

**ADDIS ABABA UNIVERSITY  
SCHOOL OF GRADUATE STUDIES  
DEPARTEMENT OF BIOLOGY**



**STUDY ON THE SURVIVAL AND REPRODUCTIVE  
PERFORMANCE OF STERILE MALE *GLOSSINA PALLIDIPES*  
(DIPTERA: GLOSSINIDAE) AT KALITY TSETSE FLY REARING  
AND IRRADIATION CENTER, ADDIS ABABA.**



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**A thesis submitted to the School of Graduate Studies of Addis Ababa University in partial fulfillment of the requirement for the Degree of Master of Science in Biology (Insect Science)**

**Jun, 2010**

## **ACKNOWLEDGEMENTS**

I would like to express my genuine appreciation to my advisors Dr. Emiru Seyoum from Addis Ababa University and Dr. Solomon Mekonnen from Kality Tsetse fly Rearing and Irradiation Center, for their excellent guidance, follow up and regular supervision during the study.

Extend my deepest thanks to the Kality Tsetse fly Rearing and Irradiation Center, staff, especially staff members working in the insectory for their full collaboration in Rearing of flies and provision of materials for research.

I would like also to extend my a acknowledgement to Addis Ababa Agricultural Bureau staff particularly Mr. Alemayehu Taye and Dr. Fasika Belete for their understanding willingness on provision of the materials and good understanding.

Many thanks go to all my colleagues and friends in particular Zwidu Areriso, Tigest Asefa, Mohamed Naser and Tibebe made this study to complete successfully. I sincerely also like to thank Addis Ababa University, Biology Department for providing me financial support to conduct the study.

Last, but not least to the Almighty God and to all my lovely parents for their moral support and encouragements throughout the study.

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## ABSTRACT

Laboratorial investigation were conducted to know the effect of irradiation (sterility) on reproductive performance of females *G. pallidipes* mated with sterile and fertile males under temperature of 23-25 °C and 75-80% relative humidity. Completely randomized designs with three replications were followed throughout the experiments.

The irradiation effects on male *G. pallidipes* at different doses of irradiation were checked using, 60Gy, 80Gy, 100Gy and 120Gy. Results showed that at highest doses the sterility effects on male *Glossina pallidipes* were significantly lower than lowest doses ( $P < 0.05$ ) on pupae production and emergence of adults. On the other hand, the highest dose (120Gy) caused abortion of egg/larvae significantly higher than the lower dose (60Gy) ( $P < 0.05$ ). In other investigation which was aimed to assessing the competitiveness of sterile males to fertile males in a cage at ratios of 9 sterile: 1 fertile males, 7 sterile to 3 fertile males, 5 sterile to 5 fertile males and 3 sterile to 7 fertile males, the results showed that the ratio with the highest number of sterile (9: 1) in the cage caused significantly low number of pupae and number of adults emerged from pupae than those with lower number of sterile males (3: 7) ( $P < 0.05$ ). Similarly, the ratio with the highest sterile males (9: 1) resulted in significantly higher number of egg/larvae abortion than those with lower number of sterile males (3: 7) ratio ( $P < 0.05$ ). In the present study, survival of male *G. pallidipes* irradiated with 120Gy three days after emergence from pupae was significantly higher than males irradiated one and ten days after emergence from pupae ( $P < 0.05$ ). This may indicate that irradiated males may need to rest up to 7 or 10 days before released to the field. The importance of sterilization using appropriate dose, the appropriate ratio of sterile to fertile males and the time at which sterility was done efficiently all are relevant.

## 1. Introduction

Tsetse flies (*Glossinidae*) are vector for transmission of the parasites causing human and animal trypanosomiasis has made them one of the most devastating insect in Africa. Apart from two known localities in the Arabian Peninsula (Elsen *et al.*, 1990), tsetse flies occur only in Africa south of the Sahara desert and north of the temperate climates of the south of the continent, over 11 million square km<sup>2</sup> of sub-Saharan Africa and the trypanosomes they transmit can cause severe illness in livestock and people (Jordan, 1995).

According to Langridge (1976) and FLDP (1989), tsetse flies in Ethiopia are confined to the southern and western regions between longitude 33° and 38° E and latitude 5° and 12° N. the total area infested by tsetse flies in 1976 and 1988 was 98,000 km<sup>2</sup> and 120,000 km<sup>2</sup>, 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 m.a.s.l. (Getachew Abebe *et al.*, 2004). In certain areas, when whether flies were caught at the highest altitudes, whether these are representative of self-sustaining population is uncertain.

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 significant economic importance. These are *Glossina 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 wooded grass land in the Southern Rift Valley of Ethiopia (Vereyzen *et al.*, 1999). Out of the nine regions of Ethiopia five (Amhara, Beneshangul-Gumuz, Gambella, Oromiya and SNNPR) are infested with more than one species of tsetse flies (Getachew Abebe *et al.*, 2004).

The morsitans group is of great importance in the transmission of animal trypanosomiasis and the *palpalis* group in the transmission of human sleeping sickness (Leak, 1998). Adult *Glossina* species are dull in appearance, varying in colour from a light yellowish brown to a dark blackish brown. In some species the abdomen may have alternate darker and lighter bands. The smallest species (*G. tachinoides*) is 6-8mm long and the largest (*G. pallidipes*) 10-14 mm (Vereyzen, 2001). The adult female produces a single egg, which hatches to a first stage larva in the uterus. After a period of development and molting a third stage larva is deposited on the ground. An adult female produces one full grown larva every 9-10 days which then pupates in light or sandy soil. The adult fly will emerge after a pupal aerial period that varies according to temperature but may be around 30 days at 24 °C (Leak, 1998).

Consequently, tsetse flies have a very low rate of reproduction, closer to that of a small mammal than to most insects. The reproductive method of tsetse flies is known as adenotropic viviparity (Vereyzen, 2001). 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. 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 fly and the infective metatrypanosomes 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 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.

Tsetse vector control methods relying on large scale bush clearing and aerial spraying methods are no longer used due to environmental concerns. Tsetse control currently relies on two bait systems: insecticide-treated traps and targets and insecticide treated livestock. Sterile Insect Technique (SIT) has also been used in efforts to eradicate tsetse flies in some areas. The SIT relies on the production of large numbers of the target insect in specialized production centers, the sterilization of the males pupae or adult fly (or sometimes both sexes), and the sustained and systematic release of the sterile males over the target area in numbers large enough in relation to the wild male population to out compete them for wild females mating of the sterile insects with virgin, native females will result in no offspring. With each generation, the ratio of sterile to wild insects will increase and the technique becomes therefore more efficient with lower population densities (inversely-density dependent) (Nagel, 1995).

## **2. LITERATURE REVIEW**

### **2.1 Biology of Tsetse Flies**

Tsetse flies (*Glossinidae*) are yellow- brownish insect, 6-13.5mm long, transmitting Nagana among domestic animals and sleeping sickness among humans (Seifert, 1996). Tsetse flies are classified as genus, *Glossina*, of the family *Glossinidae*, order Diptera (two winged flies), where 31 species and sub- species are identified, and distributed over 11 million km<sup>2</sup> of land. Tsetse flies are confined to a belt of tropical Africa extending from the southern Sahara (latitude 15<sup>0</sup>N) in the north to Zimbabwe and Mozambique in the south (latitude 20-30<sup>0</sup>S), where the species are restricted to various geographical area (Duncon *et al.*, 1992).

The prevalence of bovine trypanosomiasis, transmitted by *Glossina morsitans morsitans* (Diptera: *Glossinidae*) the only species present, is about 30% (Van den Bossche *et al.*, 2000). For their survival, tsetse flies are highly dependent on the presence of suitable habitat and hosts. The seasonal distribution of the flies is correlated with the distribution of its main host, cattle (Duncon *et al.*, 1992). However, in large parts of tsetse-infested sub-Saharan Africa the progressive clearing of the natural vegetation for cultivation, the introduction of domestic animals and the almost complete disappearance of large game animals have had important repercussions for the distribution and density of tsetse flies. In Malawi, for example, the distribution of tsetse flies is almost restricted to protected areas, where the vegetation is undisturbed whereas the extensive clearing of natural vegetation outside the protected areas has led to the disappearance of the tsetse flies and the disease they transmit (Van den Bossche *et al.*, 2000).

