood collected and stored for three years, two years, one year, and one month fresh blood, fed four times a week. To investigate irradiation effects, two groups again were allocated to feed on both irradiated and non-irradiated fresh one-month blood. Pupae were collected daily and mortality checks were done on weekly basis. The quality of the blood diet was measured by observation of microbial load in blood, tsetse production parameters including mortality/ survival, PPIF, fecundity, pupae production, and weight. One-way analysis of variance was performed to analyze data on storage time effect and the qualities of blood between irradiated and non-irradiated were analyzed by using STATA computer software (version 12). Mean separation was made using Duncan's Multiple Range Test (DMRT). The result revealed that the survival of flies fed on irradiated blood batches stored at -20ºC within two to three years' time range had lower survival compared to the rest of the irradiated blood batches stored for less than a year. Similarly, lower pupae production was also recorded among these irradiated bloods. The irradiated blood stored for two to three years range had more of small pupae (class A and B) compared to fresh one-month irradiated blood batched stored less than a year in both species. Additionally, our study showed that irradiated defibrinated bovine blood was also found to be suitable to maintain G. pallidipes and G. fuscipes fuscipes colonies compared with non- irradiated bovine blood. The result showed that the efficacy of sterilization of cobalt 60 (1.5. KGy) on blood was high. Both the survival and PPIF of flies fed on non-irradiated fresh one-month blood batches were lower compared to flies fed on fresh one-month irradiated blood in both species. Bacterial isolates of Rhudococcus equi commonly found in both non-irradiated blood feed diets and in sampled dead flies. There was a significant difference between the blood storage time and the production parameters were better among flies fed on fresh one-month blood stored less than a year. Furthermore, this study showed that irradiated defibrinated bovine blood was suitable to maintain G. pallidipes and G. f. fuscipes colonies compared with non-irradiated bovine blood. Both the survival and PPIF of flies fed on non-irradiated fresh one-month blood batches were lower compared to flies fed on fresh one-month irradiated blood in both species. Therefore, it can be recommend that fresh one-month irradiated bovine blood stored for less than a year to be an optimum storage time for rearing of both Tsetse species at KTFRC for the control of the flies using SIT.

Keywords: *G. pallidipes, G. fuscipes fuscipes*, Fecundity, Pupae per initial female, Storage time, Survival.

1. INTRODUCTION

1.1 Background of the study

Tsetse flies (Diptera: Glossinidae) are the cyclical vectors of the trypanosomes, there are about 31 known species and subspecies of tsetse flies present in Africa. They can be divided into three distinct groups or subgenera of *G. fusca* group, *G. palpalis* group and *G. morsitans* group. Only nine species and subspecies, belonging to either the *G. palpalis* or the *G. morsitans* group (leak, 2009), However, only five species *G. pallidipes, G. m. submorsitans, G. f. fuscipes, G. tachinoides* and *G. longipennis* are known in different parts of Ethiopia (Alemu *et al.,* 2007; Fedesa *et al.,* 2015). The cause of African Animal Trypanosomosis/ trypanosomiasis (AAT) or nagana in animals and Human African trypanosomiasis (HAT) or sleeping sickness in humans (Abd-Alla *et al.,* 2013; Duguma, 2016). The nagana-causing trypanosomatids, *Trypanosoma vivax* and *Trypanosoma congolense,* are foremost pathogens of cattle and other ruminants (Duguma, 2016; Ortiz-Martínez *et al.,* 2023). Human sleeping sickness is a zoonosis caused by the protozoan *Trypanosoma brucei rhodesiense* in East Africa and *Trypanosoma brucei gambiense* in West and Central Africa (Ortiz-Martínez, 2023). Vaccines are not available and are improbable to be developed due to the antigenic dissimilarity showed by the trypanosome in the mammalian host (Abd-Alla *et al.,* 2013). The drug treatment of both AAT and HAT is in a risky state and relies on old, frequently dangerous drugs and resistance is becoming an increasing problem (Duguma, 2016; Ortiz-Martínez *et al.,* 2023).

A principal component of the Sterile Insect Technique (SIT) is the sustainable production and maintenance of large numbers of high quality insects. Both male and female Tsetse flies are obligate blood feeders and one of the main challenges in tsetse fly colony maintenance is the sustained supply of high quality blood (Feldmann and Hendrichs, 2001; Duguma, 2016). Tsetse flies reproduce by adenotrophic viviparity, making the adult and larval stages dependent on the same source of food and insufficient nutrition of the female fly will lead to abortions (Benoit *et al.,* 2015). Increased abortion rates and reduced productivity resulting from inadequate diet will hamper colony growth, which is already inherently low due to the slow reproductive cycle of Tsetse flies. The membrane feeding techniques (in-vitro feeding) used for rearing of tsetse flies have advantages over the conventional methods of feeding flies on host animals.

In-vitro (membrane) feeding are now in use at several research centers, including Kality Tsetse Fly Rearing Center (KTFRC) in Addis Ababa, Ethiopia (Benoit *et al.,* 2015; Onyango, 2020). In this technique, the flies obtain blood by piercing an artificial membrane. Approximately 100 ml of blood are needed daily to feed up to 1500 flies on 45 x 45 cm surface.

An area-wide integrated pest management (AW-IPM) program with a sterile insect technique (SIT) component was proposed as a strategy to establish a tsetse free South Africa (Duguma, 2016). This strategy consisted of a phase of Tsetse population reduction using the sequential aerosol technique (SAT) followed by the release of sterile males to eradicate relic pockets. The SIT requires the production of the target insect in large numbers, the sterilization of the males with ionizing radiation followed by the sequential and area-wide release of these males in numbers sufficient to outcompete their wild counterparts for mating with fertile females. A mating of a sterile male with a virgin wild female will result in no progeny, which will lead to population reduction and the eventual eradication of the target population (parker *et al.,* 2021).

SIT as a technique of choice requires mass production of Tsetse flies in the laboratories. Therefore, quantitative amount of blood is needed for maintenance of fly colonies. In Ethiopia, establishment of Tsetse species was started in 1997 at the Animal Health Institute in Kality assisted by International Atomic Energy Agency (IAEA)-initiated a project in the Southern Rift Valley of the Southern Tsetse Eradication project (STEP) (Alemu *et al.,* 2007; Olbamo *et al*., 2024).

Mass production of good quality sterile males for SIT requires a blood diet with good quality. This is because both (males and females) flies depend on blood for their survival and nourishment. However, there are several factors that could influence the quality of the blood diet, like genetic, environmental, chemical and physical factors of the host animal (Kebede, 2012). Chemicals and microbiological contamination during collection, handling and storage also play role. This paper investigated the storage time effect of defibrinated and irradiated bovine blood feed on *G. pallidipes* and *G. fuscipes fuscipes* under laboratory conditions for the purpose of SIT.

1.2 Statement of the problem

The use of SIT in Tsetse fly control and/or eradication campaign depends on mass production of good quality sterile males in the laboratory. The mass production of the flies in turn requires a constant supply of blood diet with good quality because both males and females including the larvae within the uterus of pregnant female flies depend on the blood for their survival and reproduction. However, there are several factors that could influence the quality of the blood diet like nutritional status of animals, pathogenic bacteria, virus or chemicals contamination and storage time effect. As a result of this, an experimental based identification and response to these factors need to be given a greater emphasis in any Tsetse fly producing facilities. Nevertheless, research works on efficient use of blood diet and factors affecting the quality due to the lack of storage time in study site is lacking. Therefore, this work attempted to address storage time effect of defibrinated and irradiated bovine blood feed on *G. pallidipes* and *G. fuscipes fuscipes* under laboratory conditions for the purpose of SIT.

1.3 Objectives of the study

1.3.1 General Objective

The overall objective of this study is:

- To evaluate the effectiveness based on storage time of bovine blood on the productivity performance and mortality rate of *G. pallidipes* and *G. fuscipes fuscipes* for mass production.

1.3.2 Specific objectives

- To compare and contrast the productivity performance and mortality rate of *G. pallidipes* and *G. fuscipes fuscipes* on bovine blood diet from different storage time length.
- To analyze contaminant bacteria or pathogens in different batches of stored bovine blood.
- To evaluate the productivity and mortality rate of irradiated and non-irradiated effects and efficacy test of recent stored bovine blood diet on *G. pallidipes* and *G. f. fuscipes.*

1.4 Scope of the study

The study was conducted at the KTFRC. The scope of this study is mainly focused to be increases of laboratory Tsetse fly production, and it observes the challenges of separating selected bovine blood with different storage times fed with proper attention for 90 days. It includes improving quality of KTFRC rearing production and this is included in the sample frame.

Therefore, some limitations occurred during study time such as shortage of budget and transport. In addition to this, there were since have not been recent studies in this area.

1.5 Significance of the study

The findings of this study may provide information on the storage time effect of defibrinated and irradiated blood diets of Tsetse flies on productivity and mortality rate on *G. pallidipes* and *G. f. fuscipes* rearing for the purpose of SIT. The use of SIT in Tsetse fly control and eradication campaign depends on mass production of good quality sterile males in laboratory. The mass production of the flies in turn requires a constant supply of blood diet with good quality because both males and females including the larvae within the uterus of pregnant female flies depend on the blood for their mortality (survival) and reproduction. However, there are several factors that could influence the quality of the blood diet like nutritional status of animals, bacteria or chemicals contamination and storage time effect. Therefore, this work attempted to investigate storage time effect of defibrinated and irradiated bovine blood feed on *G. pallidipes* and *G. fuscipes fuscipes* under laboratory conditions for the purpose of SIT. This study have not been previously studied in the KTFRC.

2. LITERATURE REVIEW

2.1 General overview of Tsetse flies

Tsetse flies are obligate blood sucking flies of medical and veterinary importance because they transmit trypanosomes that cause African sleeping sickness in humans and nagana (African animal trypanosomiasis) in livestock (Ortiz-Martínez *et al.*, 2023). Generally, they are considered as one of the greatest factors affecting economic and social development in Africa. The morbidity and mortality caused by African sleeping sickness continue to be significant (Boutayeb, 2010). Nagana, which has stifled agricultural productivity for decades, still stands as a major deterrent to the development on the continent. Nagana remains the main hindrance to development of efficient and sustainable livestock production, and the presence of Tsetse is the root cause of hunger and poverty (Boutayeb, 2010; Duguma, 2016).

2.2 Classification and geographical distribution of Tsetse flies

Tsetse flies belong to the insect order Diptera, family Glossinidae and genus *Glossina.* The genus *Glossina* is divided into three different groups based on a combination of distributional and morphological characteristics (Fedesa *et al.*, 2015). These are morsitans (savannah species), fusca (forest species) and palpalis (riverine species). There are about 31 species and subspecies of *Glossina* mostly restricted to sub-Saharan Africa (O'Connell, 2008). They are distributed discretely throughout their range and each taxon is restricted to a relatively specific habitat (Fedesa *et al.*, 2015). The morsitans group species are present throughout the savannah (grassy woodland) of Africa, where climatic conditions are less severe, and their distribution may be limited by scarcity of game animals on which to feed and by lack of vegetation. *Glossina morsitans* is the most widespread species (Spinage and Spinage, 2012). The palpalis group is limited to the very humid areas of Africa, the mangrove swamps, the rain forest, the lakeshores and the gallery forests along rivers. They are found in the more humid areas of West Africa. The fusca group species are widely scattered in forest areas throughout eastern parts of Africa (Spinage and Spinage, 2012).

2.2.1 Tsetse flies in Ethiopia

In Ethiopia, Tsetse flies are confined to the western and southern regions between longitude 33^{o} and 38^{o}E and latitude 5^{o} and 12^{o}N (Fedesa,*et al.*, 2015). The total area infested by Tsetse flies in 1976 and 1988 was 98,000 km^2 and 120,000 km^2, respectively. Tsetse infested areas lie in the lowlands and in the river valleys of Abay (Blue Nile), Baro, Akobo, Didessa, Ghibe and Omo (Alembo, 2019).

The infested area extends from the southern part of the Rift valley, around the southwestern corner of the country and along the western lowlands and escarpments to the Blue Nile. Restricting a further eastward spread is the cold limit imposed by highlands that rise to the height above, which Tsetse cannot survive or the semi-desert condition along the southern border east of the Rift Valley (Kebede *et al.*, 2015). Elsewhere there have been advances of tsetse, including extension of the upper altitude limit of the fly from about 1,600 to 2,000 meters above sea level (Kebede *et al.*, 2015; Olbamo *et al*., 2024). Tsetse fronts in many places are unstable and tsetse-animal interface is constantly moving. Consequently, new areas are being invaded and settled communities are being continually evicted by the advancing tsetse. Such hot spots include the areas in upper Didessa valley, the northern and northeastern edges of Lake Abaya in the Rift Valley, the upper reaches of the Omo-Ghibe and its tributaries. According to survey result conducted by (Fedesa *et al.*, 2015), five species of *Glossina* (*G. m. submorsitans, G. pallidipes, G. tachinoides, G. f. fuscipes* and *G. longipennis*) have been recorded from Ethiopia but only five are widespread and have significant economic importance. These are *G. m. submorsitans* and *G. tachinoides*, which have across Africa south of the Sahara desert, and *G. pallidipes* and *G. f. fuscipes*, which often occur together in East Africa, although the former extends far to the south whereas the latter has essentially central African distribution. The highest catches of *G. pallidipes* were in bushes and wooden grassland in the Southern Rift Valley of Ethiopia (Duguma, 2016). Out of the nine regions of Ethiopia are infested by tsetse flies, Amhara, Beneshangul-Gumus, Gambella, Oromiya, and SNNPR, (Addis *et al.*, 2023).

2.3 Biology of Tsetse flies

Adult *Glossina* species are dull in appearance, varying in color from light yellowish brown to dark blackish brown (Spinage and Spinage, 2012). In some species, the abdomen may have alternate darker and lighter bands. The smallest species is 6-8 mm long and the largest is 10-14 mm. Tsetse flies can be distinguished from other biting flies by their forward-pointing mouthparts (proboscis) and characteristic wing venation (Duguma, 2016).

