ADDIS ABABA UNIVERSITY
COLLEGE OF VETERINARY MEDICINE AND AGRICULTURE

ASSESSMENT OF SURVIVAL AND REPRODUCTIVE PERFORMANCE OF *Glossina pallidipes* MAINTAINED UNDER BLOOD OF DIFFERENT ANIMALS AT KALITY TSETSE REARING AND IRRADIATION CENTER

MSc Thesis

BY
ASSEFA KEBEDE TSEGAY

JUNE, 2012
DEBRE ZEIT, ETHIOPIA

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A thesis submitted to School of Graduate Studies of Addis Ababa University, College of Veterinary Medicine and Agriculture in partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Pathology and Parasitology

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Board of examiners	**Signature**
1. Prof. Getachew Tilahun (Aklilu Lemma Institute of Pathobiology, Addis Ababa University)	_____
2. Dr. Genene Tefera (Institute of Biodiversity Conservation)	_____
3. Dr. Dereje Gudeta (STEP)	_____

Advisors:	**Signature**
Dr. Hagos Ashenafi (DVM, MSc, PhD)	_____
Dr. Terzu Daya (DVM, MSc)	_____

DEDICATION

This work was dedicated to my beloved mother, Sindayo Adane, who I was missed her at the beginning of this MSc program. May God rest her soul in peace!

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LIST OF ABREVIATIONS

AAT	African Animal Trypanosomosis
AW-IPM	Area-Wide Integrated Pest Management
CFU	Colony Forming Unit
Co	Cobalt
FLDP	Fourth livestock Development Project
GIS	Geographic Information System
Gy	Grey
IAEA	International Atomic Energy Agency
IPM	Integrated Pest Management
NTTICC	National Tsetse and Trypanosomosis Investigation and Control Centre
NWS	New World Screwworm
PAAT	Programme Against African Trypanosomosis
PATTEC	Pan African Tsetse and Trypanosomosis Eradication Campaign
PPIF	Pupae Per Initial Female
Rad	Radiation-absorbed dose
SADs	Stationary Attractive Devices
SAT	Sequential Aerosol Spraying Technique
SIRM	Sterile Insect Release Method
SIT	Sterile Insect Technique
SNNPR	Southern Nations and Nationalities Peoples Region
SOP	Standard Operating Procedures
SRV	Southern Rift Valley
SRVL	Sodo Regional Veterinary Laboratory
STEP	Southern Tsetse Eradication Project
TPU	Tsetse Production Unit

ABSTRACT

Survival of fertile female tsetse flies and their fecundity, the emergence rate of pupae they produce and the survival of sterile males are important for the success of SIT. In the present study the highest survival rate, mean percentage of surviving flies, and fecundity was found in flies fed on bovine and ovine blood diets. However, the emergency rate of pupae produced from these blood diets had lower than pupae produced on mixed blood diets. Moreover, the least survival rate was found in both flies maintained on porcine and mixed blood diets, the least being in mixed blood diets. The survival rate of flies maintained under caprine blood diets was slightly higher than flies maintained under porcine and mixed blood diets, however, the mean percentage of survived number of flies in this blood diets were the least of all blood diets. The fecundity of flies maintained under bovine blood expressed as pupae per initial female (PPIF) was 3.31 and 2.77 for ovine blood diets. However, the values in the rest blood groups were below the required standard for self sustaining tsetse mass rearing. In the other hand, during the production weeks, the fecundity of female *G. pallidipes* as expressed in pupae per female per 10 days (P/F/10 days) was near to the minimum average fecundity required for maintenance of a given colony in flies fed on bovine and ovine blood diets. The highest percentage of pupal quality class (class E) was recorded on ovine. However, the least was recorded in flies fed on bovine blood diets next to caprine. Extremely low mean percentage of survival, fecundity, poor pupal quality and low emergency rate recorded in flies fed on caprine blood diet. Furthermore, irradiation of adult males of *G. pallidipes* at 120 Gy gamma radiations resulted in significantly reduction of their average life span compared with non-irradiated males. Lastly, the overall emergency rate of *G. pallidipes* of the Arbaminch origin of the Center was found to be 88% and the female to male ratio was almost 1:1. Therefore, the overall performances of female *G. pallidipes* maintained under ovine blood diets were found to be almost the same or better than bovine blood. Hence, Kality tsetse rearing and irradiation center may use this blood as alternative to bovine blood. Furthermore, during releasing of sterile males as SIT component, their short survival time should be considered.

Key words: *G. pallidipes*, Fecundity, Survival, Emergency rate, Blood diets, Kality Tsetse Rearing and Irradiation Center, SIT, Ethiopia.

1. INTRODUCTION

Tsetse transmitted trypanosomosis in man and domestic animals pose a serious threat to the lives and livelihood of entire communities and constitute the greatest single constraint to livestock and crop production in sub-Saharan Africa (Hoare, 1972). The limitations imposed by tsetse and trypanosomosis remain to frustrate efforts and hampers progress in crop and livestock production there by contributing to hunger, poverty and the suffering of entire communities in Africa (PATTEC, 2001).

Tsetse flies (*Glossina* spp) can be ranked among the world‟s most destructive pests and are the vectors of the causative agents for sleeping sickness in humans and African Animal Trypanosomosis (AAT) or Nagana in livestock (Vreysen, 2001). Based on both their morphological and ecological specifics, there are about 31 tsetse species and subspecies divided into three subgenera or groups, namely the subgenus *Austenina* (*fusca* group), the subgenus *Nemorhina* (*palpalis* group) and the subgenus *Glossina* (*morsitans* group) (Jordan, 1993).

Current vector control interventions involve the use of insecticides either through sequential aerosol spraying technique (SAT); ground spraying; insecticide-treated targets or insecticide treated animals - live baits; the use of traps, and the sterile insect technique (SIT) (WHO, 2011). Insect control strategies based on chemical and biological control have had some notable successes but in many cases control has not been sustainable in the long term. This can be attributed to many reasons, including insecticide resistance, re-invasion, environmental damage and poor control program implementation (Wilke, *et al*., 2009). Moreover, some of the interventions conducted in the past such as bush clearing (tsetse habitat destruction) or elimination of wild animals (tsetse reservoir hosts) have been discarded for ecological and environmental concerns. For this reason, a species-specific, effective and environmentally friendly technique of insect control has been developed around for decades and has been widely used in the control of agricultural pests (Bushland, *et al*., 1955).

The sterile insect technique as a method of pest control integrates well into area-wide integrated pest management (AW-IPM) programmes (Dyck, *et al*., 2005). The sterile insect technique is one area-wide insect pest management method where the insect pest is controlled or eradicated by affecting its reproductive capacity. The technique relies on the production in large numbers of the target insect in mass-rearing facilities, sterilization of one of the sexes (i.e. in tsetse, male) and the release in sustained numbers in the natural habitat large enough to outnumber the wild pest population (Knipling, 1955; 1959). The Sterile Insect Technique, best known by its acronym SIT and also identified as the Sterile Insect Release Method (SIRM), is a biologically-based method for the management of key insect pests of agricultural and medical/veterinary importance (FAO/IAEA/USDA, 2003-2010). It is environmentally-friendly and sustainable methods to control major insect pests of crops and veterinary and human importance. This an area-wide integrated pest management approach, by the use of the sterile insect technique, enhances food security, introduce sustainable agricultural systems, reduce losses and pesticide use, preserve biological diversity, and facilitate international trade in food and agricultural commodities by promoting the development and application of Sanitary and Phytosanitary (FAO/IAEA, 2004). SIT is species-specific and has no effect on other „non-target" species. The technique has been effectively used for eradication of the new world screwworm (NWS) (*Cochliomyia hominivorax*) from north and then Central America to panama (Richard, 1997) and tsetse (*G. austeni*) from Unguja Island in Zanzibar (WHO, 2011). Accordingly, the sterile insect technique seems to be promising towards the eradication of tsetse flies in Africa in general and in particular in Ethiopia. SIT as a technique of choice, requires mass production of tsetse flies in the laboratories. The laboratory rearing of tsetse flies originally dependent on the availability of host animals for *in vivo* feeding (Nash, *et al.,* 1968; Van der Vloedt, 1982; Williamson, *et al*., 1983; Oladunmade, *et al*., 1990).

The frequent use of host animals as blood donors imposes the risk of over challenging them. For this reason it is necessary to develop effective and standardized tsetse fly feeding methods without using live animals for the daily blood uptake (Wetzel and Lunger, 1978). So, appropriate use of the membrane (*in vitro*) feeding technique provides a means to produce tsetse flies more economically and with less risk. *In vitro* feeding system is recommended if reliable source of quality tested blood is available (Feldmann, 1994c; Gooding, *et al*., 1997; Opiyo, *et al*., 2000).

Therefore, qualitative and quantitative amount of blood is needed for maintenance of fly colonies (IAEA, 2000) *in vitro*. For further SIT wide spread application, research projects focusing on tackling on diets for mass rearing haematophagus insects for sterile insect release and more efficient rearing techniques are necessary (IAEA, 2003). Moreover Colony maintenance in laboratory is dependent on the presence of not only qualitative and quantitative amount of blood but also in the choice of dietary requirement of a particular tsetse species (FAO/IAEA, 2006) but this has not been established for *G. Pallidipes* in Ethiopia. Moreover, survival of sterile male tsetse flies is very important for successful of SIT but not yet established for the above species in Ethiopia. Therefore the objectives of this thesis were:

General objective:

This MSc thesis was addressed the survival and reproductive performance of *G. pallidipes* reared under different animal blood sources at Kality Tsetse Rearing and Irradiation Center.

Specific objectives:

- To compare the survival and reproductive performance of non- irradiated females of *G pallidipes* maintained on bovine, ovine ,caprine, porcine, 75% bovine: 25% porcine fresh frozen blood.

- To compare the survival of irradiated males of *G. pallidipes* under sterilization dose with the non-irradiated one.

- To evaluate the overall emergency rate of *G. pallidipes* of Arbaminch origin of the center based on weekly collected pupae.

2. LITERATURE REVIEW

2. 1. Biology of tsetse flies

Adult *Glossina* species are dull in appearance, varying in color from light yellowish brown to dark blackish brown (Leak, 1999). In some species the abdomen may have alternate darker and lighter bands. The smallest species is 6-8 mm long and the largest 10-14 mm (Jordan, 1986). Tsetse flies can be distinguished from other biting flies by their forward-pointing mouthparts (proboscis) and characteristic wing venation. The adult female produces a single egg, which hatches to first stage larva in the uterus. After a period of development and moulting, a third stage larva is deposited on the ground (Figure 1). Females produce one full grown larva every 8-10 days which pupates in light clay or sandy soil. The adult fly will emerge after a puparial period that varies according to temperature but may be around 30 days at 24^0 C. Consequently, tsetse flies have a very low rate of reproduction, closer to that of a small mammal than to most insects. This reproductive method of tsetse flies is known as adenotropic viviparity (Leak, 1999).

Copulating (Mate) Larviposition (birth of the larva) Pupa

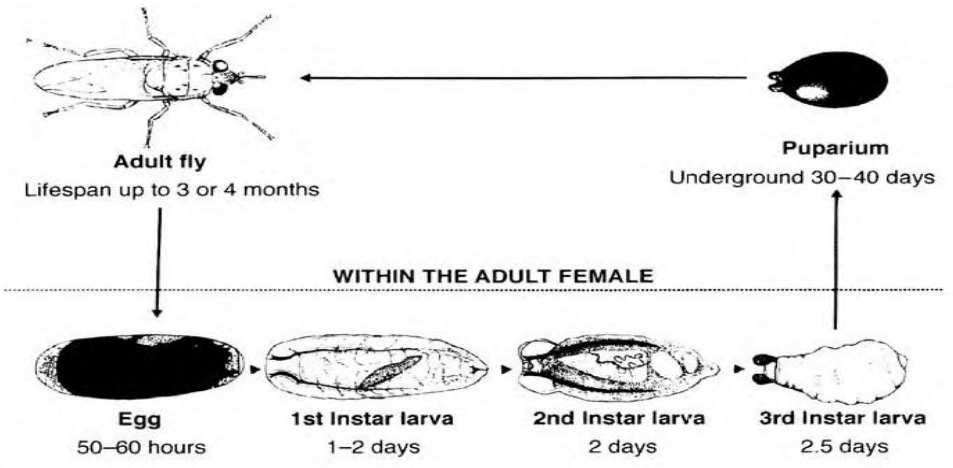


Figure 1. Life cycle of tsetse flies.

Source: (Leak, 1998).

Egg stage: Tsetse flies are unusual insects in which their egg and larval developmental stages are taken place within the female (IAEA/FAO, 1997). The egg is fertilized immediately as it enters the uterus by sperm from the spermathecae coming into contact with and penetrating the anterior part of the egg. The fertilized egg remains lying in the uterus for about four days, while development of the first instars larva takes place inside. The egg is about 1.6 mm long (*G. morsitans*) (Kettle, 1984).

Larval stages: as with other flies, the larva in *Glossina* passes through several stages or instars, as it grows. There are three larval instars in *Glossina* up to the time when the fully grown larva is dropped by the female fly: the first, second and third instars.

The larva has a mouth at the anterior end, and two posterior spiracles. The unusual feature of the *Glossina* life history is that the larva spends practically all its time, and does all its feeding; within the body of the female fly (Leak, 1998). The fly gives birth every 9-10 days to a full-grown larva, which immediately burrows into the soil and forms a pupa. Female tsetse produces at most nine larvae, and therefore has the lowest reproduction potential of any insect (IAEA/FAO, 1997).