### **2.2. Life Cycle of Tsetse Flies**

2.2.1 Mate: the mating of tsetse flies probably takes place near to or on host animals (Figure 1). Male flies settle on the back of the female, and the claspers at the posterior end of the male abdomen grip the end of the female abdomen. This position may be held for an hour or two, before the male and female flies' part company. Females are mated young, before or at

about the time of taking the first blood meal Females usually mate only once in their lives but some may mate more than once; males can mate several times. Older males are better able to mate successfully than young ones (Nagel, 1995). During mating, the male penis is inserted into the vulva, reaching into the uterus as far as the exit of the spermathecal ducts. A large ball of sperm is deposited there in a spermatophore. At the end of mating, the male releases his grip on the female and flies away. In the next few hours sperm make their way up from the spermatophore into the spermathecal ducts and the spermathecae (Roberts, 1993). Sperm remain active in the spermathecae for the rest of the female's life.

2.2.2 Egg stage: the egg is fertilized immediately 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 (*Glossina morsitans*) (Kettle, 1984).

2.2.3 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).

2.2.3.1 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).

2.2.3.2 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).

2.2.3.3 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*).

2.2.3.4 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

2.2.3.5 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.

2.2.3.6 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).

2.2.4 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 (Figure 1). 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 works itself backward out 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 1). There is no feeding by the larva after it is dropped by the female.

2.2.3. 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). 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).

2.2.4 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 works its way out of the hole so made, 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 urinates the wings begin to expand to reach their proper size.

2.2.4.1 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 semi-transparent, 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.

2.2.4.2 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.

2.2.5 Rate of reproduction: on mated, a female 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 temperatures give 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).

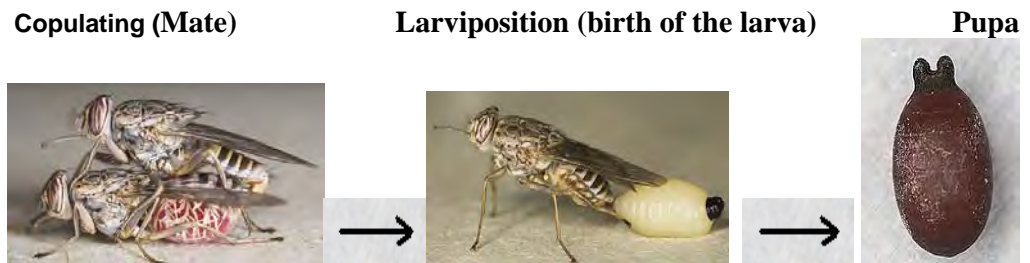


Figure 1. Life cycle of Tsetse flies. (Source Leak, 1998)

### 2.3. The Importance of *Glossina* species

In Africa, the primary vector for the three pathogenic trypanosomes (*T. congolense*, *T. vivax*, and *T. brucei*) is the tsetse fly. These trypanosomes replicate in the tsetse fly and are transmitted through tsetse fly saliva when the fly feeds on an animal (Stevens and Brisse, 2004). For this reason, the important variables in the epidemiology of trypanosomosis and probably the most important component of challenge are tsetse-related factors, particularly the density of tsetse population (Eisler *et al.*, 2004 and Hargrove, 2004). In tropical Africa, the epidemiology of animal trypanosomosis is governed especially by the distribution of tsetse flies (*Glossina* species). Here, tsetse ecology is confined roughly between 15°N- 25°S latitude where the disease as well occurs. However, the distribution of mechanically transmitted trypanosomosis (through biting flies, injections, etc.) is wider than the above limit (Bett *et al.*, 2004). When we deal with tsetse-transmitted trypanosomosis, much depends on the distribution and the vectorial capacity of *Glossina* species responsible for transmission. The three main species of tsetse flies for transmission of trypanosomes are *Glossina morsitans*, which favours the open woodland of the savanna; *G. palpalis*, which prefers the shaded habitat immediately adjacent to rivers and lakes; and *G. fusca*, which favours the high, dense forest areas (Bett *et al.*, 2004). The three groups of *Glossina*, the savannah and riverine classes are the most important ones since they inhabit areas suitable for grazing and watering (Hargrove, 2004).

Although the infection rate of *Glossina* with trypanosomes is usually low, ranging from 1 – 20 % of the flies, each is infected for life, and their presence in any number makes the rearing of cattle, pigs and horses extremely difficult. In areas where savannah tsetse is the

vector, the risk of contracting the disease is widespread. On the other hand, when the riverine species are the dominant species, transmission occurs particularly along rivers with dense vegetation along the banks. The proportion of a tsetse population found infected with pathogenic trypanosomes depends not only on its vector capability, but also on the host on which it mainly feeds (ICPTV, 2003).

From the above descriptions, it is evident that tsetse fly as the primary vector of animal trypanosomiasis in sub-Saharan Africa is incriminated as the predominant and continuing threat to the efforts aimed at improving the livelihoods of rural communities through amelioration of livestock sub-sector (Matovu *et al.*, 2003 and Sinyangwe *et al.*, 2004). For this reason, recent and ongoing international programs addressing the problem of tsetse-transmitted animal trypanosomiasis, such as the Farming in Tsetse Controlled Areas (FITCA) and the Regional Tsetse and Trypanosomiasis Control Programme (RTTCP) initiatives place great emphasis on the disease and vector control (Barret *et al.*, 2004 and Hargrove *et al.*, 2003).

### **2.3.1 Medical and veterinary important**

Human Trypanosomiasis: is a major threat to human health in Africa. Approximately 35-55 million people in 36 African countries are at risk but only about 3 million of them are under surveillance (WHO 2001). Two species of salivarian trypanosomes causes infection in humans. Both of them cause “sleeping sickness” through invasion of the central nervous system (CNS). *T.rhodesiense* usually occurs with acute syndromes while *T.gambiense* infection may be initially asymptomatic, although at a later stage it affects the CNS. Humans are infected following a fly bite which occasionally causes a skin chancre at the site. The injected trypanosomes mature and divide in the blood and lymphatic system, causing symptoms of malaise, intermittent fever, and rash and wasting. Eventually, the parasitic invasion reaches the CNS, causing behavioral and neurological changes Death may occur.

Animal trypanosomiasis: Also called *nagana* when it occurs in bovine cattle or horses or *sura* when it occurs in domestic pigs, is caused by several trypanosome species (Coetzer *et al.*, 1994). These diseases reduce the rate, milk Productivity and strength of farm Animals, generally leading to the eventual death of the infected animals. Certain species of cattle are called *trypanotolerant* because they can survive and grow even when infected with trypanosomes although they also have lower productivity rates when infected. The course of

the disease in animals is similar to the course of sleeping sickness in humans. *Trypanosoma congolense* and *Trypanosoma vivax* are the two most important species infecting bovine cattle in sub-saharan Africa. *Trypanosoma simiae* causes a virulent disease in swine. Other forms of animal trypanosomiasis are also known from other areas of the globe, caused by different species of trypanosomes and transmitted without the intervention of the tsetse fly. Tsetse vector ranges mostly in the central part of Africa (Murry and Dexter, 1988).

Livestock's have great importance to crop production due to inputs both from manure and nutrient recycling and from animal traction. It has been argued that the carrying of rangelands has been exceeded (Shaw, 2003) and increased production will come from removal of constraints to productivity by disease such as trypanosomosis. (Martin, 2001) stated that in some tsetse infested region of Africa, three to four times more livestock could be carried if trypanosomosis did not exist. In addition to the effect of trypanosomosis on livestock raised for food production, the effects on horses has frequently been referred to as one of the significant factors in isolating Africa from other parts of the world and retarding the early development (Swallow, 2000).

## **2.4 Economic Importance**

The economic impacts of trypanosomosis in Africa are diverse and complex, with direct effects on animal production and human health, as well as indirect effects on settlement patterns, land use, draught power use, animal husbandry and farming (FAO, 2004). Quantifying these wide-ranging effects has proven to be difficult, but a considerable body of evidence has been gathered through numerous studies of specific situations (Swallow, 2000 and Shaw, 2003). Aggregating from these results to a continental level is problematic because of general uncertainties about cattle numbers, infection rates and the extent of actual, as opposed to potential, tsetse infestation.