There are about 31 known species and subspecies of Tsetse flies belonging to the genus *Glossina*. They can be divided into three distinct groups or subgenera of *G. fusca* group, *G. palpalis* group and *G. morsitans* group. Only nine species and subspecies, belonging to either the *G. palpalis* or the *G. morsitans* group, are known to transmit sleeping sickness (Leak, 2009; Duguma, 2016).

2.3.1 Life cycle of tsetse flies

The female tsetse fly does not lay eggs but produces larvae, one at a time. The larva develops in the uterus over a period of 10 days and then deposited fully-grown on moist soil or sand in shaded places, usually under bushes, fallen logs, large stones and buttress roots. It buries itself immediately and turns into a pupa (Figure 1; Spinage, 2012). The fly emerges 22–60 days later, depending on the temperature that means higher temperatures shorten pupal period, lower temperatures lengthen the pupal period in some climates. Females mate only once in their life, and with optimum availability of food and breeding habitats, can produce a larva every 8-10 days (Peacock *et al.*, 2012).

Figure 1. Life cycle of Tsetse flies.

2.3.2 Feeding behaviors of tsetse flies

In all tsetse flies, both males and females feed on blood, but the species differ in their preferences for the source of blood. Most tsetse flies feed preferentially on animals and only accidentally on humans (Duguma, 2016; Marcondes and Thyssen, 2017). The most dangerous species are those that are flexible in their choice and feed on any blood source that is easily available, including humans. During the act of feeding, the fly penetrates the skin with its proboscis. The rupture of small blood vessels forms a pool of blood in the tissue and the fly injects saliva to prevent blood coagulation. Infection of the host takes place at this stage, with infective metacyclic trypanosomes in the saliva. Tsetse flies once infected with trypanosomes are likely to transmit the parasite for the remainder of their lives (Duguma, 2016; Marcondes and Thyssen, 2017). While searching for food they are attracted by large moving objects, by strikingly blue objects (Cherono, 2019).

2.4 Vector control methods

Tsetse fly control was carried out in the early days using methods that involved clearing of vegetation in large tracts of land, killing of wild game and installation of game fences to prevent the game hosts from carrying flies into the tsetse fly free areas (Wilson *et al.,* 2020).

Insecticide-based control techniques, which involve ground and aerial spraying, were introduced to complement the methods employed to destroy the vector habitat (Wilson *et al.,* 2020). Currently, Tsetse flies is controlled by the use of traps and targets, especially used of odor-baited and impregnated by insecticides. (Duguma, 2016). The flies are attracted to large blue or black cloths and chemicals such as acetone, in addition, the tsetse fly is attracted by cow breath, cow urine and when animals are treated with insecticides (Wamwiri *et al.,* 2007; Duguma, 2016). The major shortcomings in the limited size of area for which they can be economically deployed relative to the total size of the tsetse fly infested area and the continued costs associated with preventing re-invasion and also the fact that they do not lead to complete eradication (Wilson *et al.,* 2020)

SIT is birth control for insects, it is used primarily to control or eradicate insect pests, usually crop pests or human and animal pests. The target insect is reared in great numbers, the males

sterilized, and usually using gamma radiation (Klassen *et al.*, 2021). They mate with the wild females which results in infertile eggs being laid and provided certain other population management activities are properly carried out, the wild population then declines rapidly (Duguma, 2016; Stringer *et al.*, 2017).

2.5 History and principles of SIT

2.5.1 History of SIT

SIT is initiated by El-Pino Medfly Rearing Facility in Guatemala (E.M.R.F.IG), additionally, in the 1930s, Knippling had a devastating outbreak of cattle disease in North America, which was stopped by the use of SIT (Ware, 2019). The first successful use of SIT was recorded in 1953 on the island of Curacao (Klassen *et al.*, 2021). Since then SIT has been further refined and developed for number of insect pests mainly by the United States Department of Agricultural and FAO/IAEA (Bourtzis and Vreysen, 2021). SIT has been developed to suppress or eradicate more than 20 insect pests, many of them being fruit flies and other fruit pests (Reddy and Rashmi, 2016).

The infestation of livestock by *Cochilomia hominivorax* is a major economic problem and in Texas alone, in the epidemic year of 1935, there were approximately 230,000 infestations of livestock and 55 in humans (Reddy and Rashmi, 2016). As a result of the economic cost of this pest, large-scale screwworm fly control was initiated in the south-eastern states of the USA in 1957-1959. This was achieved by the release of large numbers of male *C. hominivorax*, which had been sterilized by radiation. Sterilized males mate with wild females, which are turn rendered infertile. Subsequent control operations spread the area of sterile male release and in 1966; effective control of the worm in the USA was declared (Reddy and Rashmi, 2016). In Africa started SIT technology 1988, the myiasis worms were discovered in area of 10 km south of Tripoli in Libya and causes a wide spread of myiasis and hence great economic loss. This leads to the implementation of a major international control program, which has successfully eradicated the fly from this area, again using the release of sterile males (Reddy and Rashmi, 2016).

In the island of Zanzibar, the introduction of the SIT helped in the eradication of tsetse fly in 1996 (Reichard, 2002). In Zanzibar, a sterile insect plant producing 70,000 irradiated pupae weekly was constructed that made the release of over 7.8 million sterile male flies possible. Dispersal of the irradiated males over time was done to achieve an estimated ratio of 50 sterile males for every 1 wild male in order to overwhelm the residual wild tsetse population (Reichard, 2002).

SIT was started in Ethiopia 1997, with the help of the Ethiopian Government and the International Atomic Energy Agency (IAEA). In South Rift Valley Tsetse Fly Eradication Project (STEP) was started, Tsetse fly Production in Kality started in August 2000, and wild females *G. pallidipes* and *G. fuscipes fuscipes* were collected from the Arbamanch area of the southern region (Alemu *et al.,* 2007). Among the major collaborations established are the Pan-African Regret and Trypanosomosis Eradication Campaign of the African Union (AU-PARTEC), IAEA/FAO, the Organization of the Petroleum Exporting Countries (OPEC), the Government of China, the United Nations and the Government of Ethiopia (IAEA, 1957-2007).

2.5.2 Principles of SIT

The SIT is non-intrusive to the environment, has no adverse effects on non-target organisms and it is species-specific and can easily be integrated with biological control methods such as parasitoids, predators and pathogens (Leak, 1999; Berror and Mengistu, 2023). The SIT includes the mass production, sex separation, sterilization and release of sterile males. Contemporary methods available to induce sterility in the released insects are ionizing radiation or chemo-sterilization (Duguma, 2016; Klassen *et al.,* 2021).

As a prerequisite, tsetse density has to be suppressed through the widespread application of insecticide treated SADs (Stationary Attractive Devices), live baits or fly trapping to a point where the SIT is considered feasible. The concept of sterile male release technique is based on the biology of the flies (Duguma, 2016). That tsetse female copulate only once, and if the male of a copulating pair is sterile, the female will not produce offspring during her lifetime (Berror and Mengistu, 2023). It is also estimated that ten sterile males are required per female. In order to reduce the number of sterile males required it is necessary to carry out two to three insecticide sprays and then to release 12000 sterile males per square kilometer, even in areas where the

tsetse density is low. Female tsetse produces at most nine larvae, and therefore has the lowest reproduction potential of any insect (Klassen*et al.*, 2021). A single mating provides sufficient sperm for fertilization through the female´s 90-100-day life span. Since females usually mate only once, if they are mated by a sterile male they will not produce any offspring. These features of the life cycle make the tsetse fly a good target for SIT (Kebede *et al*., 2015). However, the irradiated tsetse flies are fully capable of developing and transmitting trypanosomes to livestock (Kebede *et al*., 2015). In order to avoid the risk of disease transmission, the sterilized tsetse flies are fed on either uninfected blood meals or blood meals are medicated with trypanocidal drugs before the sterilized insects are fed.

2.6 Mass rearing of flies

Rearing of large number of insects for their continuous availability in the laboratory depends on the knowledge of insect biology, behavior, habitat and nutrition. An understanding of the mating habits, preoviposition periods, fecundity, longevity, sex ratio, environmental requirements, and food and feeding preferences of the insect is necessary in developing rearing techniques (Orozco-Davila *et al*., 2007). Conservation of space is also a major consideration, especially when several days are required from the time of eggs in rearing containers until the desired life stage has been harvested. During the process of establishing a strain, along the rearing process, and during treatment before release, the insects are subjected to highly artificial conditions, including extreme population densities, a sterilization process and sometimes genetic manipulation. These all factors highly affect the biological treated insects and their performance during SIT operation (Lux *et al.*, 2002).

The difficulties of mass rearing of an insect vary depending up on the nature of reproduction of an insect. For example, as compared to screwworm flies, moths, mass rearing of tsetse flies have more advantageous. The screwworm requires special resources and rearing conditions at all stages of its development but in the case of tsetse fly, only the pupal and adult stages have to be considered because the egg and larval stages remain within the pregnant female fly. In nature, the larvae of the screwworm fly grow with in living mammalian flesh. Therefore, for mass rearing purpose, a very complex larval medium, simulating this living tissue, had to be developed and deployed on a very large scale. For the adult stage, screwworm flies normally

feed on liquids in animal wound for protein requirements, and nectar from flowers for carbohydrates and water. The diet provided to an adult screwworm colony must take into account all three requirements (Feldmann and Hendrichs, 2001; Solano *et al.*, 2015).

The ingredients of screwworm diet, most of which have to be imported using hard currency. However, in tsetse flies, larvae do not have to be fed as they develop within the female fly. Adult tsetse flies do not required water or carbohydrates, only need high quality blood (Feldmann and Hendrichs, 2001; Solano *et al.*, 2015).

2.7 Irradiation and sterilization of biological material

When biological material is irradiated, molecular bonds are broken, ions created, and free radicals formed. The free radicals attack further molecular bonds, and when DNA is damaged, it can lead to the formation of dominant lethal mutations in the germ cells (Fong, 2016). Damage to somatic cells also occurs, especially in cells undergoing mitosis. In general, damage to the germ and somatic cells increases with dose and somatic damage decreases when irradiated later in development of the insect as the number of cells undergoing division decreases. As field competitiveness is a crucial parameter, it is important to minimize the adverse effects of irradiation (Fong, 2016). Although it is generally believed that the released males need to be fully sterile, it has been suggested that more sterility can be introduced into the field population using lower radiation doses but with more competitive insects (Robinson, 2002; Parker and Mehta, 2007). Moreover, reduced competitiveness can be partly overcome by increasing the ratio of sterile-to-wild insects (Lance and McInnis, 2021).

2.7.1 Types of irradiation

2.7.1.1 Ionizing irradiation technique

There are different types of ionizing irradiation, such as radio waves, radar, infrared, microwaves, ultraviolet, electrons, X-rays and Y-rays. Irradiation is a direct or fumigant control method of pests and genetic control is used to produce irradiated males and release males in desired places. Female pests are required to lay eggs in the laboratory (Desouky *et al.*, 2015). Irradiated male insect may result mutations in the sperm, killing spermatogonial cells, and inactivation of sperm (Coleman and Alphey, 2004). Depending on the dose and age or

physiological condition of the insect, irradiation of the actively growing stages of an insect can have several effects. Most of the genetic sterility can be caused by irradiation that may result in failure of sperm bundles to separate, lack of motility in the spermatozoa, or other malfunctions that can prevent reproduction and can be induced in sex by selecting the appropriate dose and developmental stage (Desouky *et al.,* 2015). Different types of ionizing radiations produce damage in significantly the same way and the effects are therefore equivalent if the dosage is measured in the same way (Desouky *et al.,* 2015).

2.7.1.2 Non-ionizing irradiation technique

For the irradiation of insects, gamma rays are usually used due to their high energy and penetration. The most common sources of gamma rays are the radioisotopes cobalt 60 and 137Cs as both have a long half-life and emit high-energy gamma rays (Kebede *et al.,* 2015). Cobalt 60 is more easily manufactured and is therefore more often used. In conventional self-shielded irradiators, the sample chamber is surrounded by several rods or pencils" of the isotope. The dose rate of the cell is determined by the activity of the source and the absorbed dose delivered to the insects is controlled by adjusting the exposure time (Kebede *et al.,* 2015).

The sample chamber is a vertical cylinder, approximately 150 mm diameter by 200 mm tall (volume 3.7L) and has a typical dose uniformity ratio of about 1.7. Such self-shielded isotopic irradiators (cobalt 60 or less commonly 137Cs) are the main means of insect sterilization for SIT programs worldwide (Bakri *et al.,* 2005; Kebede *et al.,* 2015). The dose rate distribution in the chamber is not uniform and accordingly, insects receive different dose rates when placed at different positions in the chamber with the dose rate being most uniform towards the center of the chamber. Besides gamma rays, x- rays and accelerated electron beams can also be used to irradiate insects. X rays of appropriate energy have similar penetration as gamma rays, and they have been used in a number of studies on *Anopheles* irradiation (Sengupta *et al*., 2022), but the use of electron beams has not been reported.

Dosimetry is used to quantify the dose received by the irradiated insects. The selection of a suitable dosimetry system depends on several considerations including dose range of interest, ease of handling, expertise available, cost, and uncertainty that is inherent in the system. For

SIT programs, a radio chromic film dosimetry system has been proposed (FAO/ IAEA/ USDA, 2003-2010).

The radiation dose absorbed by an insect to induce sterility is the main importance in sterile insect release program. Insects those receive too low dose may not be sterile efficiently and those received high dose may be uncompetitive (Parker and Mehta, 2007). The International System (SI) unit of radiation is Kilo Gray (KGY), which equals to 100 rad (radiation-absorbed dose). There is no standard radiation dose for all organisms to undergo sterility. Radiation doses that result in sterility in insects and related arthropods range widely between and within order and vary according to the species (Bakri *et al.*, 2005; Kebede *et al.*, 2015). Apart from Lepidoptera, the irradiation doses required to sterilize males of most pest specie commonly (20-150 Gy) which result in dominant lethal mutations (Sengupta *et al.*, 2022).