First instars larva: this is the stage that emerges from the egg. It breaks out of the chorion using a sharp egg tooth. The first instar grows to 1.8 mm in length (*G. morsitans*) before changing to the next stage by getting rid of its old skin. The first instars lasts for about 1 day (Vreysen, 2001)

Second instars larva: this is a stage of rapid growth and development. To either side of the posterior spiracles are swellings, and between the spiracles is an area of small spines. The second instars lasts two days, and the larva grows to a length of 4.5 mm (*G. morsitans*) (Leak, 1998).

Third instars larva: this is also a stage of rapid growth and development. The fully grown larva has a pair of large black swellings at the posterior end. These are the polypneustic lobes, which carry many small holes through which the larva breathes. The polypneustic lobes are at first white, becoming black later (Vreysen, 2001). The rest of the larva is white in colour. Most of the weight and volume of the third instars larva is due to the gut which contains large amounts of unassimilated food. The third instars lasts just over two days and the larva grows to a length of 6–7 mm (*G. morsitans*).

Feeding by the larva: Apart from the food already in the egg, all the food of the three larval instars comes from the milk gland of the mother fly. The milky secretion of this gland is poured out of the duct of the gland, at the head end of the larva. The larva sucks up this secretion and passes it straight to the midgut (Leak, 1998). Here it is slowly digested and assimilated.

Breathing by the larva: for its air supply the larva depends on air entering the vulva of the female and then passing into its posterior spiracles or polypneustic lobes.

Abortion sometimes: a larva fails to reach its full size and is expelled from the uterus before the usual time. This is called an abortion. The aborted larva dies (Vreysen, 2001).

Abortions can be caused by the mother fly not obtaining sufficient food, and may also occur when the fly is carelessly handled, or when it comes into contact with insecticide. The egg may also be aborted for the same reasons (Roberts, 1993).

Larviposition (birth of the larva): When the larva in the uterus is fully grown, the female *Glossina* flies around looking for a suitable area in which to drop it. This will usually be a place where there is a patch of loose sandy soil, sheltered by an overhanging rock, branch or twig. The female tsetse settles down either on the ground or on the overhanging object. The larva then walks itself backward cut of the vulva of the female, helped by pushing movements of the female's legs, and drops to the ground (Roberts, 1993). The larva burrows into the ground and out of sight the female flies' away within an hour or two the larva becomes barrel-shaped, darkens and may then be called a pupa (Figure 2). There is no feeding by the larva after it is dropped by the female.

Pupa: the pupa is a dark brown rounded object; at the posterior end are the polypneustic lobes the shape of which helps to distinguish the tsetse pupa from the pupae of other flies (Kettle, 1984).

Figure 2. Picture showing polypneustic lobes of pupae.
Source: (Leak, 1998).

The pupa is slightly shorter than the larva that produces it. Inside the pupa two main processes take place: the food still remaining in the midgut is digested and assimilated, the organs of the adult fly begin to form. The pupal stage usually lasts about four to five weeks, depending on the temperature. Higher temperatures shorten the pupal period; lower temperatures lengthen the pupal period (to more than 50 days in some climates) (Jordan, 1993).

Too high or too low a temperature will cause the death of the pupa. At the end of this period, the adult fly is ready to emerge (Leak, *et al*., 1993).

Adult fly: emergence of the adult fly when ready to emerge the young adult fly expands its ptilinum to burst open the end of the puparium. The body walks its way out of the hole and also gets through the surrounding soil by using the ptilinum. In this way the young fly struggles to the top of the soil and out into the open air (Leak, *et al*., 1993). At this stage, the body is very soft and the wings are small and crumpled. After a few minutes the wings begin to expand to reach their proper size. Generally, when a tsetse fly emerges from its pupal case it is free from trypanosomes. Until its first blood meal, it is called a teneral fly and after its first meal it is called nonteneral. It acquires a trypanosomal infection when it feeds on a parasitaemic mammalian host.

Teneral fly: from the time the fly emerges to the taking of its first meal, the young fly is called a teneral fly. The underside of the abdomen appears whitish and semitransparent, the ptilinum can sometimes be everted when the sides of the head are squeezed between the fingers, and the body has a soft feel to it.

Non-teneral fly: after the first blood meal has been taken, the underside of the abdomen appears more creamy yellow, and when held up to the light the dark shape of the last meal can be seen (Vreysen, 2001). The thorax feels firmer and harder, because of the greater development of muscles in it, the ptilinum cannot easily be everted. The fly is then termed a non-teneral fly.

Rate of reproduction: once mated a female fly can produce larvae for the rest of her life. At a temperature of about 25°C a female fly will produce a mature larva every 9–10 days, except for the first one which may take 18–20 days from the time of emergence of the fly from the puparium. Lower temperature gives a lower rate of breeding; higher temperatures increase the rate of breeding. However, temperatures that are too high or too low will cause breeding to stop altogether (Teesdale, 1990).

As with all insects, the tsetse fly has three main body segments: the head, thorax and abdomen (Figure 3). The wings and legs are attached to the Grayish-brown colored thorax.

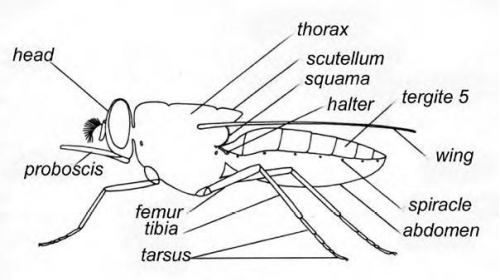
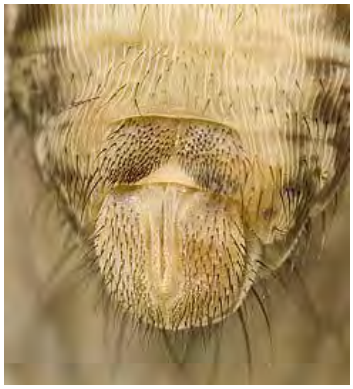


Figure 3. Diagrammatic side view of a tsetse fly: showing the three segments of fly body.
Source: (FAO, 1982).

The abdomen has seven visible segments and the male fly has a structure at the posterior tip, folded underneath the last two segments called the hypopygium (Figure 4), forming part of the external genitalia. The male has a pointed abdomen and superior claspers or external genital which are heavily pigmented. On the other hand, the female has an abdomen which is truncated and pale in its appearance and used for sexing (FAO/IAEA, 2006). Each segment of the back of the abdomen has a harder cuticle forming a plate or tergite, unlike the elastic ventral surface. The colouring and markings of the tergites are sometimes useful for species identification. There are seven pairs of spiracles, one pair for each segment, along the sides of the abdomen, and an anus at the posterior end (Pollock, 1992).

Female



Male

Figure 4. Comparison of external genitalia of male (Right) and female (Left) savannah tsetse flies (*G. m. morsitans*), showing the presence of hypopygium in males.
Source: (FAO, 2004)

The genitalia, particularly of the male, are useful features for species identification. It is easy to distinguish the sexes of tsetse by the presence of the folded hypopygium at the posterior tip of the abdomen, compared to the female in which there are no equivalent obvious structures, simply a small hole surrounded by a variable number of small flat chitinous plates (Figure 5) (Pollock,1992).

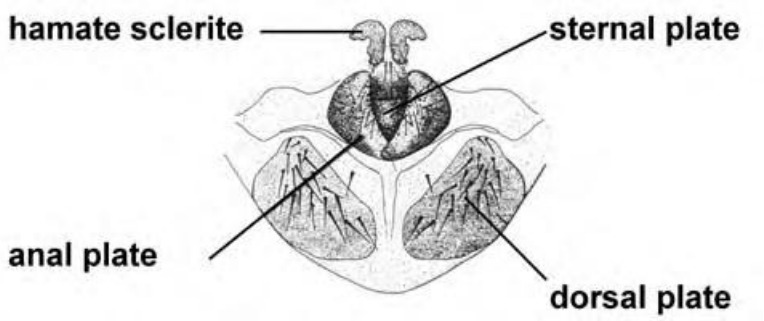


Figure 5. The external genital armature of female tsetse fly indicating the dorsal plates, the anal plates, the sternal plate and the hamate sclerite.
Source: (Mulligan, 1970).

2.2. Sterile Insect Technique (SIT)

Definition: Defined as a method of pest control using area wide inundative release of sterile insects to reduce reproduction in a field population of the same species (http://www-naweb.iaea.org/).

2.2.1. History and principles of SIT

History of SIT

The infestation of livestock by *Cochilomia hominivorax* (Figure 6) was 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 (Richard and David, 2001). 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 in 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. In 1988, the worms were discovered in area of 10 kms 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 programme which has successfully eradicated the fly from this area, again using the release of sterile males.

In the island of Zanzibar, the introduction of the SIT helped in the eradication of tsetse fly in 1996 campaign that had been commenced two years earlier. 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).

A  B 

Figure 6. A. Screw worm (*C. hominivorax*) adult fly
B. Larvae of *C. hominivorax*
Source: (FAO/IAEA/USDA, 2003-2010)

Principles of SIT

The SIT is non-intrusive to the environment, has no adverse effects on non-target organisms and it is species-specific. It can easily be integrated with biological control methods such as parasitoids, predators and pathogens (Leak, 1999). The sterile insect technique (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 (Dame, *et al*., 1981; Dame, 1985). As a prerequisite, tsetse density has to be suppressed through the wide spread 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. It is known that tsetse females copulate only once, and if the male of a copulating pair is sterile, the female will not produce offspring during her lifetime. 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 (IAEA/FAO, 1997). However, the irradiated tsetse flies are fully capable of developing and

transmitting trypanosomes to livestock (Moloo and Kutuza, 1984). 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. It is also estimated that ten sterile males are required per each female in the field. 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.

2.3. Sterile Insect Technique (SIT) in Ethiopia

Tsetse and Trypanosomosis in Ethiopia

According to Langridge (1976) and FLDP (1989), tsetse flies in Ethiopia are confined to the western and southern regions between longitude 33 ^{o} and 38^{o} E and latitude 5^{o} and 12^{o} N. 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 also in the river valleys of Abay (Blue Nile), Baro, Akobo, Didessa, Ghibe and Omo (Amare, 1995).

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. 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 seal level (Abebe, *et al*., 2004). 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 north eastern edges of Lake Abaya in the Rift Valley, the upper reaches of the Omo-Ghibe and its tributaries (Amare, 1995; NTTICC, 1996 and SRVL, 2000). According to survey result conducted by Langridge (1976) five species of *Glossina* (*G. m. submorsitans*, *G. pallidipes*, *G. tachinoides*, *G. f. fuscipes* and *G. longipennis*) have been recorded from Ethiopia but only four are widespread and have significant economic importance. These are *G. m. submorsitans* and *G. tachinoides*, which have a west to east distribution 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 grass land in the Southern Rift Valley of Ethiopia (Veryesen, *et al*., 1999). Out of the nine regions of Ethiopia five (Amhara, Beneshangul- Gumus, Gambella, Oromiya and SNNPR) are infested with more than one species of tsetse flies (Abebe, *et al*., 2004).

The smallest species (*G. tachinoides*) is 6-8mm long and the largest (*G. pallidipes*) 10-14 mm (Vreysen, 2001). The trypanosomes undergo a cycle of development and multiplication in the digestive tract of the fly until the infective metacyclic trypanosomes are produced (Stevens and Brisse, 2004). Different trypanosomes develop in different regions of the digestive tract of the tsetse fly and the infective meta-trypanosomes occur either in the biting mouthparts or the salivary gland of the fly (Leak, 1998). The period from ingesting infected blood to the appearance of the infective forms varies from one to three weeks and once infected trypomastigote are present the fly remains infective for the remainder of its life (Seifert, 1996). During the act of feeding the fly penetrates the skin with its proboscis. By the rupture of small blood vessels a pool of blood is formed 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 (Hargrove, *et al.,* 2003 and Hargrove, 2004). Tsetse flies once infected with trypanosomes are likely to transmit the parasite for the remainder of their lives.

Status of SIT in Ethiopia

In 1997, 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).

Its long-term objectives are:
(1) to create a tsetse-free zone in a 25, 000 square kilometer area under agricultural development
(2) to develop adequate national capacity for applying the concept of Area-Wide Integrated Pest management (AW-IPM) with a Sterile Insect Technique (SIT) component to the other parts of the country affected by the tsetse and trypanosomosis problem (Alemu, *et al.,* 2007).

The project was initiated with the collection and evaluation of entomological, veterinary, environmental and socio-economic baseline data which reconfirmed the presence of only one species, i.e. *G. pallidipes* Austen, in the main valley and the positive socio-economic and agro-economical impact anticipated. This situation generated international acceptance of the Southern

Rift Valley as a high priority area for the control of tsetse and trypanosomosis and for related sustainable agriculture and rural development (Alemu, *et al.,* 2007). A colony of *G. pallidipes* Austen originating from the Southern Rift Valley was also initiated in 2002. Community-based tsetse suppression was initiated in localized areas using insecticides on cattle and on blue-black blue fabric targets that attract tsetse flies. These localized tsetse suppression activities have been expanded to all operational grids of the 10500 square kilometers STEP block-1 area limited entomological and veterinary monitoring in 15 sites suggests that the apparent density of *G. pallidipes* in these localized control sites may have been reduced by 92% while the prevalence of trypanosomes in live stock in those areas decreased by 58% (Alemu, *et al.,* 2007). An analysis using geographic information system (GIS) has indicated that the community-based tsetse suppression does not cover all of the tsetse-infested areas in the STEP block-1 and it is therefore assumed that some cattle herds remain with high disease prevalence in areas that were not adequately covered by the community fly control measures. The operational programme will include the introduction of a set of implementation of rules and regulations conducive to the special needs of an operational AW-IPM campaign, i.e. an efficient management structure and the provision of adequate financial flexibility (Alemu, *et al.,* 2007).