However, direct aggregate losses due to animal trypanosomosis in the estimated 47 million cattle living in tsetse regions probably exceed the US\$1.3 billion annually calculated by which excluded losses from the reduced efficiency of draught oxen that can be substantial. To these estimates of direct losses need to be added expenditure on trypanocides, estimated at around US\$30 million per annum for some 35 million doses (Holmes *et al.*, 2004). With regard to human trypanosomosis, the World Health Organisation estimates that some 50,000 deaths occur annually from sleeping sickness, with some 300 000–500 000 people currently

infected. The annual disease burden is put at 1.6 million disability-adjusted life years (WHO, 2005).

Trypanosomosis is prevalent in two main regions of Ethiopia i.e. the northwest and the southwest regions (Getachew Abebe *et al.*, 2004). In these regions tsetse transmitted animal trypanosomosis is a major constraint to utilization of the large land resources. The potential area of tsetse infestation has been variously estimated at 66,000 km<sup>2</sup> based on the 1,500 m.a.s.l. breeding limit (Ford *et al.*, 1976); 97,855 km<sup>2</sup> based on a 1,600 m.a.s.l breeding limit (Langridge, 1976) and between 135,000-220,000 km<sup>2</sup>, based on the maximum dispersal up to 1,700 – 200,000 m.a.s.l . Inter-African Bureau for Animal Resources (IBAR, 1989) reported that at least 6 million of the 45 million heads of cattle that are raised under trypanosomosis risk in Africa, are now found in the West and Southwest Ethiopia. The northwest region of Ethiopia is also affected by tsetse and non-tsetse transmitted trypanosomosis (Getachew Abebe and Yilma Jobre, 1996; Yohannes Afework *et al.*, 2000 and Alekaw Shinishaw *et al.*, 2005).

## **2.5 Tsetse fly control measures**

### **2.5.1 Use of insecticides**

The use of special insecticide formulations applied to artificial attractive devices (insecticide-impregnated targets with or without available odour attractants) or to cattle is an efficient and sufficiently specific method to suppress tsetse target populations in most situations (Challier *et al.*, 1974 and Bauer *et al.*, 1995). Success largely depends on the density and placement of the impregnated attractive devices in the fly habitat (Vale, 1998); the availability of attractants for the target tsetse species (Torr *et al.*, 1995 and Torr *et al.*, 1997); the size of the control area; reinvasion pressure and the population dynamics of tsetse populations in adjacent areas (Van den Bosshe and Mudenge, 1999) tsetse host and pastoralist practices, i.e. the time and location of grazing and peaks of tsetse activity.

Fortunately, observations (Baylis *et al.*, 1995) show that *Glossina pallidipes* populations in Kenya apparently do not exhibit a reduced feeding response to the presence of pour-on insecticide formulations on cattle, indicating that "behavioural" insecticide resistance has not yet developed. However, it is likely that one or more of the pyrethroid resistance mechanisms already known from several other species of Diptera will manifest themselves in tsetse (Jordan, 1995) in response to the increased selection engendered by wider adoption of deltamethrin-treated targets for tsetse control at the village level. As with trypanocides, the widespread, unsupervised and insufficiently coordinated use of insecticides on targets or animals risks promoting the development of insecticide resistance.

### **2.5.2 Stationary attractive devices**

The development of insecticide impregnated, odour-baited traps (Dransfield *et al.*, 1990), targets and insecticide treated cattle as pour-on (Shereni, 1990) which attract and kills tsetse offer the prospect of cheaper alternative with less damage to the environment. On the other hand, baits (traps, targets or animals) are now-a- days used widely to replace ground broad casting of the insecticides. In Ethiopia, these techniques have been tried and are still in use

in the different tsetse infested areas. The use of insecticide impregnated target and application of pour-on on cattle in the area has suppressed the tsetse population from 4.1 to 0.9 fly/ trap/day.

As the result the prevalence of bovine trypanosomosis has dropped from 27 to 6 % in two years time (Abebe *et al.*, 2004). stressed that efficient tsetse control will lead to a reduction in use of trypanocidal drugs and this will leave their role as efficient means of curbing the disease in case of an outbreak. Several technical aspects are essential for the efficient application of this bait technology such as appropriate trap/target site selection, sufficient maintenance, periodic replacement and replenishment of the odours, appropriate reflectivity pattern of the used cloth, degradation of the insecticide deposits by UV light, etc The technique is suitable for deployment by the local farmer communities to protect small areas, but the high target densities required against certain species and in certain dense habitats make the use of these devices over large areas uneconomic (FAO, 1993).

### **2.5.3 Live bait technique**

This method is based on the insecticide treatment of livestock and exploits the blood sucking behaviors of both sexes of tsetse. Tsetse flies, attempting to feed on cattle or other treated domestic livestock are killed by picking up a lethal deposit of insecticide on the ventral tarsal spines and on pre-tarsi whilst feeding (Leak, 1998). The success of the method depends on a relatively large proportion of feeds being taken from domestic animals and a sufficient proportion of the livestock population being treated.

The use of persistent insecticides on livestock has proven to have a suppressive effect on certain tsetse populations in those areas with a high density of cattle and where adequate expert support was present, e.g. in Zimbabwe against *G. pallidipes* Austen (Thomson & Wilson, 1992), in Burkina Faso against *Glossina morsitans submorsitans* Newstead and *Glossina palpalis gambiensis* Vanderplank (Bauer *et al.*, 1995) and against *Glossina fuscipes fuscipes* Newstead and *G. pallidipes* in Ethiopia (Leak *et al.*, 1995).

Unlike with stationary attractive devices, the technique is less prone to theft and does not suffer from maintenance problems. However, several issues such as the required cattle density, the proportion of the herd that requires treatment, host preference of different tsetse

species, etc. require further research. Other disadvantages are the high treatment frequency, the high cost of the insecticides, insecticide residues in cattle dung, motivation and participation of farmers and the potential development of resistance to the insecticides in both tsetse and ticks (Oloo *et al.*, 2000).

#### **2.5.4 Sterile insect technique (SIT)**

The SIT relies on the production of large numbers of the target insect in specialized production centers, the sterilization of the males pupae or adult fly (or sometimes both sexes), and the sustained and systematic release of the sterile males over the target area in numbers large enough in relation to the wild male population to out compete them for wild females. Mating of the sterile insects with virgin, native females will result in no offspring (Nagel, 1995). With each generation, the ratio of sterile to wild insects will increase and the technique becomes therefore more efficient with lower population densities (inversely-density dependent). This novel approach has been used successfully in Burkina Faso, Tanzania, Nigeria and, most recently, in Zanzibar where it eradicated *Glossina austeni* from the 1600 km<sup>2</sup> Unguja Island (PAAT, 2003).

The SIT is non-intrusive to the environment, has no adverse effects on non-target organisms, is species-specific and can easily be integrated with biological control methods such as parasitoids, predators and pathogens. There is no development of resistance to the effects of the sterile males provided that adequate quality assurance is practised in the production process and the sterile insects cannot get established in the released areas as with other biological control programmes (Vreysen *et al.*, 2000). The release of sterile insects is however only effective when the target population density is low, it requires detailed knowledge on the biology and ecology of the target pest, and the insect should be amenable to mass rearing. In addition, the SIT necessitates efficient release and monitoring methods, which have to be applied on an area-wide basis (Vreysen *et al.*, 2000).

In Ethiopia, a major SIT project coordinated by the Ministry of Science and Technology and implemented by the Agricultural Bureau of the Southern Nations and Peoples Administrative Region of Ethiopia in the Southern Rift Valley has been initiated. Southern

Rift Valley Ethiopia Tsetse Eradication Project (STEP) is a ten-year tsetse Eradication programme of two five-year phase prepared in line with the understanding between the Ethiopian Government and the International Atomic Energy Agency (IAEA) of the United Nations. The SIT is envisaged to supplement the national effort of tsetse and trypanosomiasis management, using area wide eradication approach of the resident fly species in the Rift Valley (Getachew Abebe *et al.*, 2004). \

### **2.5.5 Biological control methods**

Biological control of tsetse flies seems to be feasible only by means of predator, parasites, parasitoids or pathogens, which are either exclusively or at least to a large extent, specialized on tsetse flies (Nagel, 1995).

#### **2.5.5.1 Predators**

Among arthropods, the most important predators of tsetse flies are probably ants, asilids (robber flies), wasps, and spiders feeding on adults (FAO, 1994). Larvae and pupae of tsetse flies are also prey of ants and insectivorous insect such as ground beetles (Carabidae e.g Cicindelinae, Carabinae). Adult tsetse flies are also assumed to the prey of insectivorous bird species (e.g. guinea fowl, francolins). Although tsetse flies have such a number of predators, they do not appear to reduce tsetse population significantly in the long term (Nagel, 1995).