2.8 In-vitro feeding techniques of Tsetse flies

In vitro feeding are now in use at several research centers, the flies obtain blood by piercing an artificial membrane. Adult tsetse flies do not require water or carbohydrate, Tsetse flies feed on a wide range of vertebrate hosts high- quality blood. Animal blood for tsetse rearing can be collected from a local abattoir and then treated with gamma radiation to eliminate any microorganisms (Feldmann and Hendrichs, 2001).

If a reliable source of quality-tested blood is available, the membrane or *in vitro* feeding system is recommended (Opiyo *et al.*, 2000). The membrane for the in vitro feeding system is made of silicone, and was developed after several attempts with animal skins failed (Feldmann and Hendrichs, 2001). A silicone membrane is reinforced with netting; the size of the netting determines the thickness of the membrane. Small tsetse species are feed using a thin membrane, and larger species a thicker membrane. Bovine or porcine blood, or a mixture of both, has been used for colony maintenance, but the choice depends on the dietary requirements of a particular tsetse species (Kebede *et al.*, 2015). Tests using only bovine blood demonstrated that most tsetse species can be maintained on bovine blood alone (Kebede *et al*., 2015).

Colonies of tsetse flies can be maintained on sterile, fresh one month frozen, defibrinated blood, or blood to which anticoagulants have been added (De Beer *et al*., 2012). The procedure for

feeding flies using the membrane system aims to ensure that flies are given sterile blood in a suitable state. Before feeding, the blood is heated to the body temperature of mammals. A quantity of 100 ml of blood is sufficient to cover a surface area of 2000–2300 cm² and to feed 1500 flies. In the trolley membrane feeding system, cages of flies are removed from the trolley and placed on membranes lying flat on a tray containing warm blood, after feeding the cages are returned to the trolley (FAO/IAEA, 2006).

The feeding system of TPU 3 is the opposite of the above, the blood moves to the flies, and the cage-holding system is stationary. The mobile blood system is moved on rails that are fixed to the floor. Two rows of cages are feed at the same time. The blood is heated by elements in the heating plates, each plate is individually controlled. Temperature sensors are attached to individual heating plates which are connected to the temperature control box. The heating plates remain switched on, but can be programmed to heat during a specified period of time. It is vital that the temperature of the feeding membrane is correct, and therefore must be checked carefully, both before and after pouring the blood onto the tray. Lower trays are filled with blood, and the blood smoothed out under the membrane, by a person standing on the floor (Opiyo *et al.*, 2000), but upper trays are filled from a working platform. When the tray of blood is at the right temperature, it is pushed under the frame of nine cages, and clips put into position to firmly press the tray onto the heating plate.

All equipment must be thoroughly washed, sterilized, and stored in aseptic conditions before being used. The equipment is rinsed with cold water before being washed by hand or in a dishwasher, and then it is sterilized. Silicone membranes are washed in a washing machine. Plastic wares are heat sterilized at 80ºC, metal and glassware at 100ºC, and feeding trays with membranes at 120ºC overnight (Feldmann and Hendrichs, 2001).

It is recommended that the in vitro feeding of flies take place in a room that is not used for other daily routine colony work. Flies should be fed in environmental conditions that are as similar as possible to those under which they are held. Lighting should be subdued, indirect, and preferably from tungsten lamps, with illumination maintained at 12–14 lux. To reduce the distraction of flies during feeding, movement in the vicinity should be minimized, and cages covered with dark cloths (O'Connell, 2008). When dealing with riverine species, e.g. *Glossina palpalis*

palpalis (9 species) to their essentially living in riverine woodland, in gallery-forest along rivers and lakeshores (RH 80-85%). Newstead, it may be necessary to increase the relative humidity (RH) in the feeding areas.

Both females and males should be fed at suitable intervals. To reduce the risk of bacterial contamination spreading throughout the colony, younger flies should be fed before older flies. If flies, after emergence, are separated using a chiller, flies should be given time to recover (2.5–3 hours) before feeding them. If flies are separated in the morning, they can be fed with a sterile feeding set-up in the afternoon or the following day, depending on the condition of the flies and their energy reserves (FAO/IAEA, 2006).

The feeding system used depends on the species and condition of the colony. However, a colony can be trained to adapt to a treatment convenient to the management of the rearing facility. The flies are feed on Mondays, Wednesdays and Fridays. On Tuesdays and Thursdays, only young flies (less than 1 week old) are feed. No feeding takes place on Saturdays and Sundays (FAO/IAEA, 2006).

When the supply of blood is not assured, and where in vitro feeding cannot be performed, it is possible to maintain tsetse colonies on an in vivo system, using live hosts such as goats, rabbits, guinea pigs and cattle. However, it should be pointed out that the husbandry of host animals impacts on the colony performance. Rabbits must be feed a high-quality diet that is free from antibiotics and insecticides. The host animals can be trained to become accustomed to being restrained during the feeding period. Rabbits are trained to sit in restraining boxes and accept cages of flies that feed on their ears (the ears rest on padded platforms). Goats and cattle are restrained, and cages of flies are strapped to the body with elastic rubber bands (O'Connell, 2008).

The frequent use of host animals to feed tsetse flies raises the risk of over challenging them. Some animals develop adverse skin reactions from tsetse bites. Antibiotics and coccidiostats in animal feed may affect the fecundity of tsetse flies, and animals undergoing treatment with antibiotics should not be used (FAO/IAEA, 2006).

In endemic Trypanosomosis areas, host animals may carry an infection at the time of purchase, escalating the probability of infecting the tsetse colony. Therefore, host animals should be checked regularly for parasites, and treated if found infected. The veterinary care of host animals is a major component of the in vivo tsetse feeding system (Hu *et al.*, 2008).

3. MATERIALS AND METHODS

3.1 Study Area

This study was conducted in Kality Tsetse Fly Research Center (KTFRC) located in Addis Ababa city, the capital city of Ethiopia. City of Addis Ababa location on 8°53' 44.9916"N and 38°47' 20.9832"E latitude and longitude, respectively and it is found at 2,140 m above sea level (Seifu and Stellmacher, 2021).

KTFRC was established in 1997 by the Ethiopian Government assisted by the International Atomic Energy Agency (IAEA) initiated a project in the Southern Rift Valley called the Southern Tsetse Eradication Project (STEP). The STEP Kality Tsetse Fly Research Center was initiated in August 2000 following the renovation and equipping of an existing building to function as a temporary insectary. It was started by wild female of *G. pallidipes* and *G. fuscipes fuscipes* that were collected from around Arbaminch (Alemu *et al*., 2007). The major partnerships for the establishment were Pan African Tsetse and Trypanosomosis Eradication Campaign of the African Union (AU-PATTEC), IAEA/FAO, Organization of the Petroleum Exporting Countries (OPEC), the Chinese Government, the United Nations and the Government of Ethiopia (IAEA, 1957-2007).

The insectary has its own microenvironment adjusted as the need of the *G. pallidipes* and *G. fuscipes fuscipes*. The experimental flies were maintained in different environmental condition as that, *G. pallidipes* were kept in the insectary at 24 - 25°C, and 75 - 80% RH and *G. fuscipes fuscipes* were kept at 24 - 25°C, and 80 - 85% RH (FAO/IAEA, 2006).

3.2 Research design

This study investigated the effect in storage time of bovine blood on the productivity and mortality/ survival rate of *G. pallidipes* and *G. fuscipes fuscipes,* between November 2022 to June 2023 at KTFRC. Accordingly, four consecutive years, different batches of bovine blood of 2020, 2021, 2022, and 2023 (1 month stored blood) kept in cold storage at -20°C were used. The blood of the most recent year in 2023 (1 month stored blood) was used. The blood batches were obtained from KTFRC collected from Addis Ababa Municipal Abattoir from cattle slaughtered

during 2020-2023 for tsetse fly mass rearing purposes, which was certified as healthy and of good quality by the slaughter house's veterinary staff.

Experimental flies: The experimental flies, *G. pallidipes* and *G. fuscipes fuscipes*, used in this study were obtained from the same production batch of pupae originated from the main colonies in the insectary of KTFRC. After emergence, teneral flies were placed separately in colony cages with a male 9 to female 32 ratio of 1:4 (FAO/ IAEA, 2006).

The two Tsetse flies species (*G. pallidipes* and *G. f. fuscipes)* used for the experiment were reared and maintained in two separate rearing rooms designed to maintain the optimum environmental laboratory conditions of each *G. pallidipes* and *G. fuscipes fuscipes* species colonies. *G. pallidipes* were kept in the insectary at 24 - 25°C, and 75 - 80% RH. *G. fuscipes fuscipes* were kept at 24 - 25°C, and 80 - 85% RH (FAO/IAEA, 2002), but, both species were under similar photoperiod of 12:12 h. (L: D), based on normal optimum environmental condition designed for these species (Berror and Mengistu, 2023). For both species, the feeding schedule was four days per week fed on the mat, during blood fed the temperature of mat at 35-37°C (Monday, Wednesday, Friday, and Saturday) (Crampton *et al.*, 2012).

3.3 Study population and study period

The study was conducted from November 2022 to June 2023, and included a total of 540 (432 females and 108 males) from each *G. pallidipes* and *G. fuscipes fuscipes* obtained from the same production batch of pupae that originated from the main colonies of the insectary at KTFRC. The cages containing the colonies of these Tsetse flies were maintained and reared under in-vitro feeding under laboratory conditions. These tsetse fly colonies have in existence since 2000 and were fed using an in-vitro silicon membrane system. All teneral flies (*G. pallidipes* and *G. fuscipes fuscipes*) of both sexes were used for both experiments and were derived randomly from the same batches of colonies of *G. pallidipes* and *G. fuscipes fuscipes* from KTFRC (FAO/ IAEA, 2006).

3.4 Study sample and sampling procedure

The required sample size depends on the type of experiment: For bovine blood feeding experiment, newly hatched (teneral) tsetse flies, 432 females and 108 males each of *G. pallidipes*

and *G. fuscipes fuscipes* were used. Experimental teneral flies were kept in cage, 45 flies per cage and in each experimental group. It was replicated three times to obtain an adequate sample size for statistical analysis. *G. pallidipes* and *G. fuscipes fuscipes* were fed for 90 days, for productivity and mortality analysis of female flies. *G. pallidipes* flies are kept at 24 - 25 °C, and 75 - 80% RH and *G. fuscipes fuscipes* are kept at 24 - 25 °C, and 80 - 85% RH (FAO/IAEA, 2006).

3.4.1 Source of blood

For all experimental studies, the frozen defibrinated bovine blood batches were obtained from KTFRC bloodstock and the test bloods were kept at - 20^{o}C. The different batches of blood stored for the study were obtained from KTFRC storage in January 2023 (1 month frozen), (1 year frozen), (2 years frozen) and (3 years frozen). It was previously assembled into KTFRC from Addis Ababa Municipal abattoir for tsetse mass rearing purpose. The collected cattle blood was confirmed to be healthy and good quality by the slaughterhouse veterinary staff. The blood was collected in a mini plastic containers from the slaughtered cattle. Then poured into 10-litre sterile polyethylene plastic container for defibrination. The goal of defibrination is to avoid rapid coagulation of the blood (YamaguchI *et al.,* 2005). A stainless steel electric paddle shaker was used to perform defibrination for 10 minutes. The collected defibrinated blood was stored at -20°C. The microbiological test does not provide definitive information about the health history of the slaughtered animals.

3.4.2 Irradiation of bovine blood meals

The four blood samples collected from each irradiated and one blood sample collected from non- irradiated frozen 1month blood were screened for bacteria by mixing the collected blood with nutrient agar in a laminar flow hood. All defibrinated blood groups collected with one-month blood before bacteriological examination were decontaminated in 1.5 KGy. Then 5 liters of different groups of blood were stored in separate polyethylene plastic containers, and the blood was stored at -20°C. Finally, the decontaminated sample blood was tested for bacterial contamination. (Asefa *et al*., 2015; Bakri *et al.,* 2021; FAO/IAEA, 2006).

Irradiation efficacy test was conducted between irradiated and non-irradiated one-month frozen defibrinated bovine blood to assess the quality of the blood between irradiated and non-irradiated as a diet for tsetse. Therefore, we exposed one group of a defibrinated and frozen blood to doses (1.5. KGy) of ionizing radiation and the other group of batch of blood left as non-irradiated (Bakri *et al.,* 2021).

3.5 Experimental Procedures

There was two sets of experiments: storage time and irradiation effect test, were conducted aiming to determine on productivity and mortality/ survival rate of *G. pallidipes* and *G. fuscipes fuscipes* colonies under laboratory condition in KTFRC. Before the execution of the actual test, a pilot test was performed to ensure the quality of all blood batches fed for 7 days and fed a few flies on *G. pallidipes* species in four groups irradiated and one group of non-irradiated blood colony in cage. If the safety of the blood was confirmed, then go to main study.

3.5.1 Bioassay feeding test on storage time effect of defibrinated and irradiated bovine blood fed in terms of productivity and mortality rate of female tsetse flies for *G. pallidipes* and *G. f. fuscipes* colonies

A newly emerged teneral flies (one day old) of both *G. pallidipes* (n = 540) and *G. fuscipes fuscipes* (n = 540) were separated into male and female, using a chiller set at +4°C. The same age of a day old, newly emerged fertile females were mated at (1: 4 ratio) male to female sex ratio. The flies were kept in cages (diameter of 20 cm and width of 5 cm) with a density of 45 (male 9 and 36 female) flies per cage with netting on top and bottom for feeding and collection of the larvae.

Four groups of experimental flies of *G. pallidipes* and *G. fuscipes fuscipes* were formed from the same production batches and each group had three replications (cages) of 45 (male 9 and female 36) flies and prepared beforehand, and fed with four types of blood batches for ninety (90) days. These four different batches (1 month, 1 year, 2 years and 3 years) of frozen blood were stored at -20°C.

The one-month frozen blood storage was chosen because it would last for one month until bioassay blood quality testing was done by following a normal or basal diet at KTFRC.