Tsetse fly infestation in the southern Rift Valley of Ethiopia has limited the expansion of mixed farming in the technical cooperation project ETH/5/012-Integrating Sterile Insect Technique for tsetse Eradication.

The project aims to create a zone free of tsetse and trypanosomosis in an area of 25 000 km^2 in the Southern Rift Valley (SRV), Ethiopia. This will free up the area to enable the introduction of mixed farming according to a land use plan being developed by the Government (IAEA, 1957-2007). The STEP Kality Tsetse Rearing and irradiation centre (Kality centre) was inaugurated on 3 February 2007. When completely equipped and operational, the new facility will have a colony capacity of approximately 7 million female flies and will be able to produce over 700 000 sterile male flies per week enough to treat approximately 7000 km^2 at a time (IAEA, 1957-2007).

The STEP is currently preparing for large-scale field operations, including the sterile insect technique. The creation of the tsetse-free zone in the entire target area is expected to be completed by 2017, including a four year verification phase. The project is currently focusing on an area of 10,500 km^2 which has considerable potential for agricultural development. Operational activities to clear this area will depend on the built-up of a sufficiently large colony of target tsetse fly species in captivity and other essential preparatory work, particularly in the field. It is likely that related field intervention work will need to continue through at least 2013(IAEA, 1957-2007).

The model project: the International Atomic Energy Agency is providing support to the Ethiopian authorities for tsetse control/eradication in an area, initially of 5,000 km^2, in the Southern Rift Valley. Over the ten-year life of the project, it is planned to extend the target area to about 25,000 km^2. Following an assessment of entomological, veterinary and socioeconomic factors which will influence subsequent project operations, a phase of tsetse suppression by appropriate conventional means will be introduced. The project area will be divided into eradication zones, each chosen to take as much advantage as possible of natural isolating features such as high or arid land. Six to eight months prior to aerial releases of sterile flies, locally made, odour-impregnated traps will be placed at a density of four per km^2 by specially trained local teams to start reducing the fly population in the target area. When fly numbers have been sufficiently suppressed, the traps will be removed and aerial release of sterile males initiated (http://tc.iaea.org/tcweb/).

As the project develops, a pattern of suppression will be followed by an airborne operation during which sterile male tsetse flies will be released over the targeted area. The flies will be produced and reproductively sterilized by irradiation at a mass rearing factory of Kality. In order to begin mass rearing, a suitable strain of tsetse fly has been collected from the project area and adapted to the artificial conditions of factory production. The Agency‟s laboratories at Seibersdorf in

Austria, and the Tsetse and Trypanosomosis Research Institute at Tanga, Tanzania, are involved in this work and will maintain a back-up colony of this strain respectively. Initially, the mass rearing facility in Ethiopia will supply 250,000 sterile males per week. Production will eventually be doubled in order to meet the project‟s needs. The flies will be chilled to immobility, avoiding the need to box them for aerial release (http://tc.iaea.org/tcweb/).

The project area has been chosen because it has high agricultural potential and is well confined from neighboring tsetse-infested areas by high escarpments and arid land. Furthermore, tsetse infestation is high and local population pressure is putting a severe strain on available fly free highlands (http://tc.iaea.org/tcweb/).

Fly releases: Unlike conventional insect control methods that are preferably applied to a high population of the target insect pest, SIT is most effective when fly populations are low. The ratio of released sterile male flies to wild fertile male flies should be as high as possible to minimize the odds that wild flies mate (http://tc.iaea.org/tcweb/).

Flies will be released over the targeted eradication zone at a rate of approximately 100 sterile males per km^2 per week over almost two years. These numbers will be adjusted as necessary in order to achieve the desired ratio of released sterile to wild fertile males. Aircraft will fly regular sorties over the area, ejecting chilled flies at a computer controlled, and specified rate. The flies warm up and become active as they reach ground level (http://tc.iaea.org/tcweb/).

2.4. Techniques of SIT

The sterile insect technique (SIT) includes the mass production, sex separation, sterilization and release of sterile males.

Mass rearing: mass rearing of tsetse flies is simplified in the laboratory because only two developmental stages need to be considered, i.e. the adult and pupal stages. New colonies of tsetse flies are established using field collected pupae after quarantine to avoid introduction of any parasitoids in to the main insectary (FAO/IAEA, 2006), collection of adult females with efficient trapping systems that attracts wild flies for collection (Bandah, 1994, Kuzoe and Schofield, 2004), collection of pupae of known age from an existing tsetse fly. Collection of female adults in the field is much easier, but the wild females may be infected with pathogenic trypanosomes leading to the risk that animals used for *in vitro* blood feeding, or the insectary staff to be infected. For this reason field collected females are usually kept separately and only the pupae they produce are

used to form the new colony. Obtaining pupae from an established colony is the easiest method, but the strain will not be exactly the same as the local strain (FAO/IAEA, 2006). In Ethiopia, in order to begin mass rearing, a suitable strain of tsetse fly *(G. pallidipes)* has been collected from the project area and adapted to the artificial conditions of factory production (http://tc.iaea.org/tcweb/).

During mass rearing, adult flies are kept either in trolley or TPU 3 holding systems. In trolley holding systems (conventional holding system), adults are held in production cages kept on shelves on trolleys with wheels where larvae and pupae are collected (FAO/IAEA, 2006). To feed the flies, cages are manually transferred to a silicone membrane covering warm blood (Figure 7) and after feeding the cages are returned to the trolleys.

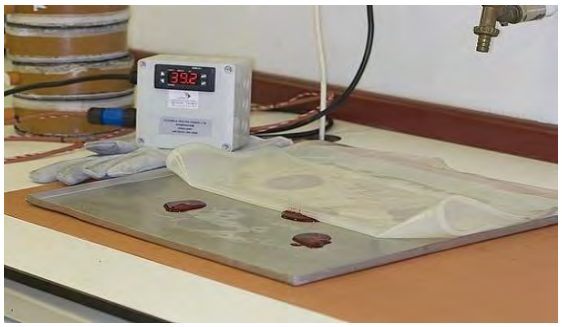


Figure 7. Trolley membrane feeding apparatus of tsetse flies.
Source: (http://www.raywilsonbirdphotography.co.uk/).

The improved colony-holding system is the tsetse production unit 3(TPU 3) (Opiyo, *et al.,* 1999; Opiyo, *et al.,* 2000; IAEA, 2003; Parker, 2005). Here fly cages are held stationary and blood in the feeding system moved on rails to the cage holding frame for fly feeding.

Routine colony maintenance: Tsetse flies are held in production cages (Figure 8) kept on shelves on trolleys in a horizontal plane. The trolleys are kept in holding rooms and are brought out during fly handling and feeding. At times due to space limitations both holding and feeding takes place in the same room. In the conventional tsetse rearing system, cages with flies are manually

transferred for feeding and then returned to the trolley for pupal collection. The number of flies introduced into 20 cm diameter cages depends on the species (or size) of the fly (FAO/IAEA, 2006). Number of flies per cage for representative species (small, intermediate and large sizes of flies) is given bellow (Table 1).

Successful 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, pre-oviposition and ovipositor periods, fecundity, longevity, sex ratio, environmental requirements, and food and feeding preferences of the insect is necessary in developing rearing techniques (Singh, 1984; Orozco-Davila, *et al*., 2007).

Table 1. Cage densities (number of adults per cage) of species of *Glossina* and cage netting size of *G. pallidipes*.

Species	Adults			Pupae for self stocking of production cage (Emergence rate=95%)		
	Females	Male	Total	Mixed	Males	Total
G. austeni	100	25	125	222	28	250
G. tachinoides	100	25	125	222	28	250
G. morsitans	64	16	80	142	16	158
G. f. fuscipes	64	16	80	142	16	158
G. p. palpalis	60	20	80	133	22	155
G. pallidipes	60	15	75	133	17	150
G.palpalis gambiensis	60	20	80	133	22	155
G. brevipalpis	45	15	60	100	17	117
	Netting size					
	Top			Bottom		
G. pallidipes	130-150 micrometer thread, 13 holes per cm^2			200-300 micrometer thread, 8 holes per cm^2		

Source: (FAO/IAEA, 2006).

Conservation of space is also a major consideration, especially when several days are required from the time of pupae 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 manes of the 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 has 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 within living mammalian flesh (Feldmann and Hendrichs, 2001). But in tsetse flies, larvae do not have to be fed as they develop within the female fly (Feldmann and Hendrichs, 2001).

Environmental conditions: Adult tsetse flies are usually maintained at 24 ^{O}C and 70-80% RH. Savannah species require less humid conditions, and riverine species may require conditions above 80% RH. Pupae can be maintained under the same conditions as adults, but experience has shown that species such as *G. tachinoides* Westwood survive better at 23^{O}C and 82.5%RH, and *G.austeni* pupae, after they are 20 days old, survive better at 85-88%RH (FAO/IAEA, 2006).

Lighting should be subdued and indirect, using tungsten lamps or high-frequency fluorescent-blue lights on a 12:12 photoperiod. Light distribution is important because it influences the distribution within a cage. Uneven distribution of light in cages may encourage crowding towards the light source, leading to increased fly mortality.

Colony feeding: larvae of tsetse fly do not have to be fed as they develop within the female fly. Adult tsetse flies do not required water or carbohydrates, only high-quality warm vertebrate blood. Originally, living animals had to be used to provide tsetse flies with a movement.

With the development of membrane feeding system, which flies accept as host skin and through which they ingest the blood, living animals are no longer required as hosts. Animal blood for tsetse rearing can be collected at a local abattoir and then treated with gamma radiation to eliminate any micro organisms (Feldmann and Hendrichs, 2001).

The membrane or *in vitro* feeding with a reliable source of quality-tested blood is recommended for tsetse (Feldmann, 1994a; Gooding, *et al*., 1997; Opiyo, *et al*., 2000). The membrane for the *in vitro* feeding system is made of silicon (Figure 8) (Bauer and Wetzel, 1976). A silicon membrane is reinforced with netting; the size of the netting determines the thickness of the membrane.

Small tsetse species are fed using a thin membrane and large species a thick membrane (Figure 8). Bovine or porcine blood or a mixture of both has been used for colony maintenance but the choice depends on the dietary requirements of particular tsetse species. Tests using only bovine blood (at the FAO/IAEA Laboratories, Seibersdorf, Australia) demonstrated that most tsetse species can be maintained on bovine blood alone (FAO/IAEA, 2006). Colonies of tsetse flies can be maintained on sterile, fresh frozen, defibrinated blood or blood to which anticoagulants have been added. The procedure for feeding flies using the membrane system (figure 8) 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.

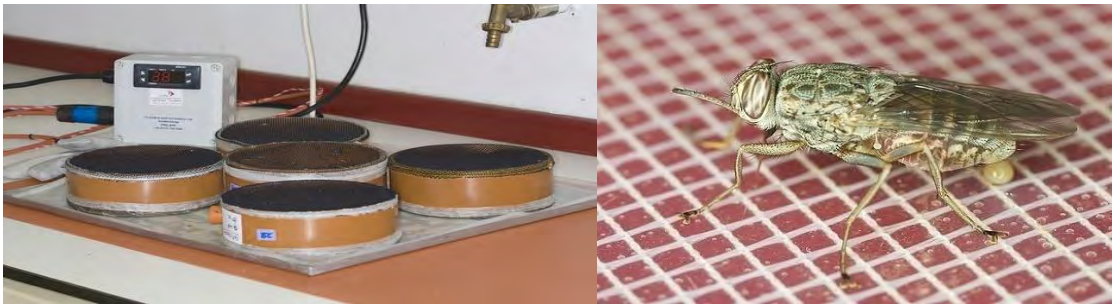


Figure 8. Cages of Tsetse flies (Left) and Male *G. m. morsitans* feeding on blood through a silicon membrane (Right).
Source: (http://www.raywilsonbirdphotography.co.uk/).

A quantity of 100 ml of blood is sufficient to cover a surface area of 2000-2300 cm^2 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).

In the tsetse production unit (TPU) 3 feeding system, the opposite occurs-blood is moved to the flies and the cage-holding system is stationary. The mobile blood system is moved on trails that are fixed to the floor. Two rows of cages are fed at the same 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 on to the tray. Lower trays are filled with blood and the blood smoothed out under the membrane by a person standing on the floor, but upper trays are filled from a working platform (FAO/IAEA, 2006).

Irradiation (Sterilization): 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 (LaChance, *et al*., 1967; Curtis, 1971). 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. 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 (Parker and Mehta, 2007; Robinson, 2002). Moreover, reduced competitiveness can be partly overcome by increasing the ratio of sterile-to-wild insects (Knipling, 1955).