#### **2.5.5.2 Parasitoids, parasites and pathogens**

To date no practicable methods have been developed for the control of tsetse flies by parasitoids and pathogens (viruses, fungi, bacteria and protozoans). However, Chrestomutilla (Hymenoptera) and Exhyalanthrax (Diptera: Bombyledae) are the most important and best known parasitoid insect of tsetse pupae (Nagel, 1995) while nematodes of the family Mermithidae are noticeable parasite of adult flies.

### **2.5.6. Trypanotolerant cattle breeds**

Certain local breeds have developed a tolerance to trypanosome infections during the centuries spent in areas strongly infested by *Glossina*. This ability, named trypanotolerance, results from several biological mechanisms under multigenic control (Hanotte *et al.*, 2003). Indeed, some breeds present the remarkable capacity to control their level of parasitemia, to resist the development of severe anemia during the infection, and to remain productive in a zone strongly infested by tsetse flies. These two characteristics (limited parasitemia and anemia) are known to be highly heritable and genetically linked to cattle productivity (Hill *et al.*, 2005). More than one single gene, it seems probable that two pools of genes, are involved but all the techniques used up to now have failed to identify them (Berthier *et al.*, 2006) such as the “normal” Boran Zebu, Indian breeds of zebu and other “exotic” breeds such as European taurine breeds. However, the resistance of West African taurine breeds appears to be considerably more pronounced.

The only surviving indigenous taurine type indigenous cattle breed in Ethiopia the Sheko exhibited better trypanotolerant attributes than the other three breeds (Abigar, Horro and Gurage), as measured lower trypanosome prevalence, less severe anemia after infection, and fewer trypanocidal treatments annum than the other breeds. Moreover, the Sheko breed maintained its physiological functions under prevailing trypanosomosis challenge and compared favorably with the other breeds in its reproductive performance. While the Abigar manifested high sensitivity and frequent death to PCV depression, Horro had strong resilience to PCV depression better response to Berenil 1 treatment assistance (Lemecha *et al.*, 2006).

#### **2.5.7. Integrated control of tsetse and trypanosomosis**

In view of the fact that tsetse and trypanosomosis has proved difficult to eradicate and the risk being compounded by emergence of drug-resistant strains of trypanosomes, it is therefore imperative to have a new and integrated approach in the control of tsetse and trypanosomosis so as to reclaim the tsetse infested lands of Africa (Holmes, 1997). There are three levels at which integrated control of tsetse and trypanosomosis can be addressed: integration with rural development, integration with other disease control measures (integrated disease management) and integration of various tsetse and trypanosomosis control measures.

The alternative tsetse control measures include method such as aerial and ground spraying with insecticides and deployment of targets, traps and screens. A particular concern with large-scale insecticide application is the pollution it may cause, as most insecticides are harmful to aquatic and terrestrial animals. If live bait animals are used without any other form of tsetse control, difficulties arise with persistence of flies in areas where the treated animals do not go (Uilenberg, 1998). In case of sterile male technique, the effect on the population only becomes apparent after a period and a substantial fly suppression has to precede the application of SIT. Traps and screens may be stolen for cloth they contain and during rainy season the rapidly growing vegetation may camouflage the trap or screen, which thus loses its visual activities for the flies. Trypanotolerant cattle that stand up to challenge in a particular region may suffer from disease when introduced into another area, and so far, all attempts at developing a vaccine against trypanosomosis have failed (Uilenberg, 1998).

In general, all of the available methods have advantages and disadvantages and the various techniques act in a complementary way; an advantage of one may off set a disadvantage of another. The economic and feasibilities of employing various control methods must be compared for any given tsetse infested area. In Ethiopia, reducing the risk of trypanosomosis by employing more effective control methods may well increase both livestock and crop production. The use of vector and parasite control in an integrated package has effectively reduced the burden of tsetse and trypanosomosis in cattle in the Ghibe (Leak *et al.* 1995) and Didessa valleys (Feyesa Regassa, 2004).

One of the major components of sustainability of these methods is the active participation of the majorities of the communities contributing to a relevant production system in a given environment or region. Successful strategies for controlling animal trypanosomosis must be based on accurate appraisals of the impacts of the disease constraints on village farming system and the development of cost-effective sustainable disease control packages which can be adapted by producers.

## **2.6 Mass Rearing Technique**

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, preoviposition 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 and Orozco-Davila *et al.*, 2007). Conservation of space is also a major consideration, especially when several days are required from the time of eggs in rearing containers until the desired life stage has been harvested.

During the process of establishing a strain, along the rearing process, and during treatment before release, the insects are subjects 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 have more advantageous. The screwworm requires special resources and rearing conditions at all stages of its development but in the case of tsetse fly, only the pupal and adult stages have to be considered because the egg and larval stages remain within the pregnant female fly. In nature, the larvae of the screwworm fly grow with in living mammalian flesh. Therefore for mass rearing purpose, a very complex larval medium, simulating this living tissue, had to be developed and

deployed on a very large scale. For the adult stage, screwworm flies normally feed on liquids in animal wound for protein requirements, and nectar from flowers for carbohydrates and water. The diet provided to an adult screwworm colony must take into account all three requirements (Feldmann and Hendrichs, 2001).

The 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 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. Unlike the ingredients of screwworm diet, most of which have to be imported using hard currency, animal blood for tsetse rearing can be collected at a local abattoir and then treated with gamma radiation to eliminate any micro organisms. Once tsetse mass rearing has reached, there will be local commercial sources in Africa selling the required quantity of sterilized blood. At presented this product is lost at the slaughterhouse without any benefit to local economies (Feldmann and Hendrichs, 2001).

## **2.7 Method of Sterile Insect Techniques**

The genetic technique is one of several methods that could be useful in providing more effective and acceptable solutions to key insect problems. Genetic control is the introduction of genetic factors into pest population's that reduce or eliminate the pest problem by mating (Curtis, 1985). It involves the rearing, radiation or chemical sterilization and release of males to introduce large amount of dominant lethal mutations into the wild pest species (Knipling *et al.*, 1968 and Coleman and Alphey, 2004) or treatment of the natural population with an agent (Chemosterilant) which will induce sterility in the native insects.

Cytoplasmic incompatibility is one of the genetic control techniques in which cytoplasmic factor transmitted through the egg, which kills the sperm of the incompatible male after its entry into the egg. In some insect species there are crossing types in which fertile females but sterile males among their progeny are produced that leads to hybrid sterility. Ionizing radiations or chemical sterilants induce dominant lethal mutation in the sperm that are used to sterilize males. Genetic control is a potential technique that may be subdivided in to

population suppression and population or replacement in which genetic traits introduced into the wild population by mating (Coleman and Alphey, 2004 and Christophides, 2005).

Sterile insect technique (SIT) is a population suppression method that eliminates a range of agricultural pests and disease vectors (including the Mediterranean fruit fly, the screwworm fly and the tsetse fly) over large areas. Populating replacement involves the development of genetically modified strain of the target vector that is may be spread throughout the wild vector population (Coleman and Alphy, 2004). (Curtis, 1985) stated that, replacement of a vector population b a harmless strain has better long-term effect than any method that serves for eradication of the insect. This is because replacement method cannot be affected by density-dependent regulatory factors and it can provide at least some degree of resistance to re-invasion of the area being immigrants of the vector genotype. Detailed knowledge of the biology, dispersal behavior and population dynamics of various insects are the most important requirements in applying the genetic techniques for insect control.

According to Tour *et al.*, (2004), the genetic control strategies in human disease vectors currently focus mainly on the sterile insect technique (SIT). In sterile-male technique, the most important step is the production of large numbers of sexually sterile male insects that can be indistinguishable from the natural males (Baumhover, 2002). Accidental release of sterile females has little impact on the efficacy of SIT but can still be harmful. For example irradiated sterile insects like Mediterranean fruit flies lay their eggs in fruit that leads to spoilage and female mosquitoes which can also able to transmit pathogens that cause disease after sterilization is not advisable to sterilize and release female insects (Alphey, 2002 and Atkinson, 2005) stated that, the major requirements in SIT are (1) a method of sterility without seriously affecting survival and mating behavior, (2) practical methods of producing large numbers of sterile insects, (3) adequate dispersal of the insects released, and (4) no danger of damage to crops, animals, or man by the insects released. The mating performance, light ability, longevity, fertility and fecundity levels serve as parameters to select a dose that minimize somatic damage to provide normal insect behavior. Gamma radiation, chemosterilants, cytoplasmic incompatibility and hybrid sterility cause sterility in insects. At the cytological level, sterilization is the result of the germ cell chromosome fragmentation leading to the production of imbalanced gametes that in turn leads to inhibition of mitosis and the death of fertilized eggs or embryos (Bakri *et al.*, 2005).