(FAO/IAEA, 2006; Berror and Mengistu, 2023). The holding room temperature and humidity was adjusted (controlled) and monitored daily from the data logger set in the rearing room. For both *G. pallidipes* and *G. fuscipes fuscipes* species, the feeding schedule was four times a week for ten minutes, Monday, Wednesday, Friday and Saturday, using defibrinated bovine blood was fed (Berror and Mengistu, 2023). Then, they were subjected of these experimental groups and the experimental parameters such as the average of productivity parameters were measured as the number of PPIF and fecundity, and survival or mortality rate were measured and analyzed for all the tested groups accordingly (Crampton *et al.*, 2012).

3.5.2 Fresh bovine blood bioassay fed test comparison on microbial, productivity and mortality rate of female tsetse flies fed on irradiated and non-irradiated bovine blood

3.5.2.1 Microbial culture from irradiated and non-irradiated bovine blood

Irradiation efficacy test conducted on defibrinated of one-month bovine blood to assess bacterial contamination on the quality of blood for diet of tsetse flies (FAO/IAEA, 2012). Therefore, 1 liter of a defibrinated, irradiated and frozen blood of 1 month was exposed to a dose of 1.5 KGy of ionizing radiation and another group of the same 1-liter blood of 1 month was not exposed to radiation (non-irradiated). Four blood samples were collected from each irradiated and one blood sample non-irradiated frozen fresh one-month blood were screened for bacteria by mixing with nutrient agar in a laminar flow hood. The mixture was incubated at 37 ºC and checked for bacterial growth after 48 hours (FAO/IAEA, 2012). Different colonies were cultured by transferring to different Petri dishes. Compassion of microbial load CFU/ml in the blood samples was observed between irradiated and non-irradiated blood.

3.5.2.2 Microbial culture from dead flies fed on irradiated and non-irradiated

In the dead flies fed on the irradiated and non- irradiated one month stored bovine blood was had been fed, for culture, isolation and identification, the taken sample was transported to Sabata Central Veterinary Laboratory (SCVL). The taken blood and dead flies sample was transported in accordance with the standard transport protocol (FAO/IAEA, 2006). Then the samples crushed and cultured for bacterial growth (FAO/IAEA, 2006).

Single pure bacterial colonies present were isolated by steaking or pouring, the colonies were tested by catalase test, oxidation test, Gram stain detection, oxidative fermentation (OF) test and Maconkey test. Based on these results, bacteria can be identified using the appropriate biochemical test, according to the reaction of the reading, and then the reaction to the Gram stain under the microscope (positive color, purple or negative, pink), size (small, medium, small, medium, small, medium) or large) and shape (rods, cocci or bacilli) (FAO/IAEA, 2006).

3.5.2.3 Comparison of irradiated and non-irradiated bovine blood bioassay feeding test in terms of productivity and mortality rate

The *G. pallidipes* (n = 270) and *G. fuscipes fuscipes* (n = 270) were separated into male and female using a chiller set at +4°C. The same age of a day old (teneral) flies, newly emerged fertile females were mated at (1:4 ratio). Female and male flies were kept in cages (diameter of 20 cm and width of 5 cm) with a density of 45 (male 9 and female 36) flies per cage with netting on top and bottom for feeding (Beker *et al.*, 2005). Two groups of experimental flies of *G. pallidipes* and *G. fuscipes fuscipes* fed with two types of blood (irradiated and non-irradiated blood). These two experimental groups of flies fed a one month frozen defibrinated bovine blood was exposed to doses (1.5 KGy) of ionizing radiation and the other group of a one month non-irradiated or don't exposed to ionizing radiation bovine blood was fed for ninety days and investigated productivity and mortality rate of flies accordingly Berror and Mengistu (2023)..

3.6 Biological data collection

Daily biological parameters were collected in order to evaluate the blood quality factor (QF). Larviposition date, number of pupae deposited, individual weight of pupae deposited, and number of dead females were all used to calculate QF (separately fed and unfed females). These variables were combined in a standard formula to produce a blood meal nutritional quality (QF) value as an indicator of colony pupae production (De Beer *et al*., 2012). The parameters were entered into the database daily. This includes the number of dead females, the number of larvae deposited and the weight of pupae, which was divided into five categories, (for *G. pallidipes*, A: 23 mg, B: 23 to 28 mg, C: 29 to 32 mg, D: 33 to 37 mg, and E: > 37 mg and *G. fuscipes fuscipes*, A: 22 mg, B: 22 to 27 mg, C: 28 to 31 mg, D: 32 to 36 mg, and E: > 36 mg), (Table 1). For the purpose of the study, QF of above 1 this value was considered acceptable and

indicates that the blood treatment is efficient and suitable for the production of quality pupae (Asefa *et al.*, 2015; Joint, 2023). The nutritional quality of each diet was established based on information collected over 90 days period.

3.6.1 Pupae Production

The mating cages were placed in individual trays with pupae was counted every morning, beginning from days 17 up to the end of the experiment. All pupae were collected from *G. pallidipes* and *G. fuscipes fuscipes* flies from different blood meals using a small Petridis (IAEA, 2012; Kapitano, 2015). The each female production of pupae were expressed as per 10 days, with considering day 17 after immediately they emerged from pupae stage as the first larviposition day (Kapitano, 2015).

3.6.2 Productivity of Pupae per initial female (PPIF)

Both male and female flies were fed 4 times a week for 10 minutes each flies. Mating cages were placed in individual trays and pupae were collected daily in the morning from day 17 until to the end of experiment from *G. pallidipes* and *G. fuscipes fuscipes* flies. PPIF and Fecundity were expressed as the Pupae per initial female (PPIF) and pupae produced per 10 days (P/f/10d) respectively (IAEA, 2012). PPIF, female productivity is measured as Pupae per initial female (PPIF), the total number of pupae produced in a given period divided by the number of initial females. The *G. pallidipes* and *G. fuscipes fuscipes* flies were commonly used to assess the PPIF of the colonies (Tsegaye *et al.*, 2020). Finally, the results of the study were classified as high and low, and total PPIF by group and number.

3.6.3 Fecundity

The fecundity of the flies is measured by the number of pupae produced by the initial females every 10 days (P/F/10d) and influenced by mortality (IAEA, 2012). Therefore, a value of 1 means that the colony would be static if this mortality and fecundity persisted indefinitely, greater than 1 the colony would grow and less than 1 the colony would shrink (IAEA, 2012). The reason for introducing this calculation is that the overall measure of colony performance and PPIF was only available after the unit is finished. Productivity was intended to give equivalent information but based on current performance (IAEA, 2012). Mating cages were

placed in individual larvipositioned Petridis and pupae were collected from all cages daily in the morning beginning from day 17 and 21 after post emergence for *G. pallidipes* and *G. fuscipes fuscipes* respectively up to the end of the experiment. All data were recorded separately per tested groups per cage and analyzed for three months. Fecundity was expressed as the fly survival (mortality) rate, number of pupae produced per initial female (PPIF) and pupae produced per 10 days, by considering day 17 after immediately they emerged from pupae stage as the first larviposition day (IAEA, 2012; Tsegaye *et al.*, 2020).

3.6.4 Pupae quality or weight

The pupae collected daily were sorted into 5 different size classes based on their weight, as they are balanced the next day. Pupae weight for *G. pallidipes* and *G. fuscipes fuscipes* were assessed by sensitive electrical balance. The weight was measured in mg unit and the pupal weight in MG was converted into different size classes (A, B, C, D and E) (FAO/IAEA, 2006; Kebede, 2012). These pupae were sorted into five different size classes. They were counted and recorded to the end of experiment by weight and pupae. The well-formed pupae and both experiment flies were placed in standard holding conditions (24-25°C and 75-85% relative humidity) (Joint, 2023), using a small Petridis for each of the different blood meals collected daily.

3.6.5 Mortality Tests

The experiment was conducted with colony flies of the same age (IAEA, 2012). Three replicates of each feeding treatment were made. For *G. pallidipes* three replicates the total had, 540 newly emerged female flies, and similarly for *G. fuscipes fuscipes* the three replicates the total had 540 newly emerged female flies. For both species and replicates, the four feeding treatments were continued for 12 weeks. Both *G. pallidipes* and *G. fuscipes fuscipes* flies had fed different batches of blood for three years (2020), two years (2021), one year (2022) and fresh one month (2023). They fed on this blood for 90 days. Mortality or survival rate was recorded weekly until the end of the experiment or for 90 days. Mortality was expressed as "percentage per week" and dead female flies were sorted into bloody and starved mortality (Joint, 2023).

3.7 Data analysis

Data were recorded separately per tested groups per cage and analyzed including their pupal size, which was graded as indicated (Table 1) (Joint, 2023). The average productivity, measured as the number of Pupae Per Initial Female (PPIF), mortality/ survival rate and the number of pupae production, were measured and analyzed about tested groups. The weight of pupae was measured by weighting using sensitive weight balance that graded the pupae based on their weight. The standard system had five pupae quality categories based on their weight labeled A (smallest) to E (largest), that has been adjusted to correspond the five weight classes previously defined to tsetse pupae of *G. pallidipes* and *G. fuscipes fusicipes* (Joint, 2023) (Table 1).

The performance of each blood batches in both experimental studies were evaluated on a weekly basis, and expressed as daily mortality (% D.M.), fecundity (P/F/10d), mean pupal weight, and % A-class pupae. Performance was reviewed at weeks and at the end of the unit life. Each flies' mortality was recorded in Excel database. Then, coding in the database was performed considering one as the occurrence of mortality recorded in a fly at a given date. Data on storage time effect and the quality of blood between irradiated and non-irradiated were analyzed by using STATA computer software (version 12). One-way analysis of variance was performed and significant means were made using Duncan's Multiple Range Test (DMRT) at 5% significance level.

Table 1. Definition of pupal weight classes for different species of *Glossina*

Tsetse species	**Wight Class (mg)**				
	A	**B**	**C**	**D**	**E**
G. austeni	< 16	16 - 18	19 - 20	21 - 23	> 23
G. tachinoides	< 14	14 - 16	17 - 18	19 - 21	> 21
G. palpalis	< 22	22 - 27	28 - 31	32 - 36	> 36
G. pallidipes	< 23	23 - 28	29 - 32	33 - 37	> 37
G. fuscipes	< 22	22 - 27	28 - 31	32 - 36	> 36
G. brevipalpis	< 56	56 - 67	68 - 75	76 - 84	> 84
G. morsitans	< 18	18 - 21	22 - 25	26 - 30	> 30

Note: < less than, > greater than

Source: Leak and Stephen (1999)

4. RESULTS

4.1 Experiment 1: Bioassay feeding test result on storage time effect of defibrinated and irradiated bovine blood fed in terms of productivity and mortality rate of female tsetse flies for *G. pallidipes* and *G. fuscipes fuscipes* colonies

4.1.1 Pupae Production

G. pallidipes and *G. fuscipes fuscipes* flies fed with different batches of bovine blood were recorded and their fed time was compared for 90 days (13 weeks) (Table 2). The results of this study showed that the storage time in *G. pallidipes* and G. *fuscipes fuscipes* had a significant effect on pupae yield. To see the productivity of the two species of flies, they were fed defibrinated irradiated bovine blood stored at -20°C for 1 year, 2 years, 3 years and one month. In *G. pallidipes* and *G. fuscipes fuscipes*, the average number of pupae produced was significantly reduced between flies fed on frozen blood stored for two years (56.3 and 29.3) and three years (43 and 17.6) respectively. Similarly pupae produced in *G. pallidipes* and *G. fuscipes fuscipes* were fed with stored blood in one month (122.3 and 79.6) and one year (126.3 and 61) showed a significant difference compared to other groups of 2 years (56.3 and 29.3) and 3 years (43 and 17.6) respectively (Table 2).

The highest pupae production in *G. pallidipes* 1 year (126.3) were also recorded among *G. fuscipes fuscipes* flies fed on fresh one month frozen blood (standard blood) stored for one month (79.6) followed by one year range (61) (Table 2).

Similarly, *G. pallidipes* flies fed on frozen blood stored for three and two years range produced significantly lower pupae production compared with other batches of blood. In contrary to *G. fuscipes fuscipes* flies, *G. pallidipes* flies fed on frozen blood stored for one year duration produced higher mean numbers of pupae compared to those flies fed on fresh one month frozen blood (standard blood) stored for one month, However, the difference was not statically significant (Table 2; Appendix 4).

Both *G. pallidipes* and *G. fuscipes fuscipes* flies fed on one-year blood produced significantly ($P < 0.05$) higher mean number of pupae than those flies fed on blood stored for two and three years.

Table 2. The mean number (± SD) pupae production of *G. pallidipes* and *G. fuscipes fuscipes*

Blood storage period	*G. pallidipes*	*G. fuscipes fuscipess*
Fresh (1 month)	122.3 ± 7.57 [a]	79.6 ± 7.37 [b]
1 year	126.3 ± 21.7 [a]	61 ± 12.12 [ab]
2 years	56.3 ± 16.56 [bc]	29.3 ± 13.65 [cd]
3 years	43 ± 11.53 [bd]	17.6 ± 10.01 [c]

Means in a column followed by the same letter(s) are not significantly different from each other at 5% level of significance (DMRT).

There was no significant difference (P > 0.05) in the mean numbers of pupae produced by both species fed on bloods stored for two years and three-years (Table 2).

4.1.2 Productivity of Pupae per initial female (PPIF)

In *G. pallidipes* average pupae per initial female (PPIF) varied between, different storage time. Average PPIF were significantly greater among one year (3.3) and fresh one month frozen or standard blood stored blood batches (3.2) compared to two years (1.5) and three years (1.16) stored blood batches.

Similarly, average pupae per initial female (PPIF) in *G. fuscipes fuscipes* female flies fed on fresh one-month blood stored for one month performed better (2.25) than the other groups of flies fed on 1 year, 2 years, and 3 years blood batches (Tables 3; Figure 2; Appendix 5).