Sterilization is induced in the male by exposing pupae or young adults to a source of gamma radiation such that the sterilizing dose does not adversely affect their mating and inseminating ability (http://www.iaea.org/). Sterilization of males is done by exposing the tsetse flies to a specific dose of gamma radiation emitted from radioisotopes (Cobalt 60 or Caesium 137) (http://tc.iaea.org/tcweb/) or Chemical compounds which can reduce or destroy the reproductive

ability of male tsetse flies to which they are applied (Borkovec, *et al*., 1968; Campion, 1971). Chemosterilants substituted for radiation believing that the chemicals should be easier to use and less costly than gamma radiation (Campion, 1971). Irradiation of male insect may result in dominant lethal mutations in the sperm, killing spermatogonial cells, inactivation of sperm and weakening of males (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, failure of the sperm to move to the spermatheca, or other malfunctions that can prevent reproduction and can be induced in either sex by selecting the appropriate dose and developmental stage (Tiltone and Brower, 1982). According to Bakri, *et al.*, (2005), cells with a high mitotic rate, with a long mitotic future under normal circumstances are most radiosensitive so the only cells that are continually dividing in an adult male fly are its sperm (the germ cell). The somatic cells of the fly are unharmed - but the radiation breaks the chromosomes in the sperm, making it genetically sterile and unable to reproduce. However, once a female fly has mated, she assumes she is fertile and will not mate again for the rest of her lifespan. As a result, the population of flies can potentially fall dramatically.

Principle of aerial release: Sustained, systematic release of Gamma-sterilized flies, packed in special paper boxes are released by air craft twice a week among the indigenous target population. For maximum effectiveness, the sterile males released must outnumber the fertile, native male flies by a considerable margin. In order to reduce populations when conditions are highly favorable for fly reproduction, the ratio of released sterile males to native males should be at least 2 to 1 (Knipling, 1955) and may, in certain circumstances, have to be as high 15 to 1.Over time, the fertile population and the reproductive capacity are progressively reduced until fertile mating do not occur and the population is eliminated (http://tc.iaea.org/tcweb).

3. MATERIALS AND METHODS

3.1. Study area

In 1997, 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 mass rearing and irradiation centre (Kality centre) was initiated in August 2000 following the renovation and equipping of an existing building to function as a temporary insectary. It was begun with wild female *G. pallidipes* that were shipped 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 present experimental study was conducted at Kality Tsetse Fly Rearing and Irradiation Center within the capital, Addis Ababa Region. Addis Ababa is the capital city of Ethiopia with geographical locations 9.03° North; 38.74° East, latitude and longitude, respectively (Figure 9). (http://www.mapsofworld.com/).

Figure 9. Map of the location of the insectary within Addis Ababa and its surrounding areas of the research area.

Source: (Dubbale, *et al*., 2010).

The insectary has its own micro environment adjusted as the need of the *G. pallidipes.* The experimental flies were maintained in similar environmental condition as that of the general colony of the center at a temperature of 23-25 ^{o}C and a RH of 75-80% (FAO/IAEA, 2006). Moreover, the study was conducted following the center's standard operating procedure for irradiation and other procedures needed to perform the experiment.

3.2. Blood collection and processing

Blood was collected from Addis Ababa Municipal Abattoir after the necessary permission and arrangement has been made with the responsible authorities of the abattoir to ensure the place, time, purpose, type and amount of blood to be collected. Blood was collected and processed separately in different days to each of the different blood type according to the slaughter program of the abattoir.

Before arrival at the abattoir, all equipments used for blood collection has been thoroughly cleaned and sterilized according to FAO/IAEA (2006). All collection materials was cleaned and sterilized over night in dry heat. All sterile equipments were packed in autoclave plastic bags during transport and until used. Blood was collected from the cut neck of the different species of animals in a plastic dish hygienically and then immediately poured into a 10 liter container while defibrinating the blood using magnetic stirrer (electrical stirrer) to prevent blood from clotting. The residual liquid was poured through a „milk" sieve in to 4 liters plastic container and then dated, coded, leveled (Annex 1), packed, and transported to the center via vehicle (Feldmann, 1994c).

3.2.1. Laboratory activities and bulk sterilization of fresh frozen blood

Preparation of proportion of blood for irradiation**:** After arrival at the center (Laboratory), blood proportion was made by dividing the 4 liters blood in to 2 liter clean heat treated PE-container that fit into the chamber of the radiation source under UV- light sterilized laminar air flow bench. Blood samples were taken for bacterial screening before irradiation and the rest blood was kept

frozen at -20 ^{O}C until further processing and testing was conducted. The samples were incubated for 48-72 hours and the results had read and recorded according to the SOP of IAEA/FAO (2006).

Bulk sterilization of fresh blood: Each frozen blood group with 2 liter container was irradiated at 1 kGy in the gamma cell 220 excel. The source of irradiation is ^{60}Cobalt irradiator (Figure 10) using the monthly radiation output of the irradiator (Annex 2) (FAO/IAEA, 2006).

Figure 10. ^{60}Cobalt irradiator: the Gamma cell 220 ®, a conventional self-shielded irradiator.
Source: (http://www.radsource.com/)

Laboratory activities: The irradiated blood was transferred in to the laboratory and thawed in water until it changed in to liquid blood at +4 ^{O}C refrigerators before test. Media were prepared according to the SOP (IAEA/FAO, 2006). While blood proportions were made in small plastic containers for next feeding of the experimental groups, samples were taken under sterile laminar air flow hood bench (UV- switched on overnight) from each group of blood in to a leveled petridish with the same code as that of the blood sampled to evaluate the level of decontamination of the irradiation. In addition, two bacteriological controls were processed, one with 1 ml plate of nutrient agar closed and the other with a plate of nutrient agar, which were left opened under the flow bench for 1 minute to confirm the sterility of the nutrient agar and the working environment respectively. The samples were incubated at 37 ^{o}C for 48-72 hrs and read at 48 hrs and 72 hrs. The results were checked for bacterial colonies after 48 and 72 hrs. Blood with accepted colony number were passed for tsetse feeding (<10 colony number) and the rest discarded (FAO/IAEA, 2006) (Annex 1). Because of lack of female flies for bioassay, the blood with accepted bacterial

load was tested on male *G.pallidipes* feeding for 10 days and no death had been recorded during that time and passed for female feeding. Bloods passed this test had prepared in small vials, labeled (Annex 3) and stored in deep freeze (-20 ^{o}C) up to the next use.

3.3. Materials

The list of materials used for the present experimental study were include: Protective clothes, 10 liter polyethylene containers with wide screw caps, along paddle that rotates by electrical power, plastic hose, wide funnel, a plastic dish, sieve, more than 50m long water proof electrical cable,4 and 2 liter polyethylene containers, plastic bags, freeze resistant labeling materials, ^{60}cobalt irradiator (Gamma cell 220), laboratory equipments, refrigerators, freezers, dry oven, autoclaves, tsetse flies(*G. pallidipes*), Pupae, Chillers (+4^{o}c), brush, holding cages (diameter of 20 cm and width of 5 cm), heating plate (with an adjustable heat source), feeding tray (anodized aluminum), silicon membrane, clips, 96% irradiated bovine, ovine, caprine, porcine and mixture of both 75% bovine and 25% porcine blood, thermoscan, air conditioners, humidifiers, aspirators, dishes, small petridishes, trolley holding system(conventional), electrical sensitive balance etc. All these materials were used following the Center's standard procedures (FAO/IAEA, 2006).

3.4. Study group/ Population

All pupae, teneral and adult flies (*G. pallidipes*) of both female and male were used for the experiments and were derived randomly from Kality tsetse fly rearing and irradiation center from the stock colonies of *G. pallidipes(* Arbaminch origin*)*. To study pupal emergency rate, pupae were collected only in one randomly selected week time of the whole colony of the center.

3.5. Study Design

Both experimental and observational study was employed beginning from September 2011 to April 2012.

Sample size determination and sampling procedures

The sample size needed was depending on the type of experiment to be executed:

a. For feeding experiment: 450 female *G. pallidipes* (Byamungu, *et al*., 2011) were used.
b. For survival experiment: 180 males (90 sterile and 90 fertile *G. pallidipes*) were used.
c. For pupal emergency rate: only pupae collected in one week time were taken.

The experimental adult flies were kept in cages (30 flies per cage) and each experimental group was replicated three times to get sample size which is adequate for statistical analysis and was fed for a period of four months (for feeding and survival experiments). For pupal emergency rate, the daily collected pupae were kept for 25 -34 days at a temperature of 23-24^{o}C and relative humidity of 75-80 % (FAO/IAEA, 2006).

Sampling method

A simple random sampling method (lottery system) was used to begin the experimental work on flies of *G. pallidipes*.

Sex separation

Adults were separated by sex for several purposes and this was done by hand on individual flies following immobilization at +4 ^{O}C chillers. Each experimental cage that contains tsetse fly populations at first day of emergence from pupae were introduced into a chiller at +4^{o}C and allowed to chill for 5-10 minutes to inactivate (chill) the flies. The whole chilled flies in one cage were dumped on to a counting plate in the chiller. Separation of sexes was visually done by

observing at the tip of the abdomen and comparing external genitalia differences of male and female flies (http://www.raywilsonbirdphotography.co.uk/), using small brush (e.g. camel-hair brushes) to manipulate flies. Males had a pointed abdomen and the superior claspers or external genitalia were heavily pigmented but female‟s abdomen was truncated and had a pale appearance (FAO/ IAEA, 2006).

3.6. Experimental Protocol

Experiment I: Survival and Reproductive performance of female *G. pallidipes*

Emerged flies were separated into male and female, using a chiller set at +4^{o}C and female flies were kept in cages (diameter of 20 cm and width of 5 cm) with a density of 30 flies per cage with netting on top and bottom for feeding and collection of the larvae (Figure 11) **(**FAO/IAEA, 2006**)**

Figure 11. Experimental flies in cages.
Source :(Tadese, 2010).

The time of matting and the ratio of male to female required for matting were adjusted as the standard of the center**.** First 5 groups were formed each with 30 female flies per cage before

matting and then matured mating was used. Even though all groups of flies were not stay up to the end of experiment, flies were fed for 4 months at different blood sources:

Group 1: with bovine blood

Group 2: with ovine blood

Group 3: with caprine blood

Group 4: with porcine blood

Group 5: with 75% bovine blood and 25% porcine blood.

All groups of the experiment were kept for the rest of time at 75-80% relative humidity and 23-25^{o}C temperature. Feeding was done three times per week using 96% irradiated blood of the above mentioned species of animals (Feldmann, 1994b). Fecundity were expressed as the number of pupae produced per initial female and pupae produced per 10 days, taking day 18 following emergence as the first larviposition day. Pupae were collected daily beginning from days 18 up to the end of the experiment and mortality were checked daily. The pupae collected from each group were maintained at 75-80% relative humidity and 23-25^{O} C for 25-30 days (Feldmann, *et al*., 1992) and their weight were graded as indicated in annex 5. The quality of the blood diet were measured by observation of tsetse production parameters including survival, fecundity(pupae production), pupal emergency and pupal weight after converted to size (Byamungu, 2011; Zelger and Russ, 1976).The number of adult flies emerged from each group were then recorded and analyzed.

Experiment II: Comparing the survival of irradiated and non-irradiated males of *G. Pallidipes (according* to the center dose and type of irradiation)

Sex separation, the population to be kept per cage and handling were similar to the experiment I but males were kept in this experiment. There were two groups here:

Group 1: Irradiated males of *G.pallidipes*

Group 2: Non-irradiated males of *G. pallidipes*

These groups were obtained from the same batches and were fed with bovine blood and were followed up for the rest of experimental time.

Experiment III: Evaluating the Emergency rate (%) of pupae

All pupae produced within one randomly selected week were collected daily and incubated in appropriate incubation room with 75-80% relative humidity and 23-25^{o}C temperature for 25 days (Feldmann, *et al*., 1992) and after that the 25-day old pupae were put into a Petri dish under the emergence cage. Each day emerged adults were sorted in to normal, crippled, male, female and normal flies were added to the colony of the center and the remaining pupae were counted until no more adults emerged from the batch of pupae and the non-emerged pupae were examined under stereomicroscope and make a note when there were partially emerged adult flies. Lastly the emergency rate was calculated as follows (FAO/IAEA, 2006).

$$\text{Emergency rate (\%)} = \frac{\text{Total number of adults emerged}}{\text{Total number of pupae}} \times 100$$

3.7. Fly and pupae maintenance

For evaluation of the survival and productivity of non- irradiated female *G.pallidipes* on different blood diets, newly emerged 450 teneral female flies were obtained kindly from Kality Tsetse Rearing and Irradiation Center. This species were initially collected from the field in and around Arbaminch and are now being reared at Kality Tsetse fly Rearing and Irradiation Center. Ninety female flies were assigned for each experimental blood diets and kept in standard cages (diameter of 20 cm and width of 5 cm) for this species. Each experimental group had 3 replications and number of flies per cage was 30 female flies up to 7 days (Tadese, 2010). To get optimal fertility in *G.pallidipes*, flies were mated at day 7 (mature mating) with a ratio of 1:4 (male: female) and the male flies were left in the cage throughout the experimental time (Olet, *et al*., 2002). The cages were set on trolley according to the center standards (Annex 4). Dead flies were removed (mortality checked up) from the rearing cages and recorded every morning and flies % of survival was calculated every day up to the end of the research week time (Annex 5).

Collection and incubating of pupae

Each day all pupae collected from flies maintained in different blood diets were collected, counted and sort into aborted, soft, and normal pupae on the basis of their morphological features (FAO/IAEA, 2006) daily. Daily collected pupae were weighed every next day according to the center"s procedure and sorted in to 5 distinct size classes based on their weight. The pupal weight classes for *G.pallidipes* were assessed by weighing individual pupae on sensitive electrical balance (Figure 12).The weight was measured in mg unit and the pupal weight in mg was converted in to different size classes (A, B, C, D and E) based on the protocol developed by Zelger and Russ (1976) (Annex 6). These pupae were sorted in to five distinct size classes based on weight frequency as indicated by (Zelger and Russ, 1976) and pupae counted and recorded on the different weight classes (Annex 5). The well-formed pupae were incubated under standard pupal holding conditions (23-25^{o}C and 75-80% relative humidity) for the pupal periods (FAO/IAEA, 2006) in a small petridish for each daily collected pupa from different blood diets.