Sterility can be induced by either interference with the formation of a zygote or interference with the development of a zygote. A zygote cannot be formed if one or both of the sexes are unable to produce a functional gamete or if the two gametes are prevented from combining. Zygote development is impaired from failure of genetic information in either or both of the gametes and from environmental conditions which do not allow normal development (Campion, 1971). From many possible procedures that bring about sexual sterility, only radiation and chemical treatments are practically possible. The basic principle is to produce sexual sterility of an insect that involves exposure of the pupal stage to a sterilizing dose of gamma radiation or application of chemical. This causes sterility to an insect when the sterilizing agents and an insect come in contact, or applied to the insect, or mixed in a bait to be eaten by an insect species.

### **2.7.1 Ionizing radiation technique**

The term radiation includes radio waves, radar, infrared, microwaves, ultraviolet, electrons, X-rays and Y-rays. Radiation may be used as direct treatment against stored-product pests as an alternative to fumigants and other control methods or by genetic control involving the release of irradiated sterile males so that most of the females will lay sterile eggs (Hasan and Khan, 1998).

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 Alpey, 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. Different types of ionizing radiations produce damage in significantly the same way and the effects are therefore equivalent if the dosage is measured in the same way (Tiltone and Brower, 1982). According to Bakri *et al.*, (2005), cells with a high mitotic rate, with a long mitotic future

(that will undergo many divisions under normal circumstances) and that of the germ cell type and most radiosensitive.

This implies that cells are most sensitive to radiation when they are dividing. Some of the earliest studies used insects for irradiation effects because of their ease of handling, short life cycles, large number of progeny produced by most species and their other favorable biological attributes. Adult insects are more radio-resistant than the other developmental stages. It may be because the cellular damage is not expressed in adult insects but some tissues such as the gonads that normally undergo cell division in the adult are radiosensitive (Tiltone and Brower, 1982).

The radiation dose absorbed by an insect to induce sterility is the main importance in sterile insect release program. Insects those receive too low dose may not be sterile efficiently and those received high dose may be uncompetitive (Parker and Mehta, 2007). The International System (SI) unit of radiation is Gray (GY) which equals to 100 rad (radiation-absorbed dose). There is no standard radiation dose for all organisms to undergo sterility. Radiation doses that result in sterility in insects and related arthropods range widely between and within order and vary also according to the species (Bakri *et al.*, 2005). Apart from Lepidoptera, the radiation doses required to sterilize males of most pest species are commonly (20-150Gy) which result in dominant lethal mutations (Curtis, 1985).

Table 1: The mean radiation doses for sterilization (Source: Bakri *et al.*, 2005).

No	order of insects/Arthropods	Mean sterilization dose in Gy
1	Lepidoptera	130-400
2	Acari	30-280
3	Coleopteran	40-200
4	Hemiptera	10-180
5	Diptera	20-160
6	Araneae	20-150
7	Dictyoptera	5-140
8	Thysanoptera,	100
9	orthoptera	4

### 2.7.2 Chemo-sterilization technique

Chemosterilants are chemical compounds, which can reduce or destroy the reproductive ability of an organism to which they are applied (Borkovec *et al.*, 1968 and Campion, 1971). Chemosterilants substituted for radiation believing that the chemicals should be easier to use and less costly than gamma radiation. Most importantly, chemosterilants have also possibility that could be used to sterilize the natural population without resorting to rearing and release of sterile specimens (Campion, 1971). The chemicals should sterilize both sexes of the pest population, or separation of male and female sterility must be developed and used simultaneously, highly selective against the target pest, and provide permanent sterilization with out serious deleterious effects on the mating behavior and mating competitiveness of the organisms sterilized. The required amount of chemosterilant doses actually administered.

The dose that administered must not kill the insect or not change its mating behavior (Borkovec *et al.*, 1968). Chemosterilants used to sterilize insects are not as hazardous as chemicals applied in the environment to control insects because small quantity is needed to sterilize insects and the release of treated insects can be delayed until most of the chemosterilant on the insects has degraded. Alkylating agents containing best known, cheap and commercially available compounds such as aziridines tepa, metepa and apholate, and have been used in much of the laboratory and field work.

These compounds have the ability to sterilize at lower doses and males are more susceptible than females (Campion, 1971). Alkylating agents are capable of replacing hydrogen in an organic molecule with an alkyl group and result in the induction of dominant lethal mutations but antimetabolites cause the insects to fail to produce ova or sperm, and may cause the death of sperm or ova after they have been produced. Antimetabolites include such compounds like 5- fluorouracil and methotrexate that interfere with nucleic acid synthesis and only have been used in the laboratory. These compounds lack specificity, have high cost, relatively scarce and generally sterilize only female insects. Miscellaneous group contains the phosphoramides, triazines, organotin, boron compounds, urea derivatives and others (Campion, 1971).

Consequently the rate of reproduction of tsetse fly is much lower than in any oviparous insects and in fact resembles that of small mammals that is why the sterile insect technique (SIT) control method is facilitated. Even though, Ethiopia is known to have a large cattle population reared and managed under traditional system unlike conventional insect control methods that are preferably applied to a high population of the target insect pest, SIT is most effective when fly population are low. The ratio of release sterile male flies should be as higher as possible to minimize the odds that wild flies mate. Studies were not so far conducted on the survival and reproductive performance of sterile to fertile *Glossina pallidipes* in Kality tsetse fly rearing and irradiation center.

### **3. OBJECTIVES OF THE STUDY**

#### **3.1 General Objective:-**

- ❖ To study on the compatibility of sterile male *Glossina pallidipes* in terms of survival and reproductive performance as a contribution to SIT focused tsetse fly management.

#### **3.2 Specific Objectives:-**

- ❖ To compare the reproductive competitiveness of sterile to fertile males under lab condition.
- ❖ To show the reproductive performance of sterile male *Glossina pallidipes* at different doses of gamma irradiation.
- ❖ To determine the effective dose of irradiations in male *Glossina pallidipes*

- ❖ To examine the survival rate of *Glossina pallidipes* versus doses of irradiation.

## **4. MATERIALS AND METHODS**

### **4.1. Location and experimental conditions**

All the investigations in the present studies were carried out at Kality Tsetse fly Rearing and Irradiation Center, Addis Ababa following the Center's standard procedure for irradiation of male flies.

#### **4.1.1 Experimental flies (*Glossina pallidipes*)**

All pupae and adult flies used for the experiments were derived from the stock colonies of *Glossina pallidipes*, which were initially collected from the field in and around Arbamech and are now being reared at Kality Tsetse fly Rearing and Irradiation Center. The pupae were placed and maintained at 75-80% relative humidity and at 23-25°C temperature regime. The pupae were kept for 25 days before being transferred into different cages where they emerged as adult after 25-30 days. The flies after emergence were kept in holding cages (diameter of 20 cm length and width of 5 cm) at a density of 30-40 flies per cage with

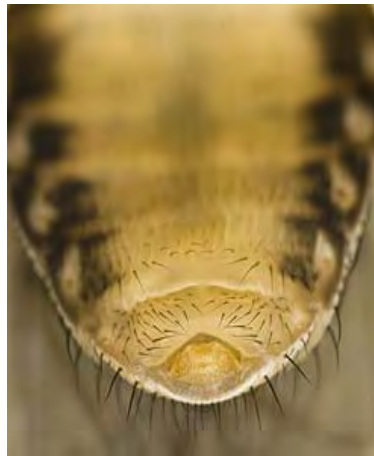
netting on top and bottom for feeding and collection of the larvae (Appendix 1). Similar to the pupae, adult flies were also maintained at a temperature regime of 23-25 °C and a relative humidity of 75-80 % (Feldmann *et al.*, 1992).