Table 3. The mean number (± SD) Productivity of Pupae per initial female (PPIF) of *G. pallidipes* and *G. fuscipes fuscipes*

Blood storage period	*G. pallidipes*	*G. fuscipes fuscipess*
Fresh (1 month)	3.2 ± 0.17 [a]	2.25 ± 0.19 [d]
1 year	3.3 ± 0.55 [a]	1.76 ± 0.39 [ad]
2 years	1.5 ± 0.40 [b]	0.81 ± 0.42 [bc]
3 years	1.16 ± 0.31 [bc]	0.50 ± 0.25 [b]

Means in a column followed by the same letter (s) are not significantly different (P > 0.05) from each other at 5% level of significance (DMRT).

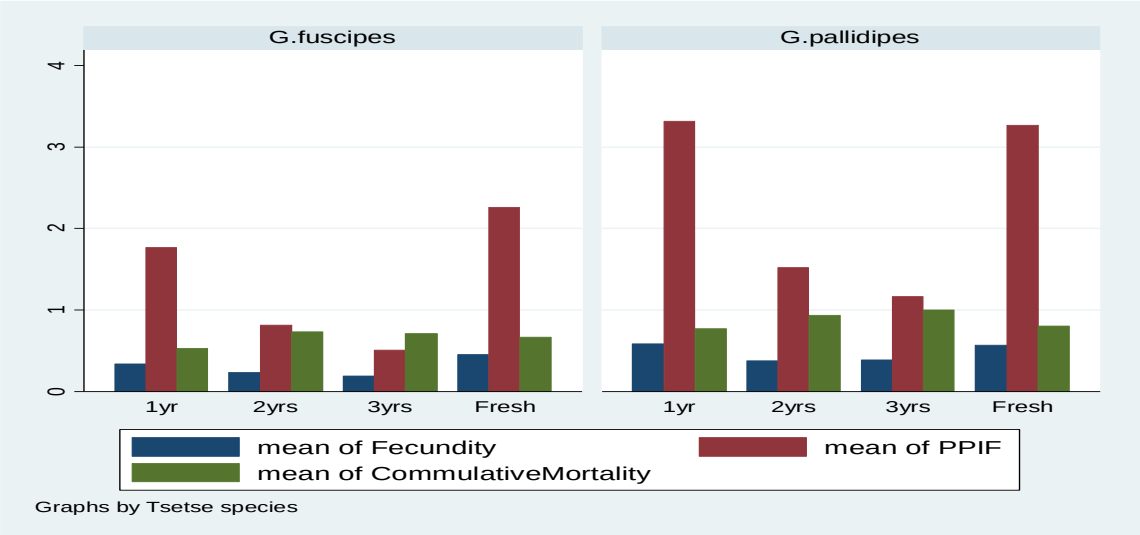


Figure 2. Mean number of PPIF, fecundity, and cumulative mortality of *G. pallidipes* and *G. fuscipes fuscipes* fed for 90 days on blood stored for different years/ times.

4.1.3. Fecundity

The fecundity of the flies was expressed as the fly mortality (survival) rate, number of pupae produced per initial female (PPIF) and pupae produced per 10 days (IAEA, 2012; Tsegaye *et al.*, 2020; Figure 2). Consequently a value of 1 means that the colony would be static if this mortality and fecundity persisted indefinitely, greater than 1 the colony would grow and less than 1 the colony would shrink, because the overall measure of colony performance and PPIF was only available after the unit was finished. Productivity was intended to give equivalent information but based on current performance (IAEA, 2012). The results of the average fecundity there was no significant difference ($P > 0.05$) in the mean numbers of pupae produced and fecundity (P/F/10d), because of the mortality rate was higher and indirectly the fecundity was lower (fecundity was influenced by mortality) in *G. pallidipes* and *G. fuscipes fuscipes* flies (Table 2; Tables 4). In *G. pallidipes*, week 13 (average) fecundity varied between, different storage time. Average fecundity were significantly greater among one year (0.58) and fresh one-month frozen blood stored blood batches (0.56) compared to two years (0.37) and three years (0.38) stored blood batches. Similarly, average fecundity in *G. fuscipes fuscipes* female flies fed on fresh one-month blood stored for one month performed better than the other groups of flies fed on 1 year, 2 years, and 3 years blood batches (Tables 4; Figure 2; Appendix 6).

In two groups ‘of batches, the proportion of flies that started as first larviposition started differed between species, day 17 and 21 after post emergence for *G. pallidipes* and *G. fuscipes fuscipes* respectively was similar among the four groups in both species. Mean Fecundity (pupae per female per 10 day cycle) was measured in *G. pallidipes* and *G. fuscipes fuscipes*, 0.56 and 0.45, 0.58 and 0.33, 0.37 and 0.23, 0.38 and 0.19 for flies fed on one month, one year, two years and three years stored blood respectively (Table 4; Figure 2; Appendix 6).

Table 4. Mean (± SD) Fecundity of *G. pallidipes* and *G. fuscipes fuscipes*

Blood storage period	*G. pallidipes*	*G. fuscipes fuscipes*
Fresh (1 month)	0.56 ± 0.06^{ab}	$0.45 + 0.035^{ad}$
1 year	0.58 ± 0.06^{b}	0.33 ± 0.068^{ac}
2 years	0.37 ± 0.069^{c}	0.23 ± 0.036^{bc}
3 years	0.38 ± 0.03^{c}	0.19 ± 0.045^{b}

Means in a column followed by the same letter(s) are not significantly different ($P > 0.05$) from each other at 5% level of significance (DMRT).

4.1.4 Pupae quality or weight

The pupae quality in *G. pallidipes* and *G. fuscipes fuscipes* flies fed on different batches of blood grouped on basis of their storage time was recorded and compared for 13 weeks. Results indicated significantly better performance in pupae quality. In *G. pallidipes* and *G. fuscipes fuscipes* flies, pupal quality basis on pupae weight in the different blood batches groups showed that the lowest production and pupae quality was recorded among flies fed for three years followed by two to one year’s storage time range.

In *G. pallidipes* and *G. fuscipes fuscipes*, there was statically significant difference in the pupal quality when the flies fed on fresh one-month compared to those flies fed for two to three years range. The irradiated blood stored for two to three years had more of small pupae (class A and B) compared to fresh one-month irradiated blood batched stored less than a year in both species (Table 1; Figure 2). To some extent, these results show a trend on the decrease of the measured parameters in relation to the storage time especially with regard to pupae number and weight (quality) (Figures 2, 3 and 4).

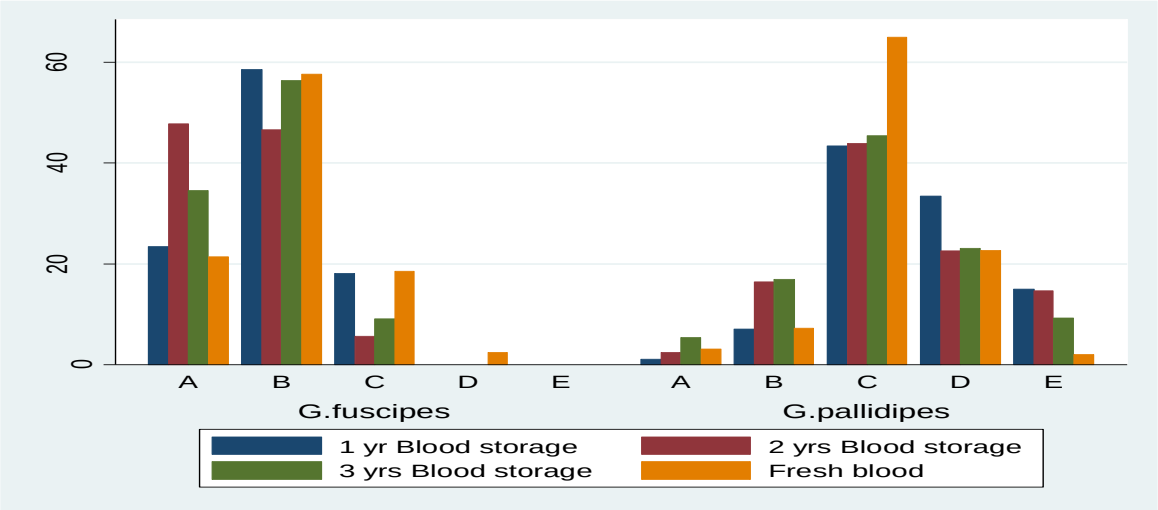


Figure 3. Mean percentage of pupae quality basis on pupae weight (mg) produced by *G. pallidipes* and *G. fuscipes fuscipes* maintained under different blood storage time.

The irradiated blood stored for less than a year had more of large size pupae (class C- E) compared to irradiated blood batched stored for more than two years in *G. pallidipes* and *G. fuscipes fuscipes* species (Table 2; Figure 4).

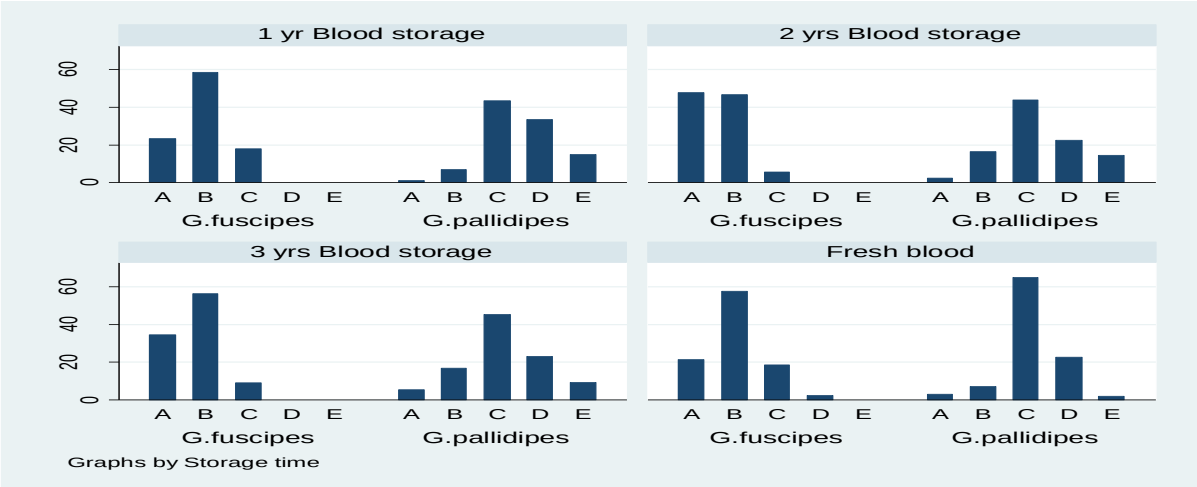
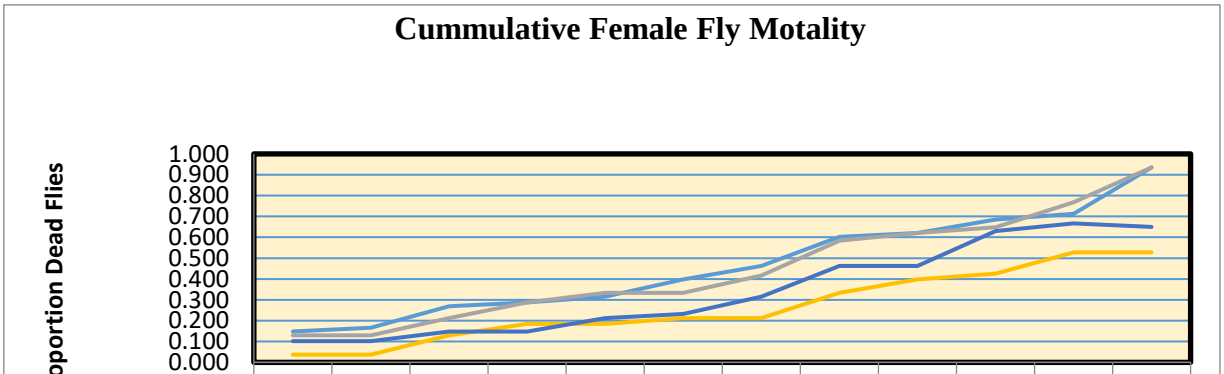


Figure 4. Mean percentage of pupa produced by female *G. pallidipes* and *G. fuscipes fuscipes* flies, sorted in to different pupae weight class fed on different blood storage time.

4.1.5 Mortality rate

The mortality in *G. pallidipes* and *G. fuscipes fuscipes* females fed on frozen defibrinated blood stored for one month, 1 year, 2 years and 3 years was above 52% at week 12 (Figure 5). In the case of *G. fuscipes fuscipes*, a one year stored blood gave the lowest mortality (52%) at week 12 (Table 5 and 6). Similarly, a one year stored blood gave the lowest mortality and the best survival rate for *G. pallidipes* (76%) at week 12. The survival of flies fed on two to three years stored blood batches had lower survival compared to the rest of the batches stored for less than a year (Figure 5). However, for *G. pallidipes* and *G. fuscipes fuscipes* species, the result showed no significant differences (P >0.05) in the survival rate between different blood storage period (Tables 5 and 6; Appendix 7). Evidently, the survival rates of these species were not affected by blood storage time.



	1	2	3	4	5	6	7	8	9	10	11	12
Three years	0.148	0.167	0.269	0.287	0.315	0.398	0.463	0.602	0.620	0.685	0.713	0.935
Two years	0.130	0.130	0.213	0.287	0.333	0.333	0.417	0.583	0.620	0.648	0.769	0.935
One year	0.037	0.037	0.130	0.185	0.185	0.213	0.213	0.333	0.398	0.426	0.528	0.528
Fresh blood	0.102	0.102	0.148	0.148	0.213	0.231	0.315	0.463	0.463	0.630	0.667	0.650

Figure 5. Weekly mean percentage mortality of female *G. pallidipes* maintained under different blood storage time.

Table 5. Mean (± SD) mortality of *G. pallidipes* and *G. fuscipes fuscipes* fed on blood of different storage time

Blood storage period	*G. pallidipes*	*G. fuscipes fuscipes*
Fresh (1month)	0.80 ± 0.027 [ac]	0.66 ± 0 [ab]
1 year	0.76 ± 0.089 [a]	0.52 ± 0.14 [b]
2 years	0.93 ± 0.057 [cb]	0.73 ± 0.089 [a]
3 years	1 ± 0 [b]	0.71 ± 0.11 [a]

Means in a column followed by the same letter(s) are not significantly different from each other at 5% level of significance (DMRT).