After this period standard emergence cages were placed on the required number of pupae until no more adults emerge from the batch of the pupae. The daily collected and emerged pupae from different diets were recorded on a format sheet (Annex 7) (FAO/IAEA, 2006).

Figure 12. Electrical sensitive balance used for weighing pupae of *G.pallidipes* at Kality tsetse fly rearing and irradiation center.

For the assessment of survival of irradiated and non-irradiated males of *G.pallidipes*, 180 male flies were taken from the center. Ninety chilled male flies for 5 minutes were irradiated in small cages containing 15 male flies per cage at a dose of 120 Gy gamma rays in air, one day after emergence (Tadese, 2010) and the rest ninety male flies were kept as control groups. After irradiation, both irradiated and non-irradiated (control group) flies were maintained on the same conditions as that of the other colonies of the center feeding on bovine blood diets and followed up till the end of the experiment. Mortality was checked in similar way as the above experimental protocol.

To evaluate the overall emergency rate of the whole *G.pallidipes* of the Arbaminch origin of the center, only one week produced pupae by the whole *G. pallidipes* colony of randomly selected

week was collected and followed up to the emergence time(FAO/IAEA,2006). Data were recorded on format sheet prepared for it (Annex 5).

Generally the experimental flies and pupae were maintained according to the center‟s rearing standard temperature(23-25 ^{O}C) and RH (75-80 %) but feeding was done 3 days per week from the beginning up to the end of the experiment for both female and male experimental groups (FAO/ IAEA 2006).

***In-vitro* feeding**

All experimental tsetse flies were kept under in vitro feeding. Silicone membranes used for the in vitro feeding of tsetse flies were used for this experiment (Bauer and Wetzel, 1976). Every feeding time, heating plates were disinfected with alcohol and feeding trays were placed on the heating plate for about 10-15 minutes after switched on the power supply. After heating, all fans, air conditioners and humidifiers were switched off and blood for each experimental group was pour on sterilized aluminum tray by lifting one end of the silicone membrane. The blood was hygienically spread using plastic roll bar to prevent contamination of the blood. The blood was allowed to warm up to body temperature (35-37 ^{O}C) and measured by using thermoscan before feeding to flies. Then all turbulences were switched on and flies were picked up from the trolley, put on to the feeding tray and allowed to feed for 10-15 minutes under off light. Then after feeding every experimental groups were turned to their trolley (FAO/IAEA, 2006). Feeding regimen was designed to be three days per week. At the end of every feeding, trays and silicone membranes had thoroughly washed and rinsed by distilled water. The membrane was placed on to a feeding tray and sterilized over night in a dry heat of 120 ^{o}C for next use (FAO/IAEA, 2006).

Mating

In the laboratory, most species of tsetse males become sexually active when about one week old and females reach sexual receptivity at about 2-3 days old. So for this reason, flies were allowed to mat in the ratio of one ten days or older male to four seven to nine days old females (1:4=male :female) for maximum insemination (Langley, 1989; Olet, *et al*., 2002) and left together for life long.

Irradiation of male *G. pallidipes*

A total of 90 males of *G. pallidipes* (1 day old) were prepared for irradiation. Then, fifteen male flies of *G. pallidipes* were placed per small cage and irradiated at 120Gy in a ^{60}Co Gamma cell 220 for 85.98 seconds. Grey (Gy) is the International System (SI) unit of radiation which equals to 100 rad (radiation-absorbed dose). The treated males were then allowed to rest for 3 hours before feeding and placed in the rearing room at a temperature of 23-24 ºC and relative humidity of 75-80 % (Feldmann, *et al*., 1992).

4. DATA ANALYSIS

Data entry and analysis were done using Microsoft Excel spread sheet and SPSS Version 15.0 respectively (SPSS, 2007). Different descriptive statistics were used to evaluate both the survival rate and survival percentage of female and the survival rate of male experimental groups. ANOVA mainly the general linear model (univariate) was used to observe the effects of the different blood diets on the average survival days and survived % of female flies. However, to see the effect of treatment (irradiation) on the average survival days of irradiated males t-test was used. Significant difference between the means was separated using Tamhane‟s and Tukey‟s honestly significant difference (HSD) test (Gomez and Gomez, 1984) and $p<0.05$ was considered significant.

5. RESULTS

5.1. Female survival and Fecundity

5.1.1. *Female survival*

The survival rate of the female *G.pallidipes* greatly varied with the type of blood supplied ($F=27.794$; $df=4,416$; $P<0.001$). The survival curve falls into two groups (Figure 13). The first group contains flies fed on bovine and ovine blood. Bovine blood and ovine blood gave average survival days of 61± (34.8) and 61± (34.8) respectively (Table 2). These values were significantly longer compared with flies fed on the other 3 types of blood diets (Tukey HSD, $P<0.05$).

The second group consists of flies fed on caprine, porcine and mixed (75% bovine and 25% porcine) blood diets. The average survival days for female flies that were reared on blood diets was 33± (19.0), 31± (17.5), and 29± (16.4), respectively. There was no significant difference in the average survival days among them. The least survival rate was recorded on flies fed on porcine blood diets, next to mixed blood diets.

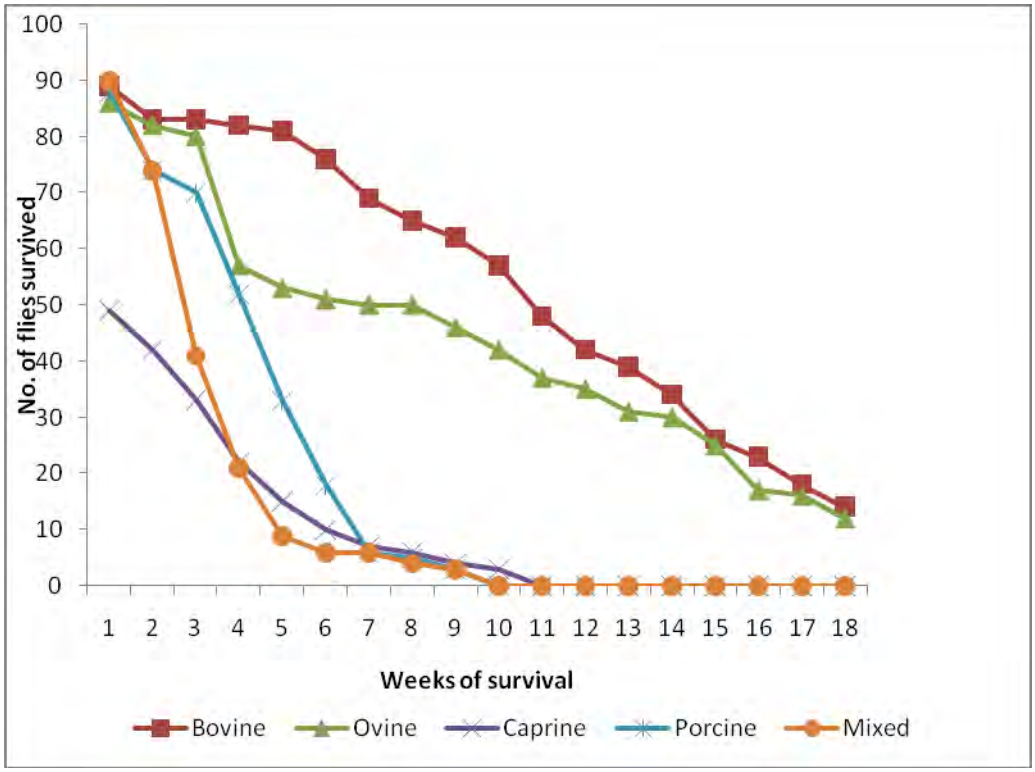


Figure 13. Weekly survival of female *G. pallidipes* on different blood diets at Kality tsetse fly rearing and irradiation center.

The overall length of survival days was also much lower for flies that were fed on blood sources from mixed (56 days), porcine (60 days), and caprine (65 days) than that were fed on bovine and ovine (120 days).

Table 2. Minimum, maximum and average survival days of female *G. pallidipes* reared under different blood diets at Kality tsetse fly rearing and irradiation center.

Blood diets	Survival days			
	Minimum survival	Maximum survival days	Average survival (days) ±SD	Std. Error of Mean
Bovine	1	120	61±34.8[a]	2.623
Ovine	1	120	61±34.8[bcd]	2.623
Caprine	1	65	33±19.0[ab]	3.564
Porcine	1	60	31±17.5[ad]	2.710
Mixed*	1	56	29±16.4[ac]	3.840

Where mixed* refers to 75% bovine and 25% porcine blood diets.

Means along the same column depicted as superscripts with the same letters are significantly different (Turkey HSD, $p<0.05$).

Furthermore, mean percentage of female flies survival among different blood groups had strong significant differences ($F= 12.497$; $df=4, 416$; $P< 0.001$). Flies maintained on bovine blood diet had higher mean percentage of survival in compared to flies fed on other blood diets (Figure 14) but there had no significant difference between flies fed on bovine and ovine blood diets ($P>0.05$). Bovine blood diet had strong significant difference on mean percentage of female flies‟ survival in compare to the rest blood groups ($P<0.001$) but ovine blood diet didn‟t show any significant difference from flies fed on porcine blood diet ($P>0.05$).

The least mean percentage of survival had recorded in flies fed on caprine blood diets. There was no significant difference between flies fed on caprine and mixed, and mixed with porcine blood diets but there was significant difference between caprine and porcine blood die.

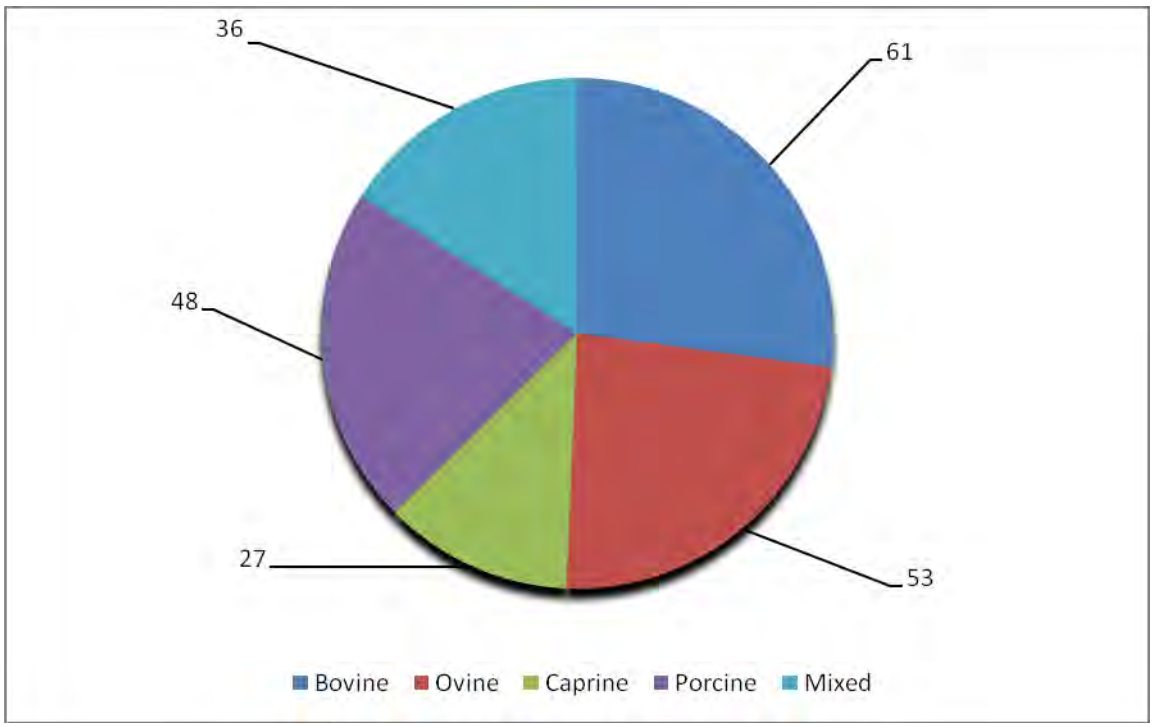


Figure 14. Mean percentage of the number of female *G. pallidipes* flies survived maintained under different blood diets at Kality tsetse fly rearing and irradiation center.

5.1.2. Female Fecundity

Pupae per initial female (PPIF): The PPIF of flies maintained on bovine, ovine, caprine, porcine and mixed blood diets was found to be 3.31, 2.77, 0.08, 0.53 and 0.3, respectively. Flies maintained on bovine blood diets had higher production of pupae in compare to other blood diets but no significant differences in fecundity between bovine and ovine blood diets. Moreover, flies maintained on bovine and ovine blood were produced significantly more pupae than those fed on caprine, porcine and mixed blood diets (Tukey HSD, $P<0.001$) but the rest had no any significant difference among them. A mixture of bovine and porcine blood diets diminished pupal production next to caprine blood when compared to bovine, ovine and porcine blood.

Total pupal production: Overall higher production of pupae was recorded on flies fed on bovine and ovine blood diets but in flies fed on caprine, Porcine and mixed was extremely lower with least in caprine (Figure 15).