#### 4.1.2 Sex separation

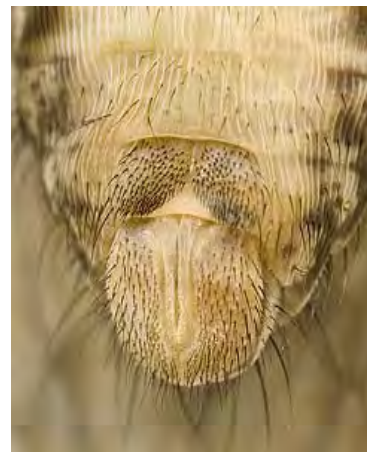
A cage that contains tsetse fly populations (30-40) at first day of emergence from pupae was introduced into a refrigerator of 4°C for 5-10 min. to inactivate (chill) the flies. Each fly was taken out of the cage one at a time and observed at the tip of the abdomen under compound microscope to determine their sex. 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 (Figure 2).

#### 4.1.3 Irradiation of male *Glossina*

A total of 60 male tsetse flies (1 day old) were chilled at 4°C for 5 -10 min. Fifteen male flies were placed in a small cage and irradiated in bulk in a pre-chilled vacuum flask with 120Gy in a <sup>60</sup>Co Gammacell 200 for 56.4 seconds. Gray (Gy) is the International System (SI) unit of radiation which equals to 100 rad (radiation-absorbed dose). The treated males then allowed to rest for 3 hour before feeding and placed in the rearing room at a temperature of 23-25 °C and relative humidity of 75-80 % (Feldmann *et al.*, 1992).



Female



Male

Figure 2. Comparison of external genitalia of male and female savanna tsetse flies (*Glossina morsitans morsitans*) (Source: FAO, 2004)

#### 4.2. MATERIALS

The materials used for the experiments in the present work included, holding cages (diameter of 20 cm and width of 5 cm) with netting on top and bottom for feeding and collection of the larvae, feeding tray (anodized aluminum), heated mat (with an adjustable heat source), 96% irradiated bovine blood and silicon membrane to cover warm blood. All these materials were used following the Center's standard procedure.

### **4.3 METHODOLOGY**

#### **4.3.1 Experiment I: Reproductive performance of sterile male *G. pallidipes* at different dose of irradiation.**

Emerged flies were separated into male and female, using a chiller set at 4°C. The flies were kept in cages (diameter of 20 cm and width of 5 cm) with a density of 30-40 flies per cage with netting on top and bottom for feeding and collection of the larvae. The newly emerged males were given irradiation treatments (60Gy with 28.2sec., 80Gy with 37.6sec., and 100Gy with 47 sec. and 120Gy 56.4 sec.) on day one following emergence and then allowed to mix with the newly emerged 30 female adults in a cage. Sterile males were allowed to mate at a ratio of 10:30 (1:3) male to female per cage. One group of untreated males with females of the same ratio as those treated was kept as control. The experiment was designed in a completely randomized design (CRD) in three replications (Appendix 2). All groups of the experiment were kept for up to 30 days at 75-80%Rh and 23-25°C. Abortion of eggs/larvae and production of pupae were recorded from days 20-30 and 18-30 days, respectively at the time of feeding. Feeding was done three times in a week using 96% irradiated bovine blood (Feldmann, 1994). Females were dissected to observe blockages of pupae in the uterus and findings were recorded as number of pupae produced. Fecundity was expressed as the number of pupae produced per mature female per day, taking day 18 following emergence as the first larviposition day. The pupae collected from each group were maintained at 75-80% Rh and 23-25°C for 25-30 days (Feldmann *et al.*, 1992). The number of adult flies emerged from each group were then recorded and analyzed.

#### **4.3.2 Experiment II: Reproductive competitiveness of sterile and fertile male *G. pallidipes***

As described above (experiment I), emerged flies were separated into male and female using a chiller set at 4°C. The flies were kept in cages (diameter of 20 cm and width of 5 cm)

with a density of 30-40 flies per cage with netting on top and bottom for feeding and collection of the larvae. the experiment was conducted in such a way that newly emerged males were given irradiation with dose of 120Gy and same age of newly fertile males were mated sterile to fertile males at different ratios (9 sterile to 1 fertile male, 7 sterile to 3 fertile males, 5 sterile to 5 fertile males and 3 sterile to 7 fertile males) were allowed to mate with females in different cages that contain 30 females each. On the other hand, 10 fertile males and 10 sterile males were allowed to mate into 30 females as a control. Mating was hence done at a 1:3 (male: female) ratio. The experiment was designed in a completely randomized design (CRD) in three replications (Appendix 3). Insects were kept for 30 days at 75-80%Rh and 23-25<sup>o</sup>c. Abortion of egg/larvae from days 20- 30 and number of pupae produced which started from day 18-30 day were recorded at time of feeding. Feeding was done three times a week using 96% irradiated bovine blood (Feldmann, 1994). After day 30, female flies were dissected to observe blockages of pupae in the uterus and findings were recorded from each group as number of pupae produced. Similar to experiment I, the pupae collected from each group were maintained at 75-80% Rh and 23-25<sup>o</sup>c for 25-30 days (Feldmann *et al.*, 1992). The number of adult flies emerged from each group were recorded and analyzed.

#### **4.3.3 Experiment III: Survival of sterile male *G. pallidipes* versus age of emergence**

Similar to experiments I and II, emerged flies were separated into male and female using a chiller set at 4<sup>o</sup>c. The flies were kept in cages (diameter of 20 cm length and width of 5 cm) at a density of 30-40 flies per cage with netting on top and bottom for feeding and collection of the larvae. The emerged males were divided into groups of 20 insects each. The males were irradiated at different ages of emergence using a dose 120Gy. The 1st groups of 20 male flies were kept in a cage at the 1st day of emergence and irradiated with 120Gy while the 2nd and 3rd groups of 20 male flies were irradiated after three and ten days of emergence, respectively. As a control group, 20 non-irradiated (fertile) male flies were used. Feeding was done three times a week using 96% irradiated bovine blood (Feldmann, 1994). Feeding was finally quitted and survival was recorded on days 2, 5 and 8 after 10 days of

emergence. The experiment was designed in a completely randomized design (CRD) in three replications (Appendix 4).

#### **4.4 Statistical Analysis**

Data entry and analysis were done using Microsoft Excel and SPSS Version 15.0 respectively. Data were transformed using Arcsine transformation when necessary. one way ANOVA was used to observe the effects of treatment (irradiation) on number of pupae, number of F1 progeny, number of abortion of egg/larvae and the effect of treatment (irradiation) on survival of male fly. Significant difference between the means was separated using Tukey's honestly significant difference (HSD) test (Gomez and Gomez, 1984).

## **5. RESULTS**

### **5.1 Effects of gamma irradiation on reproductive performance of male *G. pallidipes* against dose.**

#### **5.1.1 Study on irradiation versus abortion of egg/larvae.**

Results on the rate of aborted egg/larvae on females mated with males irradiated with different doses over a period of 20- 30 days are presented in Table 2. The number of aborted egg/larvae in females mated with males irradiated at dose of 80Gy was not significantly different from those treated with 100Gy, 60Gy and the controls over 20 days of observation after treatment ( $P > 0.05$ ) whereas, Males irradiated with 100Gy and mated with females

however caused significantly higher abortion than those treated with 60Gy and the controls (P< 0.05) (Table 2).

The number of aborted egg/larvae in cages following irradiation using doses of 80Gy, 100Gy, and 120Gy for 30 days of observation after inseminating /mating females with sterile male in the female cages caused significantly higher than those irradiated with 60Gy dose and the controls (P< 0.05) (Appendix 5). However, the numbers of aborted egg/larvae were not significantly different in males treated with doses of 80Gy, 100Gy and 120Gy mated to females over 30 days of observation after treatment (P> 0.05) (Table 2).

As presented in Table 2, abortion of egg/larvae in females mated with males treated with doses, 100Gy and 120Gy was not significantly different over 20, 25 and 30 days of observation after treatments (P> 0.05). Similarly, females mated with males irradiated at a dose of 60Gy did not show significant different in abortion of egg/larvae over 20, 25 and 30 days of observation after treatment (P> 0.05). Flies irradiated with doses including 80Gy, 100Gy and 120Gy caused abortion of egg/larvae significantly higher than those treated with 60Gy and the controls for 30 day of observation after treatment. In the present study, the highest rate of aborted egg/larvae was observed at highest dose (120Gy) (Table 2).

Table 2: The magnitude of egg/larvae abortion in female flies mated with sterile male at different dose of irradiation over days of observation after treatment.