4.2 Experiment II: Fresh bovine blood bioassay fed test result comparison on microbial, productivity and mortality rate of female tsetse flies fed on irradiated and non-irradiated bovine blood

4.2.1 Microbial test results under irradiated and non-irradiated bovine blood and dead flies

To assess the quality of blood between irradiated and non-irradiated as a diet for tsetse, blood was screened for the presence of pathogenic bacteria (Figure 6).

The result revealed that the efficacy of sterilization of cobalt 60 (1.5. KGy) irradiated blood was high. The overall average bacterial count in non-irradiated blood (22 CFU/ml blood) was significantly higher than in irradiated blood (0 CFU/ml), and blood samples from the gamma irradiated bovine blood showed significantly a lower bacterial colony growth (Table 6). Bacterial isolate *Rhudococcus equi* (63.1%) was dominantly detected in non-irradiated blood than irradiated blood (Table 6; Appendixes 1-3). However, at KTFRC, there was four bacterial genera were identified such as *R. equi, Actinobaccilus, Staphylococcus* and *Streptococcus* in non-irradiated blood, examine under compound microscopy with oil immersion but not all bacteria were identified to the species level (Appendixes 1-3).

Table 6. Microbial summary of irradiated and non-irradiated blood samples, (colony factor unit per ml blood CFU/ml), bloody and starved mortality rate of *G. pallidipes* and *G. fuscipes fuscipes* colony

ID	Spe.	IS	QF	NOBM	NOSM	FBM%	FSM%	TCCF	ACFPMB
1	*G. p*	IR	108	9	81	8	75	1	-
2	*G. f. f*	IR	108	2	88	2	81	1	-
3	*G. p*	NI	108	57	49	53	45	3	-
4	*G. f. f*	NI	108	14	88	13	81	3	-
5	FOMBB	IR	-	-	-	-	-	-	0
6	FOMBB	NI	-	-	-	-	-	-	22

Spe = Specimen, *Gp = Glossina pallidipes*, *Gff = Glossina fuscipes fuscipes*, IR = irradiated, NI = non-irradiated, FOMBB = fresh one month bovine blood, IS = irradiation states, QF = Quantity of fly, NOBM = No. of bloody mortality, NOSM = No. of starved mortality, FBM = Female bloody mortality by percentage, FSM = starved mortality by percentage, ACFPMB = Average of colony factor unit per ml of blood.

Similarly, dead flies due to blood mortality were collected and sampled immediately after wards, and bacteria densities were also quantified in colony factor per unit of one (1) flies (CFU) growth was recorded and compared among flies fed irradiated and non-irradiated blood. In 33% and 100% of sampled flies from irradiated and non-irradiated batch respectively bacterial were identified (Figure 6).

Similarly, bacterial isolates of *R. equi* (63.1%) were also commonly found along with *Actinobaccilus, Streptococcus* and others *Staphylococcus* genera, and on sampled dead flies fed on the same non-irradiated blood, and in theses flies there were more bacterial species in the non- irradiated blood than irradiated blood batch (Table 6). However, in the insectaries at KTFRC, there was only four bacterial genera such as *R. equi*, *Actinobaccilus, Staphylococcus* and *Streptococcus* bacteria identified in sampled blood, but all bacteria were not identified to the species level (Appendixes 1-3).

4.2.2 Comparison of irradiated and non-irradiated bovine blood bioassay feeding test result on *G. pallidipes* and *G. fuscipes fuscipes*

In *Glossina pallidipes* and *Glossina fuscipes fuscipes* survival and PPIF of flies fed on non-irradiated fresh one-month blood batches were lower compared to flies fed on fresh one-month irradiated blood (Table 7). The non-irradiated fed on fresh one-month blood accumulated mortality on week 13 of *G. pallidipes* (77%) and *G. fuscipes fuscipes* (85%) was the highest for female flies that had fed on non-irradiated defibrinated blood compared with flies that fed on irradiated blood (64% and 67%) respectively (Figure 7). However, statistical significant difference was recorded in their PPIF, fecundity and pupae production in *G. pallidipes* and *G. fuscipes fuscipes* species. Both pupae production and PPIF were highest among flies fed on irradiated blood compared to non-irradiated blood batch in *G. pallidipes* and *G. fuscipes fuscipes* (Table 7; Appendixes 4-6).

Table 7. Mean summary of PPIF, fecundity and pupae production of *G. pallidipes* and *G. fuscipes fuscipes* fed on irradiated and non-irradiated blood

IR and NI Spp.	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
NIFOMBF*Gp*	0.274 ± 0.0393 ^{a}	33 ± 4.51 ^{a}	0.898 ± 0.130 ^{a}	0.9074 ± 0.0697 ^{a}
NIFOMBF*Gff*	0.298 ± 0.0393 ^{ab}	35.33 ± 4.51 ^{a}	0.916 ± 0.130 ^{a}	0.7592 ± 0.0697 ^{a}
IFOMBF*Gff*	0.455 ± 0.0393 ^{bc}	79.66 ± 4.51	2.259 ± 0.130	0.6666 ± 0.0697 ^{a}
IFOMBF*Gp*	0.566 ± 0.0393 ^{c}	122.33 ± 4.51	3.26 ± 0.130	0.0.64 ± 0.0697 ^{a}

NIFOMBF*Gp* = non-irradiated fresh one month blood fed to *G. pallidipes*, NIFOMBF*Gff* = non-irradiated fresh one month blood fed to *G. fuscipes fuscipes*, IFOMBF*Gff* = irradiated fresh one month blood fed to *G. fuscipes fuscipes*, IFOMBF*Gp* = irradiated fresh one month blood fed to *G. pallidipes*, SD = standard deviation.

Means in a column followed by the same letter(s) are not significantly different from each other at 5% level of significance (DMRT).

Flies fed on non-irradiated blood were more susceptible to bloody mortality (the dead flies abdomen they have a blood) than flies fed on irradiated blood in *G. pallidipes* and *G. fuscipes fuscipes*. Higher mortality of *G. pallidipes* due to blood mortality (52.7%) was recorded in non-irradiated blood batch group compared to irradiated group (8.3%). Similarly, the results showed that irradiation exposure had significant impact on the densities of bacterial colony growth in frozen defibrinated bovine blood and in flies (Table 6; Figure 6).

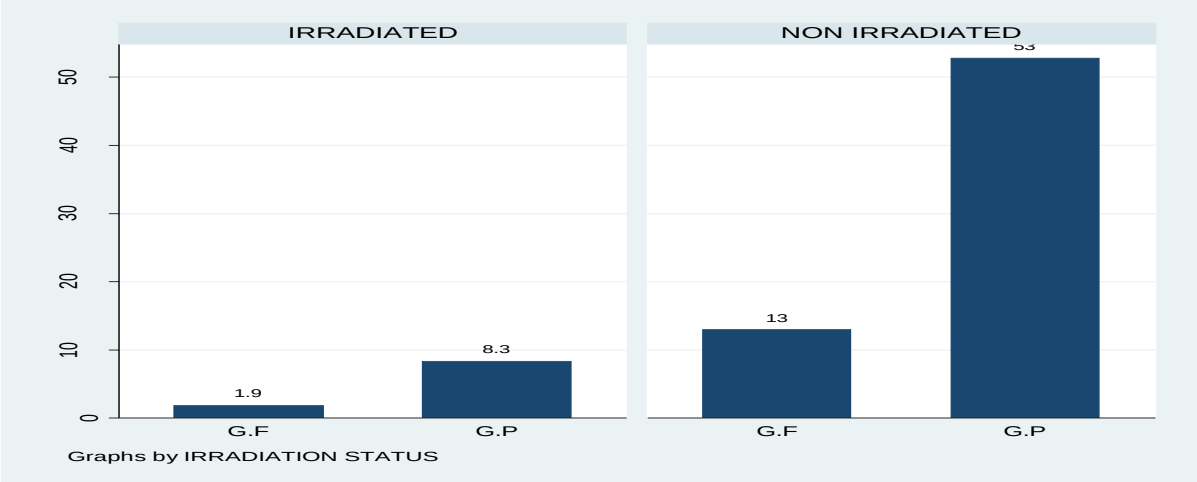


Figure 6. Mean of cumulative bloody mortality of *G. pallidipes* and *G. fuscipes fuscipes* fed on irradiated and non-irradiated fresh one-month blood.

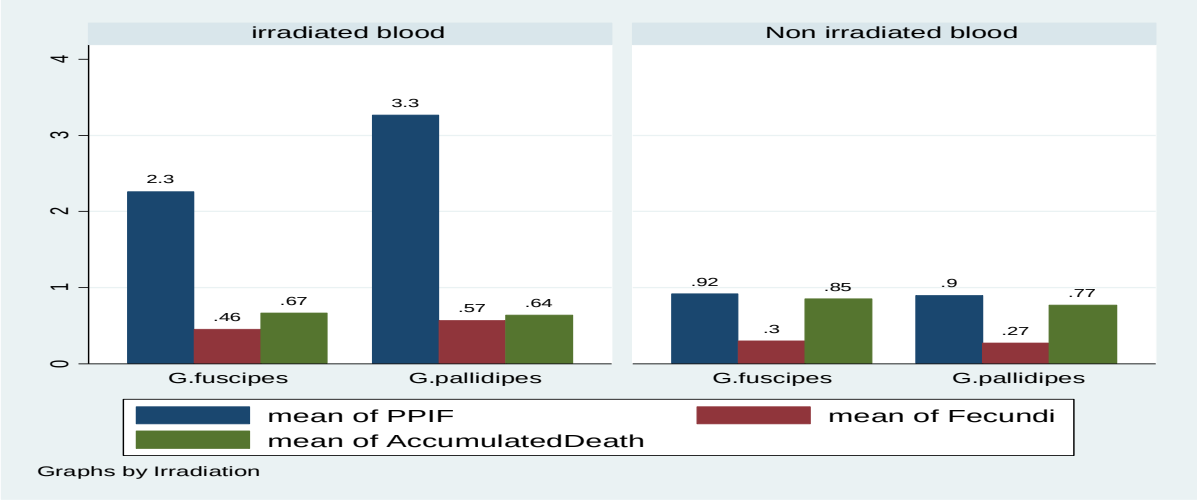


Figure 7. Mean of PPIF, mortality, and fecundity of *G. pallidipes* and *G. f. fuscipes* flies fed on irradiated and non-irradiated fresh one-month blood.

5. DISCUSSION

Insect mass rearing is an essential requirement for the sterile insect technique. Production at a large scale requires the development of standardized rearing procedures to produce good quality males that are able to compete with wild males to mate with wild females. High quality blood as a rearing diet is critical to ensure the growth and sustainability of tsetse fly colonies. Although irradiated, freshly frozen, and defibrinated blood used with a silicon membrane is considered the most efficient way of maintaining larger tsetse colonies, obtaining sterile, high quality blood required for tsetse rearing in vitro feeding system can be challenging (De Beer *et al.*, 2012). Two sets of experiments were conducted to determine both storage time and irradiation effect of bovine blood on productivity and mortality/ survival rate of *G. pallidipes* and *G. fuscipes fuscipes* colonies under laboratory condition. Mortality/survival, reproductive performance and pupae quality are the three essential comprehensive parameters of female tsetse flies routinely used for assessing colony performance.

In this study, *G. pallidipes* and *G. fuscipes fuscipes* flies fed on irradiated blood batches stored at -20 ºC for two to three years had lower survival or higher mortality compared to the rest of the irradiated blood batches stored for one month to a year, although the differences were not significant. This finding is consistent with the finding of Byamungu *et al.* (2011) who found that there was a significant difference in fly survival for different batches of blood used as diet to rear *Glossina austeni*. According to Byamungu *et al.* (2011), the production including flies survival parameters were also better in the last three years, i.e. 2008, 2007 and 2006. According to Galun and Kabayo (1988), the variation in nutritional value of the collected blood could be influenced by genetic, environmental, chemical and physical factors of the host animal. Chemicals and microbiological contamination during collection, handling and storage would also play a role (Galun and Kabayo, 1988).

The findings of the study revealed that storage time had a significant effect on reproductive performances, including pupae production, pupae per initial female (PPIF) and fecundity in both *G. pallidipes* and *G. fuscipes fuscipes.* Average pupae per initial female PPIF/ fecundity were significantly greater among *G. pallidipes* colonies maintained under bovine blood stored for one year 3.3/ 0.58 and one month 3.2/ 0.56 compared to two years 1.5/ 0.37 and three years 1.16/

0.38. Similarly, the results obtained in *G. fuscipes fuscipes* colonies were also consistent with the results of *G. pallidipes* colonies that both PPIF higher in one month 2.25/ 3.2 and one year stored 1.76/ 3.3 blood batches compared with two and three years batches although value obtained rather low compared with *G. pallidipes* colonies. Several studies have revealed that for a colony to sustain the minimum PPIF should be above 2.1 for 13 weeks (Opiyo *et al.*, 2000; Berror and Mengistu, 2023).

The results of this study indicated that blood diets frozen at -20^{o}C and stored for two and three years had the lowest mean number of PPIF and fecundity in both species. This result in the lowest mean PPIF and fecundity which were found below the minimum standard PPIF (PPIF = 2.1) and fecundity (F = 0.6) of the colony. In other words, this result shows that one-month or more than one year stored blood affects PPIF and fecundity (less than a year is better for PPIF and fecundity) in *G. pallidipes* and *G. fuscipes fuscipes* flies (Byamungu *et al.*, 2011). Hence, the nutritional quality and overall performance of fresh one-month blood stored at -20^{0}C for less than a year is better in both colonies. In both colonies, the overall results obtained from the reproductive test were also consistent with the findings of pupae quality test (Byamungu *et al.*, 2011).