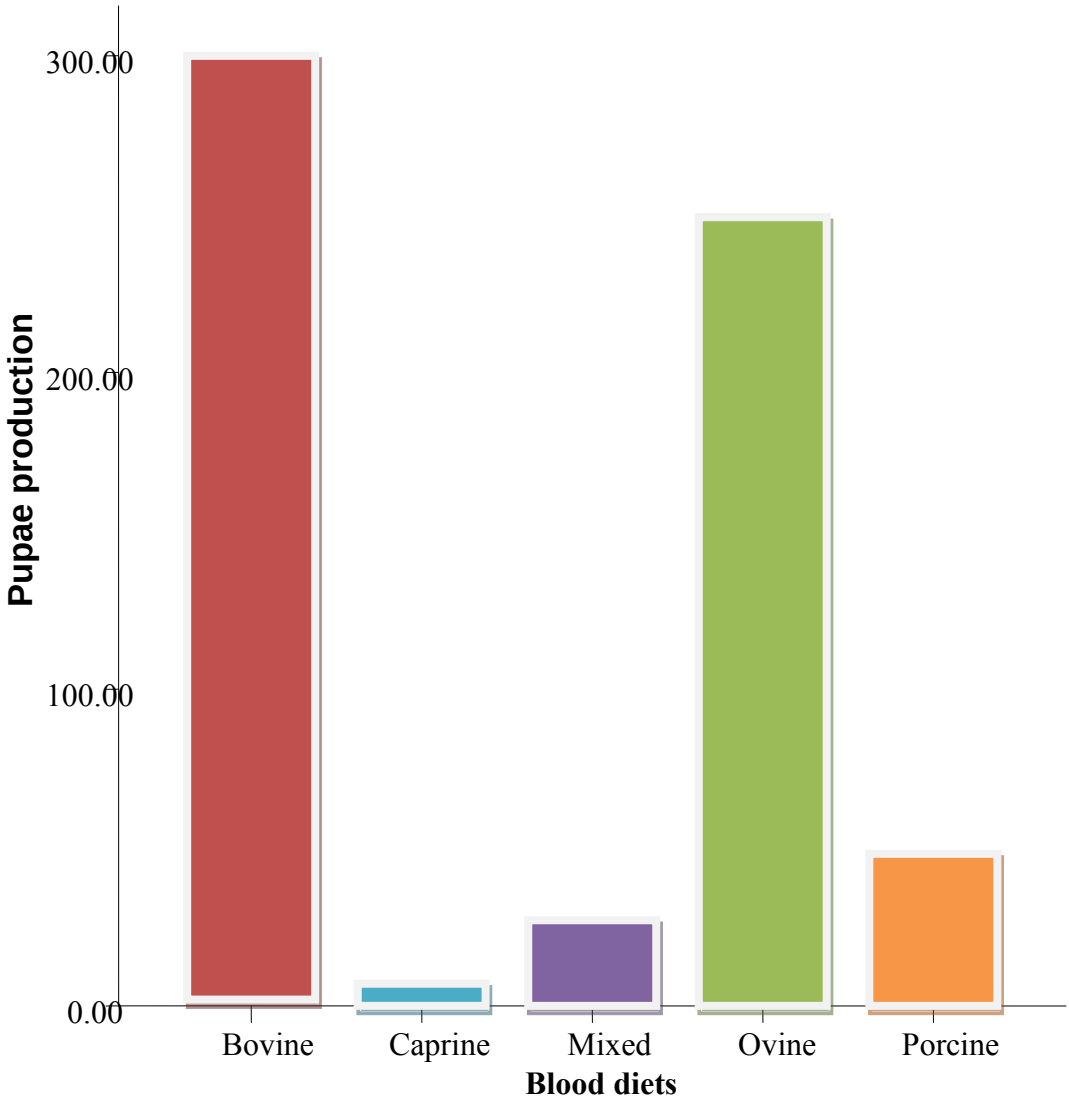


Figure 15. The overall Pupal production of female *G.pallidipes* on different blood diets during the survival days at Kality tsetse fly rearing and irradiation center.

Pupae per female per 10 days (P/F/10d): The fecundity of flies that survived in each blood group was expressed as follow for each of survival week as number of pupae per female per 10 days (P/F/10 days) (Table 3).

The fecundity of survived flies was found to be slightly higher in bovine and ovine blood diets, however, it was extremely lower in the rest blood diets but not significantly different ($F=0.611$; $df=2, 1$; $p>0.05$).

Table 3. Weekly production of pupae per female of *G. pallidipes* per 10 days in different experimental blood diets at Kality tsetse fly rearing and irradiation center.

Blood diets	Weeks													
	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Bovine	0.1	0.76	0.59	0.51	0.64	0.56	0.54	0.59	0.37	0.37	0.43	0.34	0.26	0.08
Ovine	0.45	0.45	0.59	0.40	0.62	0.73	0.4	0.4	0.59	0.51	0.54	0.37	0.18	0.10
Caprine	--	0.12	0.10											
Porcine	0.10	0.31	0.31	0.19	0.09	0.16								
Mixed	0.03	0.23	0.17	0.14	0.48	0.71								

Pupal classes: The percent of pupal classes in weight of pupae produced by the different flies maintained on different blood diets was found to be as follow (Table 4).

Table 4. Size classes of pupae on basis of weight (mg) produced by *G. pallidipes* maintained under different blood diets for about 18 weeks at Kality tsetse fly rearing and irradiation center.

Blood diets	Pupal classes (%)				
	A	B	C	D	E
Bovine	1.34	6.04	18.46	38.6	35.6
Ovine	1.6	4.1	10.2	21.1	63
Caprine	0	28.57	28.57	28.57	14.29
Porcine	6.3	12.5	18.8	20.8	41.7
Mixed	3.7	3.7	14.8	25.9	51.9

Pupal classes represented by letters A to E indicate size in ascending order where A refers to small pupae while E refers to large pupae.

Pupal quality as measured by weight and converted to size classes showed that the distribution of the pupae (%) in the different size classes produced by females *G.pallidipes* maintained on different diets was found to be different in all blood diets but not statistically significant. The highest percentage of pupal quality class (class E) was recorded on ovine and mixed blood diets than other blood diets (Table 4). The value was 63% for ovine and 51.9% for mixed blood diets. However, the difference was not statistically significant by the Tukey HSD test ($P>0.05$). This implies that, flies fed on ovine blood diets gave high quality of pupae than flies fed on other blood diets including bovine, porcine and mixed blood diets. However, mixed blood diets had a superior effect on the production of quality pupae than bovine and porcine blood alone.

Unlike flies fed on the other blood diets, flies fed on caprine blood diets had produced very low high quality pupae (class E) than other blood diets. Lastly, the inferior quality (class-A) was in the normal range which was < 10%.

Emergency rate of the newly produced pupae and percentage of emerged females

The overall emergency rate of newly produced pupae in different experimental blood diets was found to be 88.9, 74.2, 73.9, 64.6 and 42.9% in flies fed on mixed, bovine, ovine, porcine and caprine blood, respectively. In the present study, the highest emergency rate was recorded in pupae produced from flies fed on mixed blood diet, followed by pupae produced from bovine and ovine blood diets respectively but there were not significant differences among them. The values were almost similar in pupae collected from flies that had fed on bovine and ovine blood diets. The least emergency rate was recorded in caprine blood diets and there was slightly significant difference in compared to mixed blood diets ($P<0.05$) but was not significantly different from the rest blood diets. The percentage of emerged female flies was found to be slightly higher in most experimental groups except in pupae produced by flies fed on bovine blood which was slightly lower than 50% and in mixed blood diets was extremely lower (29.2%) (Table 5).

Table 5. Emergency rate and sex ratio of *G. pallidipes* pupae produced from females fed on different blood diets at Kality tsetse fly rearing and irradiation center.

Blood diets	Total number of pupae collected	Total emerged adult female flies	Total emerged adult male flies	Total emerged adult flies (Female and Male)	Total emergency rate (%)	Emergency rate of females (%)
Bovine	298	107	114	221	74.2	48.4
Ovine	249	100	84	184	73.9	54.3
Caprine	7	2	1	3	42.9	66.7
Porcine	48	17	14	31	64.6	54.8
Mixed	27	7	17	24	88.9	29.2

5.2. Survival of irradiated and non-irradiated male tsetse flies

The average life span of irradiated male *G.pallidipes* with a dose of 120 Gy gamma rays was found to be 37.4±21.1 days but that of the Non-irradiated (control) group was 59.2±34.4 days (Table 6). Treating males with 120 Gy caused a strong significant reduction in their average survival days (t-test, $P<0.001$). Generally, the numbers of survived flies fall below 50% within 43 days in irradiated male flies but within 73 days in non-irradiated male flies.

Table 6. Comparison of the average survival days of irradiated and non-irradiated males of *G. pallidipes* at Kality tsetse fly rearing and irradiation center.

Experimental groups	Survival days	
	Mean±SD (Days)	Maximum
Irradiated Male flies	37.4±21.1	72
Non-irradiated Male flies	59.2±34.4	116

5.3. Emergency rate of the whole colony of Kality tsetse fly rearing and irradiation center for *G. pallidipes* of Arbaminch origin.

The overall emergency rate of the *G. pallidipes* colony was found to be 88% and the female to male ratio was approximately 1:1 (Table 7).

Table 7. Emergency rate and sex ratio of *G. pallidipes* pupae (Arbaminch origin) produced from females of the whole colony Kality tsetse fly rearing and irradiation center.

	Total no. of pupae collected per day	No. of Females Emerged	No. of Males emerged	Total (male and female)		Emergency rate (%)	Female : Male (ratio)
	515	241	236	477		92.6	1.02
	555	227	271	498		89.7	0.84
	406	187	167	354		87.2	1.1
	383	162	203	365		95.3	0.8
	410	168	167	335		81.7	1.0
	491	214	195	409		83.3	1.1
	495	225	202	427		86.3	1.1
Total	3255	1424	1441	2865	**Average**	88%	0.99

Out of 3255 pupae collected per one week, a total of 1424 females and 1441 males were emerged. The overall emergency rate in the one week time was found to be 88% obtained by dividing the total number of adults emerged (2865) out of pupae collected per week by total number of pupae collected per week (3255) multiplied by 100.

6. DISCUSSION

Tsetse flies (*Glossinidae*) are important vectors for transmission of the parasites trypanosome causing a disease known as Nagana among domestic animals and sleeping sickness among human (Vreysen, 2001) has made them one of the most devastating insect in Africa in general and in Ethiopia, in particular.

The present study was performed to evaluate the survival rate, mean survival percentage and reproductive performance of non- irradiated females of *G. pallidipes* maintained on bovine, ovine, caprine, porcine and 75% bovine to 25% of porcine fresh frozen blood. According to the present study, female *G. pallidipes* fed bovine and ovine blood diets showed higher average survival days than flies fed on other blood types. By contrast, studies carried out in other tsetse fly species, *Stomoxys calcitrans* Linneaus and horn flies which were also obligate blood feeders, had shown great differences in average survival days among flies reared on cattle or other types of blood, such as pig, horse, sheep, goat, chicken or rabbit (Suenaga, 1965; Ohshio, 1965; Bailey and Meifert, 1975; Doutoit, 1975; Langley, 1977; Sutherland, 1978; Kuramochi, 2000).

On the other side, the average survival days of female flies fed on porcine, caprine and mixed blood was found to be lower than the flies fed on bovine and ovine blood diets (Table, 2). Generally these values were found to be below the average survival days of female *G. pallidipes* that had been reported to be 35 days or more in the field level (FAO, 1982). This contradicts with the findings on *G. m. morsitans* and *G. p. palpalis* fed on defibrinated bovine, equine or porcine blood which was reported to be equal (Wetzel and Luger, 1978). The low average survival rate in case of caprine blood may be due to the differences in host specificity of *G. pallidipes* and a range of physiological adaptations to the specific host blood feeding. However, in Porcine and

mixed blood diets it may be due to degradation in blood quality because of bad storage conditions (repeated freeze-thaw cycle) and the inconsistent maintenance of deep freeze by the refrigerators, mat contamination and easily perishable nature of porcine blood than the other blood diets may

favor multiplication of the very few residual bacteria post laboratory screening and this could easily affect the survival of these flies. Furthermore, there may be some non-specific chemicals (drug and insecticides residues) in the caprine/porcine blood diets in low concentration that can kill easily the fertile females (Nogge, 1976; Langley and Roe, 1984; FAO/IAEA, 2006).

On the top of that, in the present study female *G. pallidipes* maintained on irradiated fresh frozen bovine blood had better mean percentage of survival (61%) compared to other experimental blood diets. However, this was found to be lower compared with the data obtained on *G. pallidipes* (85.8%) and *G. austei* (75.5%) fed on similar bovine blood diets (FAO /IAEA, 1997) but it was almost similar with the work of Byamungu (http://au-ibar.org/) on effect of male: female ratios (1:4) on performance of *G. austeni* which was found to be 63.75%.

Nevertheless, previous study showed that porcine blood alone or mixed 75% bovine and 25% porcine fresh frozen irradiated blood to have a better or equal effect on mean survival percentage of female *G. pallidipes* and *G. austeni* as that of irradiated frozen bovine blood *(*FAO/IAEA, 1997). On the contrary, the present finding showed an inferior effect of this blood diet on the mean survival percentage of female *G. pallidipes* in comparison with bovine and ovine blood diets but better than caprine blood diets. This may be due to degradation in blood quality of porcine/ mixed blood diet because of bad storage condition (repeated freeze-thaw cycle) and the inconsistent maintenance of deep freeze by the refrigerators, mat contamination and easily perishable nature of porcine blood than other blood diets may favor multiplication of the very few residual bacteria and this could easily affect the survival of these flies.