Treatments	Abortion of egg/larvae over days after treatment (mean± se)		
	20	25	30
60Gy	0.33± 0.33c	1± 0.58cd	0.33± 0.33b
80Gy	1.33± 0.33bc	2.33± 0.33bc	2.67± 0.67a
100Gy	2.33± 0.33ab	3.67± 0.33b	3.67± 0.33a
120Gy	3.00± 0.58a	5.33± 0.33a	4.33± 0.33a

Control	0.00± 0.00c	0.33± 0.33d	0.00± 0.00b
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Mean within the column followed by the same letters are not significantly different at  $\alpha = 0.05$ , Tukey Student Test (HSD).

### 5.1.2 Effects of irradiation on pupae production over observation periods.

The number of pupae produced by females mated with males irradiated with different doses over a period of 18- 30 days and the blocked pupae found during dissecting females at 30 days after treatment are presented in Table 3. Results showed that for 18 day of observation after treatment, insects (males) treated with irradiations doses of 60Gy, 80Gy, 100Gy, 120Gy mated with females resulted in significantly lower number of pupae than the controls ( $P < 0.05$ ) (Appendix 6). However, there was no significant between insects (males) treated with different doses mated to females in terms of pupae production over a period of 18 days observation after treatment ( $P > 0.05$ ).

The number of pupae produced in female mated with males treated by 80Gy, 100Gy and 120Gy doses were significantly lower than the controls over 18, 22, 26 and 30 days of observation after treatments ( $P < 0.05$ ). However, the number of pupae produced by females mated with males treated with doses of 80Gy, 100Gy and 120Gy were not significantly different on the same days of observation after treatment ( $P > 0.05$ ) (Table 3).

The results showed that there was no significant difference in pupae production in flies treated with doses 60Gy, 80Gy, 100Gy, and 120Gy over 26 days of observation after treatment. But the highest pupae reduction was observed in females mated with males treated with dose of 120Gy (Table 3) as expected.

As presented in Table 5, the number of pupae produced by females mated with males irradiated at dose of 60Gy was not significantly different from 80Gy, 100Gy and the controls over 22 days of observation after treatment ( $P > 0.05$ ) whereas, in males treated with 80Gy and 100Gy mated with females, the number of pupae produced were significantly lower than the control over 22 day of observation after treatment ( $P < 0.05$ ) (Table 3).

Moreover, numbers of blocked pupae during dissecting was recorded in females mated with males irradiated at a dose of 80Gy was not significantly different from those males treated with 120Gy, 100Gy and 60Gy ( $P>0.05$ ). On the other hand, the number of blocked pupae in insects treated with 60Gy mated with females was significantly higher than 120Gy and 100Gy irradiated males ( $P<0.05$ ).

Results also showed that, the number of blocked pupae found in females during dissecting recorded in males treated with 80Gy, 100Gy and 120Gy mated with females were significant lower than the controls ( $P<0.05$ ). However, the number of blocked pupae found in females mated with males treated with 80Gy, 100Gy and 120Gy were not significantly different ( $P>0.05$ ). In general, results of these experiments showed that production of pupae was inversely proportional to doses.

Table 3: Mean number of pupae produced by females after mating with treated males against doses of irradiation.

Treatments	Pupae produced over days after treatment (mean± se)				Blockage of pupae
	18	22	26	30	
60Gy	0.67±0.33b	3.33±0.33ab	3.33±0.88b	4.33±0.33a	2.67±0.33ab
80Gy	0.33± 0.33b	2± 0.58bc	1.67±0.33b	2± 0.58b	1± 0.58bc

100Gy	0.00± 0.00b	0.33±0.33bc	1.33±0.33b	0.67±0.33b	0.67±0.33c
120Gy	0.00± 0.00b	0.00± 0.00c	0.67±0.33b	0.33±0.33b	0.33± 0.33c
control	3± 0.58a	5.33± 1.45a	6.33±0.88a	6±1a	4± 0.58a

Mean within the same column followed by the same letters are not significantly different at  $\alpha = 0.05$ , Tukey Student Test (HSD).

### 5.1.3 Effects of irradiation on adults fly emergence.

Results on number of emerged adults from pupae collected after treated males with different doses mated with females from 25-30 days of observation are presented in Table 4. The number of emerged adults from all treated males with doses, 60Gy, 80Gy, 100Gy, 120Gy mated with females and the number of collected pupae were significantly lower than the control over 25 days of observation after treatment ( $P < 0.05$ ) (Appendix 7). But the comparison on the number of emerged adults emerged from pupae produced by females mated with males treated by different doses were not significantly different over 25 days of observation after treatment ( $P > 0.05$ ). Similarly, for all days of observation after treatment the number of emerged adults from collected pupae, produced by females mated with males treated by 80Gy, 100Gy and 120Gy were not significantly different ( $P > 0.05$ ) (Table 4).

Emergence of adults from collected pupae produced by females mated with males treated with dose of 80Gy over 30 day of observation after treatment were not significantly different than males treated with doses of 60Gy, 100Gy and 120Gy ( $P > 0.05$ ). However, males treated with 60Gy mated with female, adult emerged from pupae were significantly higher than from those treated with 100Gy and 120Gy over 30 days of observation after treatment ( $P < 0.05$ ). Results showed that emergence of adults from pupae produced by treated males were inversely proportional to doses.

Table 4: Mean number of adults flies emergence from pupae produced by female mated with males subjected to different irradiations doses.

Treatments	Adult emergence versus days after treatment (mean± se)		
	25	27	30
60Gy	1.33± 0.33b	4.33± 0.33b	2.33± 0.33b

80Gy	0.00± 0.00b	1.67± 0.33c	1.67± 0.33bc
100Gy	0.00± 0.00b	0.33±0.33c	0.33± 0.33c
120Gy	0.00± 0.00b	0.33± 0.33c	0.00± 0.00c
control	5± 0.57a	8.33± 0.33a	3.67± 0.33a

Means within the same column followed by the same letters are not significantly different at  $\alpha = 0.05$ , Tukey Student Test (HSD).

## 5.2 Reproductive competitiveness of sterile to fertile male *G. pallidipes*.

### 5.2.1 Effects of sterility (irradiation) on abortion of egg/larvae.

The number of aborted egg/larvae at different ratios of sterile to fertile males mated with females at different days of observations is presented in Table 5. Results showed that number of aborted egg/larvae in females mated with groups contains 9 sterile to 1 fertile male, 7 sterile to 3 fertile males and 5 sterile to 5 fertile males for 25 and 30 days of observation after treatment were significantly higher than the batch consisting 3 sterile to 7 fertile males and all fertile positive control males ( $P < 0.05$ ) (Appendix 8).

Comparison of two groups, (3 sterile to 7 fertile males and all fertile positive control) for all days of observations after treatment in terms of number of aborted eggs/larvae were not significantly difference ( $P > 0.05$ ). Similarly, for all days of observation after treatment, groups containing 7 sterile to 3 fertile males and 5 sterile to 5 fertile males were not significantly difference ( $P > 0.05$ ) in number of aborted egg/larvae (Table 5).

The number of aborted egg/larvae in females mated with groups contains 9 sterile to 1 fertile male were not significantly different from those with 7 sterile to 3 fertile males, 5 sterile to 5 fertile males and all sterile negative control over 20 day of observation after treatment ( $P > 0.05$ ) whereas, 7 sterile to 3 fertile males and 5 sterile to 5 fertile males were significantly lower than all sterile negative control (10 sterile males with 30 females) over 20 day of observation after treatment ( $P < 0.05$ ). This experiment showed that the number of aborted egg/larvae was observed to be higher in groups containing greater numbers of sterile males than fertile males.

Table 5: Abortion of egg/larvae at different ratio of sterile to fertile males mated with females against days of observation post treatment.

Treatments (Sterile : fertile males ratio)	Abortion of egg/larvae by day after treatment (mean± se)		
	20	25	30
9 : 1	3.33±0.33ab	4±0.58a	4.33± 0.33b
7 : 3	1.67± 0.33bc	4± 0.58a	3.67± 0.33b
5 : 5	1.67±0.33bc	3.67±0.33a	3.33± 0.33b
3 : 7	0.00± 0.00c	1± 0.00b	0.67±0.33c
0: 10	0.00± 0.00c	0.33± 0.33b	0.00± 0.00c
10: 0	3.67±0.67a	5±0.58a	6± 0.58a

Mean within the same column followed by the same letters are not significantly different at  $\alpha = 0.05$ , Tukey Student Test (HSD).