The irradiated blood stored for two to three years had also more of small pupae or lightweight (class A and B) of poor quality compared to fresh one-month irradiated blood batched stored less than a year in both species. According to Byamungu *et al.* (2011), the weight of a pupa depends on the amount of blood taken by a female during pregnancy, with a highly significant correlation between pupa weight and quality of blood ingested. High fecundity and low mortality is correlated with pupal size and weight (Byamungu *et al*., 2011). Very small and light pupae results in low emergency rate, a low number of strong and viable flies. The size and weight of pupae are reflection of the caring and feeding of female flies, and her ability to transfer the nutrients to her offspring (Macmanus *et al.*, 2009).

The lack of significant improvement in reproductive performance of both *G. pallidipes* and *G. fuscipes fuscipes* colony maintained and reared under bovine blood diets stored for two to three years range at -20^{o}C in the current experiments indicated that the nutritional value of the blood meal was lost after two to three years period of freezing at -20^{o}C. The nutritional value of the

blood meal was lost after a long period of freezing, according to QF analysis (Wetzel and Luger, 1978). According to Byamunguet *al.*, (2011), a poor PPIF of 0.94 pupae per female was recorded after 30 days test with *G. austeni* after feeding with 5 years-old frozen blood. As an alternative to decreasing the nutritional value of frozen blood with negative consequences on tsetse fly reproduction, the study conducted by (Wetzel and Luger, 1978) shows that an addition of 10-3 moles of ATP to the blood meal improves tsetse fly fecundity. This suggests that, even under stable storage conditions, blood could be stored for only one year without losing its quality. Therefore, according to this study, blood stored for 1 year should not be discarded, blood stored at -20^{o}C for at least 1 year can be used for fly feed. Repeated thawing and freezing of blood is detrimental to blood quality (De Beer *et al.*, 2012).

Another aspect of the research was on the effect of gamma irradiation on quality and nutritional value of bovine blood for tsetse diet. Freshly collected and cobalt 60 (1.5 KGy) defibrinated bovine's blood, usually fed to tsetse flies after a one month bioassay quality test, was tested to assess the efficacy of radiation on microbial load comparing with non-irradiated blood and blood was screened for the presence of bacteria, and the pathogens were identified. The tests were performed on the day of irradiation and after storage at -20°C, thirty days later. The results of this study show that cobalt 60 (1.5 KGy dosage) irradiation is highly effective in sterilizing blood from bacteria. The overall bacterial count in non-irradiated blood (22 CFU/ml blood) was significantly higher than in irradiated blood (0 CFU/ml), and 100% of microbial contamination was eliminated by irradiation at 1.5 kGy. There were more bacterial species examined in the non-irradiated blood than irradiated blood. In an earlier study, Baker *et al.* (1983) showed that a dose of 100 rad (radiation-absorbed dose) was effective against the most pathogenic bacteria of flies, *Pseudomonas aerooinosa* inoculated into the blood. Furthermore, in the present work, in the insectaries at KTFRC, there were only four bacterial genera of *R. equi*, *Actinobaccilus*, *Staphylococcus* and *Streptococcus* identified in non-irradiated blood, but no bacteria were identified in irradiated blood. According to Kaaya and Darji (1989), screening of bacteria in collected blood and in rearing rooms, bacterial species belonging to seven genera were identified, *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Streptococcus*, *Acinetobacter*, *Enterobacter* and *Corynebacterium*. *Staphylococcus* spp. and *Streptococcus* spp. are normal flora, but can cause problems if ingested in abundance. Experiments (Kaaya and Darji, 1989)

done elsewhere revealed that some *Bacillus* spp. and *Pseudomonas* spp. caused mortality to laboratory reared flies. In this current study, some *Actinobacillus* were isolated; suggesting that more standards that was hygienic was required in handling blood. In addition, *R. equi* (63.1%) was isolated in large occurrence in non-irradiated blood. These bacteria of *R. equi* also were isolated from sampled majority of dead flies due to blood mortality in non-irradiated batches. These bacteria were also isolated from both dead flies and non-irradiated blood diet they fed. This implies that bacteria was being transferred from blood to flies during feeding but it would not be possible to establish as a cause of mortality. Control of bacteria in the blood would probably minimize the contamination. In view of the above-mentioned health issues not elucidated in this study, research efforts in the insectaria colony should focus on the detection of pathogens and chemical residues that may be present in blood meals before feeding. Certain species of tsetse flies are more sensitive to bacterial contamination than others (Wetzel and Thiemann, 1979).

To assess the quality of the blood between irradiated and non-irradiated as a diet for *G. pallidipes* and *G. f. fuscipes* species, a ninety-day feeding test was carried out. These findings showed that irradiated defibrinated bovine blood was found to be suitable to maintain *G. pallidipes* and *G. fuscipes fuscipes* colonies survival and PPIF of both species fed on non-irradiated blood were also more susceptible to bloody mortality than flies fed on irradiated blood in both species. Evidently, the difference was due to microbial load in non-irradiated blood. In *G. pallidipes* large number of fly's death due to blood mortality (52.7%) was recorded in non-irradiated blood batch group compared to irradiated group (8.3%). However, this difference was not statically significant in this survival rate in both species. Probably the effect of bacteria on the quality of the blood depends on the virulence of the bacteria, and ingestion the time of feeding frequency (Kaaya and Darji, 1989). Bacterial colonies could also affect the quality of the blood through their by-products. Explain the poor performance of a colony fed with a non-irradiated frozen blood meal in this way if we consider the health problems concerning bacterial contamination (Bauer and Aigner, 1978; Wetzel and Luger, 1978) and/or the presence of antibiotic residues at low doses in the collected blood (Pooda *et al*., 2013). Using non-irradiated blood for colony rearing may therefore expose the flies to health risks. In a former investigation of Baker *et al.* (1983), the feasibility of using Gama irradiated blood for the *in-vitro* feeding of

G. morsitans morsitans was demonstrated that the blood irradiated with I00 rad was accepted by *G. morsitans morsitans* without injury to colony performance over a continuous period of 9 months.

In spite of the slight radiation effects on nutritional quality of blood (Baker *et al.*, 1983; Hamann and Bickhardt, 1983), the blood irradiated with 1.5 KGy was accepted by both *G. pallidipes* and *G. fuscipes fuscipes* without injury to colony performance over a continuous period of 3 months comparing with non-irradiated blood. At least within the applied storage time the dietary quality of the blood for the *in-vitro* feeding of the two tsetse species used has not affected. In rearing tsetse flies, irradiation of blood in decontamination of bacteria has a role in reducing abortion and mortality rate in flies. In rearing tsetse flies bacterial contamination is crucial, can cause a high abortion rate and high mortalities and deterioration of blood quality, this underscores the importance of precautionary measures taken during blood collection and storage, and during the feeding process, including irradiating and freezing blood, and conducting bioassays before exposing colonies to new batches of blood (Feldmann, 1994).

6. CONCLUSION AND RECOMMENDATIONS

In conclusions, the present study demonstrated that blood storage time had significant effect on growth performances of *G. pallidipes* and *G. fuscipes fuscipes*. In particular, overall colony performances are batter among flies fed on fresh one-month blood stored less than one year. Additionally, this study showed that irradiated defibrinated bovine blood was found to be suitable to maintain the colonies of *G. pallidipes* and *G. fuscipes fuscipes* compared to the non-irradiated bovine blood.

The blood under stable storage conditions blood diet could be stored for at most one year without affecting the quality. This could be adopted as an optimum storage time under regional conditions. It is important that optimum storage conditions (temperature -20^{o}C) should be maintained throughout the storage period. In addition to this, blood should be kept intact for that period, no thawing and freezing unless it is to be used at once. Therefore, the size of storage containers should be considered when intending to keep the blood for that long in relation to the fly colony size. An added advantage of the establishment storage time is for easier management of tsetse colonies and plans could be made to collect and have enough amount of blood in stock for a specific time and thus reduce inconveniences and minimize the rearing costs.

This study proved also a template for the possible creation of stored at most one year a frozen blood bank that could conceivably a continuous supply of slaughter blood for feeding the tsetse fly colony. To ensure mass production of pupae, the blood bank must be renewed within a period not exceeding one month after the feeding test. Nevertheless, additional studies during the microbiological examination *R. equie* was found in non-irradiated feed blood and dead flies, this was a new discovery in this study. Whether or not *R. equie* is harmful to tsetse flies and the chemical residues found in the blood meal collected at KTFRC require further research in the future.

Therefore, this study recommends frozen irradiated blood stored at -20^{o}C for less than a year to be an optimum storage time for blood diet under existing conditions.

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8. APPENDEXES

Appendix 1. Record sheet of microbial screening and bacterial identification test result from bovine whole blood

ID	Spp.	ST	SS	SOS	DIFB	DI	CFU/Ml	QDF	BCT				MOB	BGI		
									CT	OT	OF	Mck		G- ve	G+ ve	
1	IBB	3ys	A	B	9/2/23	1.5 KGy	1	Non	+	-	UNR	Ng	Cocci	x	√	
			B	B	14/3/23	1.5 KGy	1	Non	+	-	UNR	Ng	Cocci	x	√	
			C	B	4/4/23	1.5 KGy	0	Non	NG	NG	NG	ng	NG	x	NG	
2	IBB	2ys	A	B			1	Non	+	-	UNR	ng	S/R	x	√	
			B	B			0	Non	NG	NG	NG	ng	NG	x	NG	
			C	B			0	Non	NG	NG	NG	ng	NG	x	NG	
3	IBB	1y	A	B			0	Non	NG	NG	NG	ng	NG	x	NG	
			B	B			0	Non	NG	NG	NG	ng	NG	x	NG	
			C	B			0	Non	NG	NG	NG	ng	NG	x	NG	
4	FIBB	1M	A	B			0	Non	NG	NG	NG	ng	NG	x	NG	
			B	B			0	Non	NG	NG	NG	ng	NG	x	NG	
			C	B			0	Non	NG	NG	NG	ng	NG	x	NG	
5	FNIBB	1M	A	B			23	Non	+	-	F	ng	Cocci	x	√	*S*
			B	B			26	Non	+	-	F	ng	Cocci	x	√	*S*
			C	B			18	Non	+	-	UNR	ng	Cocci	x	√	

Appendix 2. Record sheet of microbial screening and bacterial identification test result from dead fly of *G. pallidipes*

ID	Spp.	ST	SS	SOS	DIFB	DI	CFU/Ml	QDF	BCT				MOB	BGI		B
									CT	OT	OF	Mck		G- ve	G+ ve	
1	*Gp*	3ys	A	DF	9/2/23	1.5 KGy	1	29	+	-	UNR	ng	Cocci	x	√	*R*
			B	DF	14/3/23	1.5 KGy	1		+	-	UNR	ng	Cocci	x	√	*R*
			C	DF	4/4/23	1.5 KGy	0		NG	NG	NG	ng	NG	x	NG	N
2	*Gp*	2ys	A	DF			1	44	+	-	UNR	ng	S/R	x	√	*R*
			B	DF			0		NG	NG	NG	ng	NG	x	NG	N
			C	DF			0		NG	NG	NG	ng	NG	x	NG	N
3	*Gp*	1y	A	DF			0	18	NG	NG	NG	ng	NG	x	NG	N
			B	DF			0		NG	NG	NG	ng	NG	x	NG	N
			C	DF			0		NG	NG	NG	ng	NG	x	NG	N
4	*Gp*	1M	A	DF			1	9	+	-	F	ng	Cocci	x	√	*St*
			B	DF			0		NG	NG	NG	ng	NG	x	NG	N
			C	DF			0		NG	NG	NG	ng	NG	x	NG	N
5	*Gp*	1M	A	DF			1		+	-	UNR	ng	Cocci	x	√	*R*
			B	DF			1		+	-	UNR	ng	Cocci	x	√	*R*
			C	DF			1	57	+	-	UNR	ng	Cocci	x	√	*R*

Appendix 3. Record sheet of microbial screening and bacterial identification test result from dead fly of *G. fuscipes fuscipes*

ID	Spp.	ST	SS	SOS	DIFB	DI	CFU/Ml	QDF	BCT				MOB	BGI		BSI
									CT	OT	OF	Mck		G- ve	G+ ve	
1	*Gff*	3ys	A	DF	9/2/23	1.5 KGy	1		+	-	UNR	ng	Cocci	x	√	*R. Equ*
			B	DF	14/3/23	1.5 KGy	0		NG	NG	NG	ng	NG	x	NG	NG
			C	DF	4/4/23	1.5 KGy	0		NG	NG	NG	ng	NG	x	NG	NG
2	*Gff*	2ys	A	DF			1	7	+	-	F	Gr	Rod	√	X	*Acb.sp*
			B	DF			0		NG	NG	NG	ng	NG	x	NG	NG
			C	DF			0	6	NG	NG	NG	ng	NG	x	NG	NG
3	*Gff*	1y	A	DF			0		NG	NG	NG	ng	NG	x	NG	NG
			B	DF			0		NG	NG	NG	ng	NG	x	NG	NG
			C	DF			0	9	NG	NG	NG	ng	NG	x	NG	NG
4	*Gff*	1M	A	DF			1	2	+	-	F	ng	Cocci	x	√	*Stap. s*
			B	DF			0		NG	NG	NG	ng	NG	x	NG	NG
			C	DF			0		NG	NG	NG	ng	NG	x	NG	NG
5	*Gff*	1M	A	DF			1		-	-	F	ng	Cocci	x	√	*Stre sp*
			B	DF			1		+	-	F	ng	Cocci	x	√	*Stap. s*
			C	DF			1	14	+	-	UNR	ng	Cocci	x	√	*R. Equ*

Keywords: Spp. = Species, ST = Storage Time, Gp = *Glossina pallidipes*, Gff = *Glossina fuscipes fuscipes*, SS = sample size, B = blood, S0S = sample of specimen, DIFB = day of irradiation fed blood, DI = Dose of irradiation, CFU/Ml refers to ″colony factor unit per ml″, QDF = quantity of died flies, BCT = bio chemical test, CT = catalase test, OT = oxidase test, OF = oxidase fermentative, Mck = MacConkey, MOB = morphology of bacteria, BGI = bacterial gram identification, G +ve = Gram positive, G –ve = Gram negative, BGLI = bacteria species identification, FIBB = fresh one month irradiated bovine blood, FNIBB = fresh one

month non-irradiated bovine blood, 1M = one month, NG = no growth, Gr = growth, DF = dead flies , Acb spp = Actinobacillus species, Stap. Spp. = Staphylococcus species, = Stre. Spp. = streptococcus Species, UNR = UN reactive and F = fermentative.