Unlike the survival rate, female *G. pallidipes* flies fed on caprine blood noticed to have the least mean percentage of survival (27%). Majority of dead female flies in this experimental study were found to be with large amount of undigested blood in their abdomen (blood type death), such deaths are claimed to be due to too high relative humidity or insufficient aeration in the breeding room, excessive diet ingestion, in vitro feeding membranes too hot, microbial contamination and toxicity / drug residues in the host animal feed such as ivermectin (Nogge, 1976; Langley and Roe, 1984; FAO/IAEA, 2006). However, among others, the death in this experiment most likely

to be due to *in vitro* feeding membranes too hot, microbial contamination (mal functioning of the refrigerators to maintain deep freeze and mat contamination) and toxin such as aflatoxins/ insecticides/ivermectins in the host animal from were blood diets were collected. It might be also due to the fact that *G. pallidipes* female flies couldn‟t prefer to feed on goat blood both at the field level (ancestors) or insectary (experimental flies) in ideal ecology. This might also explain the reason why prevalence of trypanosomosis among goats at field level is lower than other domestic animals which are the first preferable hosts to *G. pallidipes* such as bovidae. For instance, in Ethiopia the prevalence of trypanosomosis in goats was found to be lower up to 3.5 % according to study conducted by (Hunduma, 2003) in Didessa and Ghibe valley of Ethiopia and it was also reported to be 0.4% by Lemecha, *et al.* (2002). However, in this experiment they may forced to take the blood due to abscence of choice but they couldn‟t have enzyme that digest caprine blood diets, as a result they die.

Another possible reason for the observed lower mean percentage of survival of female flies of *G. pallidipes* may be attributed to some toxic plant derivative residues which could present in caprine blood owing to the browsing nature of the animals on different leaves of plants. It may also be associated with the recent treatment of the animals with drugs like ivermectin which was claimed to cause 100% mortality in *G. m. morsitans* in fertile female flies even in low doses (>1.6 μg ml^{-1}) (Langley and Roe, 1984).

It is well known that due to their adenotrophic viviparity nature, tsetse flies have a very low reproductive capacity, closer to that of small mammals than to most insects (Leak, 1999). For a colony to survive, the minimum PPIF should be above 2.1 for 13 weeks (Opiyo, *et al*, unpublished). However, Pupal production below 3 per initial female results in no effective colony growth, or even decline (IAEA, 2003). In the present study the fecundity of female *G. pallidipes* expressed as the number of pupa per initial female was found to be higher in flies fed on bovine blood (PPIF=3.31) than flies fed on other blood diets. However, the PPIF female *G. pallidipes* fed on bovine blood was almost comparable to that of ovine blood fed flies (PPIF=2.77). The PPIF required standard value for self colony maintenance among female flies of *Glossina* species fed on bovine blood is above 2.1 (Opiyo, *et al*., unpublished). Thus, from the results of the

present study, the PPIF value of flies fed on bovine and ovine is too higher than the standard. Similarly, it was found out that the PPIF for *G. p. palpalis* reared *in vitro* on fresh guinea-pig blood to be 3.0 (Deloach and Taher, 2009), this was in lined with the PPIF value of flies maintained on bovine blood diets. In agreement with the present finding of PPIF value of 2.77 for *G. pallidipes* maintained on ovine blood, similar result was found for *G. pallidipes* and *G. Austeni* with PPIF values of 2.51, 2.79 maintained on fresh frozen bovine blood, respectively (FAO/IAEA, 1997).

On the other hand, the PPIF value for *G. austeni* was reported to be 4.71 (FAO /IAEA, 1997). This lower PPIF value as revealed in the current study by *G. pallidipes* (Arbaminich origin) fed on bovine and ovine blood diets as compared to *G. austeni* may be linked to the high prevalence of salivary gland hypertrophy virus (SGHV) in the insectary which was reported to be more than 85% (Abd-Alla, *et al*.,2007). This virus highly diminished the productive capacity of tsetse flies. Besides, the variation in the value of PPIF between *G. pallidipes* (Arbaminich origin) and *G. austeni* may also be associated with species differences in reproduction capacity.

On the contrary, the PPIF of *G. pallidipes* flies maintained on porcine, mixed and caprine blood was found to be below 2.0 which was far below the requirements for self sustaining colony maintenance of a given tsetse insectary. This agrees with the PPIF reported on generation F3 *G. austeni* and *G. pallidipes* fed on reconstituted freeze-dried bovine blood (Opiyo, *et al*., un-published). However, the PPIF of flies maintained on mixed fresh frozen bovine: porcine blood (75%: 25%), caprine and porcine disagrees with other reports on the same species of flies fed on porcine blood (FAO/IAEA, 1997). This may be due to the high daily mortality rate occurred on flies fed on these blood diets which lowers the number of productive flies and productivity.

In addition to the above it may be due to the presence of drug residue in the animals‟ blood such as coccidiostats, antibiotics and/ or presence of some toxins such as aflatoxin/insecticides may adversely affect the fecundity of the female flies (Nogge, 1976). The antibiotics applied against bacteria residing in the tsetse equally affects also the flies leading to reduction in productivity. Parental females as well as females which emerged from larvae deposited by these flies (F1-

generation) 6 days after the administration of the drug to the pregnant females showed a similar loss in productivity. This corresponds with a degeneration of mesenteric symbionts (Wetzel and Thiemann, 1979). In the present study flies that had reached the production period, produced very low numbers of pupae as compared to the surviving number of flies fed on caprine blood diet. This showed that the caprine blood diet highly reduces *G. pallidipes* (Arbaminch origin) female fly production. This may be due to low nutritional composition of caprine blood for *G. pallidipes* but this may need further verification.

Another method for assessing the fecundity of tsetse flies is the number of pupae per female per 10 days (P/F/10d). According to the present study, a relatively higher fecundity (P/F/10d) was found on flies fed on bovine and ovine blood diets (Table 3). This was almost in agreement with the minimum average fecundity standard value set by FAO/IAEA (2006) for the production weeks. However, as age increased the P/F/10 days was found to decrease. This may be due the effect of age on survival and productivity of tsetse flies. On the other side, it was found that the P/F/10 days were extremely lower in porcine, caprine and mixed blood diets throughout the productive time. These obtained values were disagreed with the standard value (0.6) required to maintain tsetse colony (FAO/IAEA, 2006). This variation can be explained with the same reasons as mentioned for that of the PPIF. Specifically, high daily mortality and the presence of drug residue/toxins in the host animals‟ blood may be among the important factors.

In order to maintain a colony at a constant size each female invested in to the colony should not only have a good fecundity potential rather the proportion of poor pupal quality (class A) pupae and emergency rate should remain at acceptable levels (Opiyo, *et al*., unpublished; FAO/IAEA, 2006).

The present study agreed with the above idea in that the class “A” pupae in all blood diets were found to be in the acceptable range which was less than 10% (FAO/IAEA, 2006). On the other hand, in the present study, the highest percentage of pupal quality class (class E) was recorded in ovine and mixed blood diets than other blood diets. This implies that flies fed on ovine blood yield the highest quality of pupae than flies fed on other blood diets including bovine, porcine

and mixed blood diets. This may be due to presence of better nutritive value in ovine blood diets that supports the development of large weighed pupae and hence size of pupae as well. However, mixed blood (75% bovine: 25% porcine) diets had a superior effect on the production of quality pupae than bovine and porcine blood alone. This agreed with the report of Opiyo, *et al.* (Unpublished) on female *G. pallidipes* maintained on mixed blood diets to have higher % of large pupae (Class E) than produced by females maintained on bovine blood alone. The least E class pupae were found on flies that had maintained under caprine blood diets. This may be due to low nutritional value of caprine blood to *G.pallidipes* (Arbaminch origin) resulting in lower sized pupae.

As mentioned above, good fecundity, production of acceptable level of poor size class pupae and emergency rate of adult tsetse flies are the main important factors necessary to maintain a colony at a constant size (FAO/IAEA, 2006).

In the present study, the highest emergency rate was recorded in pupae produced from flies fed on mixed, followed by bovine and ovine blood diets, respectively. The emergency rate of the pupae produced by flies maintained on mixed blood diets had only slight difference with the present finding on the whole *G. pallidipes* (Arbaminch origin) colony of the center (Table 7).

This was slightly higher than the minimum standard emergency rate required in mass rearing tsetse flies which was described to be above 85% (FAO/IAEA, 2006). However, it disagreed with the previous result reported on the same species by FAO/ IAEA (1997, 2002) which had a value of 46.36%, 64% for each production years, respectively. The reason why flies maintained under mixed blood diets had a better emergency rate than flies fed on other blood diets may be due to the large percentage of high quality pupae (class E) production by flies maintained under mixed blood diets than in other blood diets except in ovine blood. In addition to that, females under mixed blood diets were stay only for short time and produced these quality pupae while they were young. This may be due to the Large sized and heavily weighed pupae resulted in high emergency rate than the small sized and lightly weighed pupae (FAO/IAEA, 2006) produced by other blood diets. In addition to the above reason pupae produced by young female flies may

have a better chance of emerging than pupae produced by old female flies. However, in ovine blood diets the large percentage of high quality class pupae were produced by both young and old female flies those were kept for long time. These pupae produced by old flies may have very low emergency rate and hence this reduced the overall emergency rate of the pupae in ovine blood diets than that of the mixed blood diet. Unlike the finding on mixed blood diets, the emergency rate of pupae collected from flies fed on caprine and porcine blood was found to be almost similar with the above FAO/IAEA (1997, 2002) reports, respectively (Table 5). The emergency rate of pupae collected from flies that had fed on bovine and ovine blood diets were almost similar. This agreed neither with the present emergency rate finding of the whole colony of the center nor with the past reports of FAO/IAEA (1997, 2002). Moreover, these values were below the minimum emergency rate required standard set by FAO/IAEA (2006). Up on dissecting all non-enclosed pupae showed the presence of wasp parasites in few fully developed adults. This may have a certain impact in reducing the emergency rate of these pupae. In addition to the above reason, there may be a seasonal variation in pupal emergency rate related to different factors.

The percentage of emerged female flies was found to be slightly higher in most experimental groups except in pupae produced by flies fed on bovine blood which was slightly lower than 50% and in mixed blood diet was extremely lower (29.2%). From the results of the present study, except bovine and mixed diets, all others were found to be in accepted range which was reported to be close to 50% (FAO, 1982). The deviation of the percentage of female emergency on flies maintained on bovine and mixed blood diets may be due to genetic variation in individual flies or associated to some procedural effects (FAO/IAEA, 2006).

Previous studies indicate that, radiation may be used as genetic control involving the release of irradiated sterile males so that most of the wild females will lay sterile eggs (Hasan and Khan, 1998). The somatic damage caused by the irradiation of adult males is expressed by a reduction in average longevity compared with non-irradiated males (Vreysen, *et al*. 1995). The present laboratory based results showed that irradiation of male *G. pallidipes* at a sterilization dose of 120 Gy for *G. pallidipes* (Opiyo, *et al*., unpublished) significantly reduced the average life span of male flies (37 days) as compared to non-irradiated (control) males (59 days). However, the

average survival days of irradiated males remained above 35 and this was comparable with data obtained for *G. tachinoides*, *G. f. fuscipes* and *G. austeni* males treated with 120Gy, 80-100Gy and 120Gy, respectively (Vreysen, *et al*., 1992; 1995).

In contrast to the present findings, radiation induced increase in average life span in male flies of *G. brevipalpis* treated with doses of 10 to 40 Gy (Vreysen, *et al*., 1995). This can be due to the lower doses of irradiation were resulted in no significant damage of somatic cells of the flies and hence no significant differences in their life span. Furthermore, Dean and Wortham (1969) observed an increase in average longevity of *G. morsitans* males treated in the pupal stage with doses ranging from 10 to 20 Gy and 120 to 150 Gy. Likewise, an increase of male mean longevity was found for pupae irradiated in nitrogen with 70 Gy in the late pupal phase (Curtis & Langley, 1972). These observations contradict with the present result. The somatic damage caused by treating pupae with 120 - 150 Gy in air seems substantial and Dean and Wortham's observations cannot be explained by the present result. This radiation induced increase in survival of Diptera species has been attributed to several phenomena as (i) the apparent absence in this group of somatic cell renewal (Ducoff, 1972), (ii) a significant elimination of contaminating micro-organisms (Atlan, *et al.,* 1970), and (iii) the fact that certain physiological processes are slowed down by the radiation and/or that repair mechanisms in the insects are stimulated sufficiently by the slight destruction of certain tissues incurred by the radiation (Cork, 1957).

In the present study, the overall emergency rate of *G.pallidipes* of the Arbaminch origin of kality tsetse fly rearing and irradiation center was found to be 88%. This was higher than the previous result reported on the same species by FAO/IAEA (1997, 2002) which had a value of 46.36% and 64%, respectively. Similarly, the overall emergency rate of *G. pallidipes* of the Arbaminch origin of kality tsetse fly rearing and irradiation center was higher than the present results found on the experimental groups maintained on different blood diets which was found to be 74.2, 73.9, 64.6 and 42.9% in flies fed on bovine, ovine, porcine and caprine blood, respectively. However, it was slightly different from the present findings particularly on flies that were maintained on mixed blood diet which had a value of 88.9%. The female to male ratio was approximately 1:1. This was in lined with the finding in FAO/IAEA (2006) and FAO (1982).