Appendix 4. Duncan multiple mean range test (DMRT) for pupae in *G. pallidipes* and *G. fuscipes* *fuscipes*

Pupae	Contrast	Std. Err.	Duncan t	Duncan P>|t|	Duncan [95% Conf.	Interval]
Spps#Storagetime						
(1 1) vs (1 0)	4	10.90489	0.37	0.719	-19.11734	27.11734
(1 2) vs (1 0)	-66	10.90489	-6.05	0.000	-90.94441	-41.05559
(1 3) vs (1 0)	-79.33333	10.90489	-7.28	0.000	-104.7597	-53.90699
(2 0) vs (1 0)	-42.66667	10.90489	-3.91	0.001	-65.784	-19.54933
(2 1) vs (1 0)	-61.33333	10.90489	-5.62	0.000	-85.57495	-37.09171
(2 2) vs (1 0)	-93	10.90489	-8.53	0.000	-118.7745	-67.22548
(2 3) vs (1 0)	-104.6667	10.90489	-9.60	0.000	-130.7009	-78.63241
(1 2) vs (1 1)	-70	10.90489	-6.42	0.000	-95.42634	-44.57366
(1 3) vs (1 1)	-83.33333	10.90489	-7.64	0.000	-109.1079	-57.55881
(2 0) vs (1 1)	-46.66667	10.90489	-4.28	0.001	-70.90829	-22.42505
(2 1) vs (1 1)	-65.33333	10.90489	-5.99	0.000	-90.27774	-40.38892
(2 2) vs (1 1)	-97	10.90489	-8.90	0.000	-123.0343	-70.96574
(2 3) vs (1 1)	-108.6667	10.90489	-9.96	0.000	-134.8986	-82.43474
(1 3) vs (1 2)	-13.33333	10.90489	-1.22	0.239	-36.45067	9.784005
(2 0) vs (1 2)	23.33333	10.90489	2.14	0.058	-.9082856	47.57495
(2 1) vs (1 2)	4.666667	10.90489	0.43	0.674	-18.45067	27.784
(2 2) vs (1 2)	-27	10.90489	-2.48	0.031	-51.24162	-2.758381
(2 3) vs (1 2)	-38.66667	10.90489	-3.55	0.004	-63.61108	-13.72226
(2 0) vs (1 3)	36.66667	10.90489	3.36	0.006	11.72226	61.61108
(2 1) vs (1 3)	18	10.90489	1.65	0.136	-6.241619	42.24162
(2 2) vs (1 3)	-13.66667	10.90489	-1.25	0.228	-36.784	9.450671
(2 3) vs (1 3)	-25.33333	10.90489	-2.32	0.041	-49.57495	-1.091714
(2 1) vs (2 0)	-18.66667	10.90489	-1.71	0.106	-41.784	4.450671
(2 2) vs (2 0)	-50.33333	10.90489	-4.62	0.001	-75.75967	-24.90699
(2 3) vs (2 0)	-62	10.90489	-5.69	0.000	-87.77452	-36.22548
(2 2) vs (2 1)	-31.66667	10.90489	-2.90	0.016	-56.61108	-6.722258
(2 3) vs (2 1)	-43.33333	10.90489	-3.97	0.002	-68.75967	-17.90699
(2 3) vs (2 2)	-11.66667	10.90489	-1.07	0.301	-34.784	11.45067

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. pwmean Pupae, over(Spps Storagetime) mcompare(duncan) effects cimeans groups

Pairwise comparisons of means with equal variances
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Appendix 5. Duncan multiple mean range test (DMRT) for PPIF in *G. pallidipes* and *G. fuscipes fuscipes.*

PPIF	Contrast	Std. Err.	Duncan t	Duncan P>|t|	Duncan [95% Conf.	Interval]
Spps#Storagetime						
(1 1) vs (1 0)	.0462963	.2955358	0.16	0.877	-.5802116	.6728041
(1 2) vs (1 0)	-1.75	.2955358	-5.92	0.000	-2.426024	-1.073976
(1 3) vs (1 0)	-2.101852	.2955358	-7.11	0.000	-2.790936	-1.412767
(2 0) vs (1 0)	-1.009259	.2955358	-3.42	0.004	-1.635767	-.3827514
(2 1) vs (1 0)	-1.5	.2955358	-5.08	0.000	-2.156977	-.8430228
(2 2) vs (1 0)	-2.453704	.2955358	-8.30	0.000	-3.152224	-1.755183
(2 3) vs (1 0)	-2.759259	.2955358	-9.34	0.000	-3.464819	-2.053699
(1 2) vs (1 1)	-1.796296	.2955358	-6.08	0.000	-2.485381	-1.107212
(1 3) vs (1 1)	-2.148148	.2955358	-7.27	0.000	-2.846669	-1.449627
(2 0) vs (1 1)	-1.055556	.2955358	-3.57	0.003	-1.712533	-.3985783
(2 1) vs (1 1)	-1.546296	.2955358	-5.23	0.000	-2.22232	-.8702726
(2 2) vs (1 1)	-2.5	.2955358	-8.46	0.000	-3.20556	-1.79444
(2 3) vs (1 1)	-2.805556	.2955358	-9.49	0.000	-3.516472	-2.094639
(1 3) vs (1 2)	-.3518519	.2955358	-1.19	0.251	-.9783597	.274656
(2 0) vs (1 2)	.7407407	.2955358	2.51	0.029	.0837635	1.397718
(2 1) vs (1 2)	.25	.2955358	0.85	0.410	-.3765078	.8765078
(2 2) vs (1 2)	-.7037037	.2955358	-2.38	0.037	-1.360681	-.0467265
(2 3) vs (1 2)	-1.009259	.2955358	-3.42	0.006	-1.685283	-.3332356
(2 0) vs (1 3)	1.092593	.2955358	3.70	0.003	.4165689	1.768616
(2 1) vs (1 3)	.6018519	.2955358	2.04	0.070	-.0551254	1.258829
(2 2) vs (1 3)	-.3518519	.2955358	-1.19	0.251	-.9783597	.274656
(2 3) vs (1 3)	-.6574074	.2955358	-2.22	0.050	-1.314385	-.0004302
(2 1) vs (2 0)	-.4907407	.2955358	-1.66	0.116	-1.117249	.1357671
(2 2) vs (2 0)	-1.444444	.2955358	-4.89	0.000	-2.133529	-.7553598
(2 3) vs (2 0)	-1.75	.2955358	-5.92	0.000	-2.448521	-1.051479
(2 2) vs (2 1)	-.9537037	.2955358	-3.23	0.008	-1.629727	-.27768
(2 3) vs (2 1)	-1.259259	.2955358	-4.26	0.001	-1.948344	-.5701746
(2 3) vs (2 2)	-.3055556	.2955358	-1.03	0.317	-.9320634	.3209523

Appendix 6. Duncan multiple mean range test (DMRT) for fecundity in *G. pallidipes* and *G. fuscipes fuscipes.*

Fecundi	Contrast	Std. Err.	Duncan t	Duncan P>|t|	Duncan [95% Conf.	Interval]
Spps#Storagetime						
(1 1) vs (1 0)	.019872	.0550295	0.36	0.723	-.0967854	.1365293
(1 2) vs (1 0)	-.1914274	.0550295	-3.48	0.005	-.3173047	-.0655501
(1 3) vs (1 0)	-.1779439	.0550295	-3.23	0.007	-.3002747	-.0556131
(2 0) vs (1 0)	-.1108983	.0550295	-2.02	0.061	-.2275556	.0057591
(2 1) vs (1 0)	-.2284412	.0550295	-4.15	0.001	-.3567505	-.1001319
(2 2) vs (1 0)	-.3341	.0550295	-6.07	0.000	-.4641663	-.2040337
(2 3) vs (1 0)	-.3748557	.0550295	-6.81	0.000	-.5062328	-.2434787
(1 2) vs (1 1)	-.2112994	.0550295	-3.84	0.003	-.3396087	-.08299
(1 3) vs (1 1)	-.1978159	.0550295	-3.59	0.004	-.3236932	-.0719385
(2 0) vs (1 1)	-.1307703	.0550295	-2.38	0.037	-.2531011	-.0084394
(2 1) vs (1 1)	-.2483132	.0550295	-4.51	0.001	-.3783795	-.1182468
(2 2) vs (1 1)	-.353972	.0550295	-6.43	0.000	-.485349	-.2225949
(2 3) vs (1 1)	-.3947277	.0550295	-7.17	0.000	-.5271022	-.2623532
(1 3) vs (1 2)	.0134835	.0550295	0.25	0.810	-.1031739	.1301408
(2 0) vs (1 2)	.0805291	.0550295	1.46	0.184	-.0418017	.2028599
(2 1) vs (1 2)	-.0370138	.0550295	-0.67	0.511	-.1536712	.0796435
(2 2) vs (1 2)	-.1426726	.0550295	-2.59	0.025	-.2650035	-.0203418
(2 3) vs (1 2)	-.1834284	.0550295	-3.33	0.007	-.3093057	-.057551
(2 0) vs (1 3)	.0670456	.0550295	1.22	0.241	-.0496118	.1837029
(2 1) vs (1 3)	-.0504973	.0550295	-0.92	0.398	-.1728281	.0718335
(2 2) vs (1 3)	-.1561561	.0550295	-2.84	0.018	-.2820334	-.0302788
(2 3) vs (1 3)	-.1969118	.0550295	-3.58	0.005	-.3252212	-.0686025
(2 1) vs (2 0)	-.1175429	.0550295	-2.14	0.066	-.2434202	.0083344
(2 2) vs (2 0)	-.2232017	.0550295	-4.06	0.002	-.351511	-.0948924
(2 3) vs (2 0)	-.2639574	.0550295	-4.80	0.000	-.3940238	-.1338911
(2 2) vs (2 1)	-.1056588	.0550295	-1.92	0.073	-.2223162	.0109985
(2 3) vs (2 1)	-.1464145	.0550295	-2.66	0.022	-.2687454	-.0240837
(2 3) vs (2 2)	-.0407557	.0550295	-0.74	0.470	-.1574131	.0759016

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. pwmean Fecundi, over(Spps Storagetime) mcompare(duncan) effects cimeans groups

Pairwise comparisons of means with equal variances
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Appendix 7. Duncan multiple mean range test (DMRT) for mortality in *G. pallidipes* and *G. fuscipes fuscipes.*

AccumulatedDeath	Contrast	Std. Err.	Duncan t	Duncan P>|t|	Duncan [95% Conf.	Interval]
Spps#Storagetime						
(1 1) vs (1 0)	-.037037	.0677256	-0.55	0.592	-.180609	.1065349
(1 2) vs (1 0)	.1296296	.0677256	1.91	0.074	-.0139423	.2732016
(1 3) vs (1 0)	.1944444	.0677256	2.87	0.014	.0438901	.3449988
(2 0) vs (1 0)	-.1388889	.0677256	-2.05	0.081	-.2968011	.0190233
(2 1) vs (1 0)	-.2777778	.0677256	-4.10	0.002	-.4378524	-.1177032
(2 2) vs (1 0)	-.0740741	.0677256	-1.09	0.315	-.2246285	.0764803
(2 3) vs (1 0)	-.0925926	.0677256	-1.37	0.226	-.2475117	.0623265
(1 2) vs (1 1)	.1666667	.0677256	2.46	0.032	.0161123	.317221
(1 3) vs (1 1)	.2314815	.0677256	3.42	0.006	.0765624	.3864006
(2 0) vs (1 1)	-.1018519	.0677256	-1.50	0.185	-.256771	.0530673
(2 1) vs (1 1)	-.2407407	.0677256	-3.55	0.005	-.3986529	-.0828286
(2 2) vs (1 1)	-.037037	.0677256	-0.55	0.592	-.180609	.1065349
(2 3) vs (1 1)	-.0555556	.0677256	-0.82	0.449	-.2061099	.0949988
(1 3) vs (1 2)	.0648148	.0677256	0.96	0.353	-.0787571	.2083868
(2 0) vs (1 2)	-.2685185	.0677256	-3.96	0.002	-.4285931	-.1084439
(2 1) vs (1 2)	-.4074074	.0677256	-6.02	0.000	-.5690951	-.2457197
(2 2) vs (1 2)	-.2037037	.0677256	-3.01	0.013	-.3586228	-.0487846
(2 3) vs (1 2)	-.2222222	.0677256	-3.28	0.008	-.3801344	-.06431
(2 0) vs (1 3)	-.3333333	.0677256	-4.92	0.000	-.495021	-.1716456
(2 1) vs (1 3)	-.4722222	.0677256	-6.97	0.000	-.6351375	-.3093069
(2 2) vs (1 3)	-.2685185	.0677256	-3.96	0.002	-.4264307	-.1106063
(2 3) vs (1 3)	-.287037	.0677256	-4.24	0.001	-.4471116	-.1269625
(2 1) vs (2 0)	-.1388889	.0677256	-2.05	0.057	-.2824608	.0046831
(2 2) vs (2 0)	.0648148	.0677256	0.96	0.378	-.0857396	.2153692
(2 3) vs (2 0)	.0462963	.0677256	0.68	0.504	-.0972757	.1898682
(2 2) vs (2 1)	.2037037	.0677256	3.01	0.013	.0487846	.3586228
(2 3) vs (2 1)	.1851852	.0677256	2.73	0.019	.0346308	.3357396
(2 3) vs (2 2)	-.0185185	.0677256	-0.27	0.788	-.1620905	.1250534

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. pwmean AccumulatedDeath, over(Spps Storagetime) mcompare(duncan) effects cimeans groups

Pairwise comparisons of means with equal variances
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