7. CONCLUSIONS AND RECOMMONDATIONS

The sterile insect technique is one area wide insect pest management method where the insect pest is controlled or eradicated by affecting its reproductive capacity. This technique relies on the production in large numbers of the target insect in mass-rearing facilities, sterilization of one sex and the release in sustained numbers in the natural habitat large enough to outnumber the wild pest population. To support the already established program in Ethiopia a research has been conducted on survival and fecundity of *G. pallidipes* (the target fly for SIT) maintained on different blood diets.

The present study disclosed a relatively highest survival rate and fecundity in bovine and ovine blood diets. Furthermore, flies maintained on bovine and ovine blood diets had higher mean percentage of survival than flies fed on other blood diets. The least mean percentage of survival was found in flies fed on caprine blood diets. According to the present study, overall higher production of pupae was recorded on flies fed on bovine and ovine blood diets. However, in mixed blood diets pupal production was found to be lowest next to caprine blood. Moreover, the highest percentage of pupal quality class (class E) was recorded in flies fed on ovine blood diets followed by mixed blood diets. But, the least was recorded on flies fed on caprine blood diets. The inferior quality (class A) was in the normal range, which was < 10% in all blood diets. The overall emergency rate of newly produced pupae in different experimental blood diets was found to be higher on flies fed on mixed blood diets. However, the least emergency rate was recorded in caprine blood diets. In the present study the percentage of emerged female flies was found to be slightly higher than 50% in all blood diets except in bovine and mixed blood diets. The overall emergency rate of the whole colony *G. pallidipes* of Arbaminch origin in the one week time was found to be 88% and the female to male ratio was almost 1:1. Thus the present research work brought vital information about mass rearing of tsetse flies in kality and alternative feed sources for enhancing SIT not only to Ethiopia but also to whole African countries which are in progress to establish SIT. In the present experimental study it was found that irradiation of male *G. pallidipes* with the sterilizing dose of 120 Gy, highly reduced the average life span of irradiated males compared to non-irradiated males.

In order to strengthen the fight against tsetse and trypanosomosis, such a detailed study on the biology, survival and emergency rate of the major vectors like *G. pallidipes* should be carried out and assessed. Moreover, field adaptability studies before the actual implementation of SIT is mandatory.

Therefore, based on the above highlights, the following recommendations are forwarded:

- The overall survival rate and fecundity of *G. pallidipes* maintained on ovine blood was found to be better or the same with flies fed on bovine blood. Hence, the center may alternatively adopt the ovine blood in order to assure better production.

- The life span of irradiated males in laboratory was highly reduced; this may also be exacerbated under field conditions. This indicates that irradiated males have lesser chance to stay alive and inseminate wild female flies. Therefore, this situation should be emphasized and considered in SIT programs and field survival studies must be conducted.

- Even though the overall emergence rate of the whole colony of Arbaminch origin *G. pallidipes* was found to be in the acceptable range of value, the emergence rate of pupae collected from the different experimental blood diets was below the standard. This particular condition may be associated with seasonal rise in papal parasites like wasp. Thus, further study should be conducted to assess the effect of wasp and if any, other pupal parasites at the center.

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9. ANNEXES

Annex 1. Record sheet for tsetse blood diet collection and processing (Modified from the IAEA/FAO standard operating procedures for mass-rearing tsetse flies (2006).

Blood code	Date collected	Storage	Test no.	Date and Year
FBB				
FOB				
FCB				
FPB				
Mixed				
Date processed				
MICROBIAL SCREENING				
Diet before $^{\gamma}$		Diet after$^{\gamma}$		
CFU/ml		CFU/ml		

Where: FBB=Frozen bovine blood

FOB=Frozen ovine blood

FCB= Frozen caprine blood

FPB=Frozen porcine blood

Mixed=75% bovine blood: 25%Porcne blood

Annex 2. Monthly radiation output for bulk blood decontamination of the center.

Month	A=initial Mon Kgy/hr	B=Decay Factor	C=A*B Month Kgy/hr	Sec @ 0.5 Kgy/hr	Sec @ 1.0 KG/hr	Sec @ 1.5 Kgy/hr	Remark
Jan-09	19.391	0.3764	7.30	246.6	493.2	739.9	
Feb-09	19.391	0.3723	7.22	249.3	498.7	748.0	
Mar-09	19.391	0.3682	7.14	252.1	504.2	756.3	
Apr-09	19.391	0.3642	7.06	254.9	509.8	764.6	
May-09	19.391	0.3602	6.98	257.7	515.4	773.1	
Jun-09	19.391	0.3563	6.91	260.5	521.1	781.6	
Jul-09	19.391	0.3524	6.83	263.4	526.8	790.2	
Aug-09	19.391	0.3485	6.76	266.4	532.7	799.1	
Sep-09	19.391	0.3447	6.68	269.3	538.6	807.9	
Oct-09	19.391	0.3410	6.61	272.2	544.4	816.7	
Nov-09	19.391	0.3372	6.54	275.3	550.6	825.9	
-09Dec	19.391	0.3336	6.47	278.3	556.5	834.8	
Jan-10	19.391	0.3299	6.40	281.4	562.8	844.1	
Feb-10	19.391	0.3263	6.33	284.5	569.0	853.4	
Mar-10	19.391	0.3228	6.26	287.6	575.1	862.7	
Apr-10	19.391	0.3192	6.19	290.8	581.6	872.4	
May-10	19.391	0.3157	6.12	294.0	588.1	882.1	
Jun-10	19.391	0.3123	6.06	297.2	594.5	891.7	
Jul-10	19.391	0.3089	5.99	300.5	601.0	901.5	
Aug-	19.391	0.3055	5.92	303.9	607.7	911.6	

10						
Sep-10	19.391	0.3022	5.86	307.2	614.3	921.5
Oct-10	19.391	0.2989	5.80	310.6	621.1	931.7
Nov-10	19.391	0.2956	5.73	314.0	628.1	942.1
Dec-10	19.391	0.2924	5.67	317.5	634.9	952.4
Jan-11	19.391	0.2892	5.61	321.0	642.0	962.9
Feb-11	19.391	0.2860	5.55	324.6	649.1	973.7
Mar-11	19.391	0.2829	5.49	328.1	656.3	984.4
Apr-11	19.391	0.2798	5.43	331.8	663.5	995.3
May-11	19.391	0.2768	5.37	335.4	670.7	1006.1
Jun-11	19.391	0.2737	5.31	339.2	678.3	1017.5
Jul-11	19.391	0.2708	5.25	342.8	685.6	1028.4
Aug-11	19.391	0.2678	5.19	346.6	693.3	1039.9
Sep-11	19.391	0.2649	5.14	350.4	700.8	1051.3
Oct-11	19.391	0.2620	5.08	354.3	708.6	1062.9
Nov-11	19.391	0.2591	5.02	358.3	716.5	1074.8
Dec-11	19.391	0.2563	4.97	362.2	724.4	1086.5
Jan-12	19.391	0.2535	4.92	366.2	732.4	1098.5
Feb-12	19.391	0.2507	4.86	370.3	740.5	1110.8
Mar-12	19.391	0.2480	4.81	374.3	748.6	1122.9
Apr-12	19.391	0.2453	4.76	378.4	756.8	1135.3
May-12	19.391	0.2426	4.70	382.6	765.3	1147.9
Jun-12	19.391	0.2400	4.65	386.8	773.6	1160.3
Jul-12	19.391	0.2373	4.60	391.2	782.4	1173.5

Aug-12	19.391	0.2347	4.55	395.5	791.0	1186.5
Sep-12	19.391	0.2322	4.50	399.8	799.5	1199.3
Oct-12	19.391	0.2296	4.45	404.3	808.6	1212.9
Nov-12	19.391	0.2271	4.40	408.7	817.5	1226.2
Dec-12	19.391	0.2247	4.36	413.1	826.2	1239.3

Annex 3. Preparation and labeling of tsetse flies blood diets from different blood source under UV- light Sterilized hood.

Annex 4. Setting of the experimental groups of *G. pallidipes* on trolley during experimental times.

Annex 5. Data collection format to study the survival and fecundity of *G. pallidipes* at Kality tsetse rearing and irradiation center.

Weekly summary		Blood code: FOB						Pupae production status		
Week	No. of flies	Mortality			Total surviving flies at the end of the week	Daily mortality Percentage	Survival rate	Normal	Soft	P/F/10 days
		Starved	Bloody	Total						
1	90									
2										
3										
4										
5										
6										
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										

Annex 6. Definition of pupal weight classes for different species of *Glossina*.

Species	Weight class (mg)				
	A	B	C	D	E
G.austeni	<16	16-<19	19-<21	21-<23	>23
G.tachinoides	<14	14-<17	17-<19	19-<21	>21
G.palpalis	<22	22-<28	28-<32	32-<36	>36
G.pallidipes	<23	23-<29	29-<33	33-<37	>37
G.fuscipes	<22	22-<28	28-<32	32-<36	>36
G.brevipalpis	<56	56-<68	68-<76	76-<84	>84
G.morsitaans	<18	18-<22	22-<26	26-<30	>30

Key ‘‘-<’’: refers to “less than or equal to”

Source: Zelger and Russ (1976).

Annex 7. Daily record sheet for pupae production, emergency and emerged sexes.

Experimental group	Larvi-position date (L.P.D)	Emergency date	♀	♂
FBB				
FOB				
FCB				
FPB				
Mixed				

10. CURRICULUM VITAE

PERSONAL INFORMATION

- Name — Assefa Kebede (Dr.)
- Date of birth — September 1983 G.C.
- Birth place — Samre, Tigray
- Sex — Male
- Marital status — Single
- Religion — Orthodox Tewahdo
- Nationality — Ethiopian
- Profession — Veterinarian
- Occupation — Lecturer
- Contact address: — Mobile: 0922462581
  E-male: assefakebede82@yahoo.com

EDUCATIONAL BACKGROUND

- 2012: **Tropical Veterinary Parasitology and Pathology** from Addis Ababa University College of Veterinary Medicine and Agriculture, Debre Zeit, Ethiopia.
- 2002/2003-2006/2007: Addis Ababa University Faculty of Veterinary Medicine, Debre Zeit, Ethiopia
- 2001/2002: Addis Ababa University, Science Faculty, Arat Kilo.
- 1999/2000-2000/2001: Atse-Yohaness High School, Mekelle, Tigray
- 1998/1999: Meserete Secondary School
- 1997/1998: Fre-Abyot Secondary School
- 1990 /1991– 1996/1997: Samre Elementary School

RESEARCHES

Second Degree:

- **MSc-Thesis**: ASSESSMENT OF SURVIVAL AND REPRODUCTIVE PERFORMANCE OF *Glossina pallidipes* MAINTAINED UNDER BLOOD OF DIFFERENT ANIMALS AT KALITY TSETSE REARING AND IRRADIATION CENTER
- **MSc-Seminar:** A REVIEW ON STERILE INSECT TECHNIQUE (SIT) AND ITS POTENTIAL TOWARDS TSETSE ERADICATION IN ETHIOPIA.

Fist Degree:

- **DVM-Thesis**: A RETROSPECTIVE STUDY OF CALF MORTALITY IN WOLAITA SODDO JERSEY CATTLE BREEDING AND MULTIPLICATION CENTER, WOLAITA SODDO, SOUTHERN ETHIOPIA.
- **DVM-Seminar Paper**: DIAGNOSTIC AND THERAPEUTIC APPLICATIONS OF CYTOKINES.
- A certificate on Basic Pedagogical Courses from Hawassa Teachers Training College.
- A diploma in Basic computer knowledge (Introduction to computers, Ms-Dos, Ms-windows 98/2000/XP, Ms-Word 2003, Ms-Excel 2003 and Ms-Access-2003) from Rehoboth Computer Center, Debre Zeit, Ethiopia.
- A Certificate on Basic Computer Knowledge from Ministry of Agriculture and Rural Development, Alage Agricultural and Vocational Education Training College.

WORK EXPERIENCE:

- 5 years experiences of teaching as an instructor at Alagae ATVET College.

LANGUAGE

	Writing	Reading	Speaking
Tigrigna	Excellent	Excellent	Excellent
English	Excellent	Excellent	Excellent
Amharic	Excellent	Excellent	Excellent

REFERENCES:

- AAU, FVM registrar telephone number- **0014338533**
- Dr. Hagos Ashenafi (DVM, MSc, PhD, Assistant Professor, Department Head of Pathology and Parasitology): Department of Pathology and Parasitology, Colllege of Veterinary Medicine and Agriculture, Addis Ababa University.
  Telephone: 00251911405318, Fax: 00251114339933,E-mail:hagos83@yahoo.com

  P.O.Box:34, Debre Zeit, Ethiopia.

- Dr. Getachew Terefe (DVM, PhD, Assistant Professor, Dean of post Graduate Studies)
  Department of Pathology and Parasitology: College of Veterinary Medicine and Agriculture, Addis Ababa University.

  Telephone: 00251913171784, Fax: 00251114339933, E-mail: getachew_ terefe@yahoo.com

  P. O. Box: 34, Debre Zeit Ethiopia.

- Dr. Dinka Ayana (DVM, MSc, Dean of the College).
  Department of Pathology and Parasitology: College of Veterinary Medicine and Agriculture, Addis Ababa University.

  E-mail: dinka_ayana@yahoo.com

DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for a Degree in any other University and that all sources of materials used for this thesis have been duly acknowledged

Name _____

Signature _____

Date of Submission_____

This thesis has been submitted for examination with my approval as University advisor

_____

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