### 5.2.2 Effects of sterile to fertile males mated with females on pupae production

Results on the number of pupae produced by females mated with sterile to fertile males at different ratios over a period of 18- 30 days and the numbers of blocked pupae from dissected females after 30 days are presented in Table 6. The number of pupae produced by females mated with sterile to fertile males at a ratio of 7 sterile to 3 fertile males was not significantly different from a ratios of 9 sterile to 1 fertile male, 5 sterile to 5 fertile males, 3 sterile to 7 fertile males and all sterile negative control over 26 and 30 days of observation after treatment ( $P > 0.05$ ). On the other hand, 5 sterile to 5 fertile males and 3 sterile to 7

fertile males mated with females produced pupae significantly higher than group contain 9 sterile to 1 fertile male and all sterile negative control over 26 and 30 days of observation after treatment ( $P < 0.05$ ) (Table 6).

The number of pupae produced by females mated with males in the ratio of 9 sterile to 1 fertile male and all sterile negative control for all days of observation after treatment were not significantly different ( $P > 0.05$ ). Similarly, the number of pupae produced by females mated with males for groups containing 3 sterile to 7 fertile males and the fertile positive control were not significantly different over 18 and 30 days of observation after treatment ( $P > 0.05$ ) (Table 6). Even though, groups containing 9 sterile to 1 fertile male mated with females caused reduction of pupae, the difference was not statistically different from those groups containing 7 sterile to 3 fertile males and 5 sterile to 5 fertile males over 18 day of observation after treatment.

Moreover, the number of blocked pupae found in females groups contain 9 sterile to 1 fertile male, 7 sterile to 3 fertile males and 5 sterile to 5 fertile males groups had no significant difference as compared with all sterile negative controls ( $P > 0.05$ ) (Appendix 9). Results showed that the number of blocked pupae observed by dissecting females mated with males at a ratio of 5 sterile to 5 fertile males was not significantly different from the other groups including, 9 sterile to 1 fertile males, 7 sterile to 3 fertile males, 3 sterile and 7 fertile males and all sterile negative control ( $P > 0.05$ ). However, similar to the batch containing 3 sterile to 7 fertile males mated to females, the number of blocked pupae during dissecting was significantly higher than the batches consisting 9 sterile to 1 fertile male, 7 sterile to 3 fertile males and all sterile negative control ( $P < 0.05$ ). In sum, the results in this experiment showed that the increased number of pupae produced by females was directly related with groups containing greater number of fertile males mated with females.

Table 6: Mean number of pupae produced by females mated with fertile and sterile males at different ratios against days of observation after treatment.

Treatments (Sterile : fertile males ratio)	Pupae produced over days after treatment (mean ± se)				Blockage of pupae
	18	22	26	30	
9 : 1					

7 : 3	0.00± 0.00b	0.67±0.33de	0.67±0.33c	0.33± 0.33c	0.33± 0.33c
5 : 5	0.33±0.33ab	2± 0.00d	2± 0.58bc	1.33±0.33bc	0.67±0.33c
3 : 7	0.67±0.33ab	4.33±0.33c	3.67±0.33b	2.33±0.33ab	1.33±0.33bc
0: 10	2± 0.58a	6.33± 0.33b	3.67±0.88b	2.67±0.33ab	3± 0.58ab
10: 0	2± 0.58a	9.00± 58a	7±1a	3.33±0.33ab	4± 0.58a
	0.00± 0.00b	0.33±0.33e	0.67±0.33c	0.00± 0.00c	0.33± 0.33c

Mean within the same column followed by the same letters are not significantly different at  $\alpha = 0.05$ , Tukey Student Test (HSD).

### 5.2.3 Effects of sterile to fertile males mated with females on emergence of adults from pupae.

The emergence of adults from pupae produced by females after mating with different ratio of sterile to fertile males from day 25-30 is presented in Table 7. Results showed that the number of adults emerged from pupae produced by females mated with all groups of sterile to fertile males ratio and all sterile negative control were significantly lower than all fertile positive control(10 fertile males allowed to mate 30 females) over 30 and 27 days of observation after treatment ( $P < 0.05$ )(Appendix 10).

Over 27 and 30 days of observations after treatment, the emergence of adults from pupae produced by groups containing lower number of fertile males (9 sterile to 1 fertile male and 7 sterile to 3 fertile males) were significantly lower than groups containing greater number of fertile males (5 sterile to 5 fertile males and 3 sterile to 7 fertile males) ( $P < 0.05$ ). But, comparing each ratio with in the groups (in lower number of fertile male ratios and higher number of fertile males ratios), showed not significant difference in number of emerged adults ( $P > 0.05$ ) (Table 7).

Results in this part of the experiments showed that number of emerged adults from pupae produced by females mated with males at the ratio of 5 sterile to 5 fertile males and 3 sterile to 7 fertile males were not significantly different from those with 9 sterile to 1 fertile male, 7 sterile to 3 fertile males, all sterile negative control and all positive fertile control over 25 days of observation after treatment ( $P > 0.05$ ) (Table 7). In the contrary, batches consisting of

9 sterile to 1 fertile male, 7 sterile to 3 fertile males and all sterile negative control were significantly lower than all fertile positive control over the same days of observation after treatment ( $P < 0.05$ ). This experiment showed that emergence of adults from pupae produced by large number of sterile males ratio mated with females was inversely proportional.

Table 7: Adult flies emergence from pupae produced by sterile to fertile males mated to females versus day of observation.

Treatments (Sterile : fertile males ratio)	Adult emergence day after treatment (mean± se)		
	25	27	30
9 : 1	0.00± 0.00b	0.33±0.33c	0.00± 0.00c
7 : 3	0.33± 0.33b	1.33± 0.33c	1.33± 0.33c
5 : 5	0.67±0.33ab	4.67±0.33b	4± 0.58b
3 : 7	1.33± 0.33ab	5.33± 0.67b	5.33±0.33b
0: 10	2± 0.58a	8.33± 0.33a	9± 1.16a
10: 0	0.00± 0.00b	0.33±0.33c	0.00± 0.00c

Mean within the same column followed by the same letters are not significantly different at  $\alpha = 0.05$ , Tukey Student Test (HSD).

### 5.3 Effects of gamma irradiation on survivals of male *G. pallidipes* over days of emergence following treatment

Results on survivals of adult male *G. pallidipes* irradiated with the dose of 120Gy at different ages of emergence over a period of 2-8 days are presented in Table 8. The number of survived sterile males following irradiation at the dose of 120Gy males irradiated one day after emergence and males irradiated ten days after emergence were significantly lower than males irradiated three days emergence after emergence and the control over 2 and 8 days of

observation after treatment ( $P < 0.05$ ). However, the number of survived sterile males after irradiation at 120Gy males irradiated one day after emergence was not significantly different from males irradiated ten days after emergence. Similarly, the number of those emerged on day three sterile males were not significantly different from the control for 2 and 8 days of observation after treatment ( $P > 0.05$ ) (Table 8 ).

The number of survived sterile males in all treatments (males irradiated one days after emergence, males irradiated three days after emergence and males irradiated ten days after emergence) were not significantly different from the control over 5 days of observations after treatment ( $P > 0.05$ ) (Appendix 11). In general, this experiment showed that adult males irradiated three days post emergence survived more than males irradiated one aged and ten aged after emergence.

Table 8: Mean number of survival males after ten day of feeding by irradiation at different age of emergence.

Treatments	mean± se Survival by days after treatment		
	2	5	8
Males irradiated one days after emergence	17± 0.58a	9.67±0.33a	2.33± 0.33a
Males irradiated three days after emergence	19.33±0.33b	9.33±0.33a	4.67± 0.67b
Males irradiated ten days after emergence	17±0.58a	9.00±.58a	1± 0.56a
control	19.67±0.33b	9.67±1.20a	5.33±0.33b

Mean within the same column followed by the same letters are not significantly different at  $\alpha = 0.05$ , Tukey Student Test (HSD).

## 6. DISCUSSION

Tsetse flies (Glossinidae) are vector for transmission of the parasites trypanosome causing Nagana among domestic animals and sleeping sickness among human has made them one of the most devastating insect in Africa and Ethiopia in particular.

The results in the present work revealed that the highest dose (120Gy) treated males mated with females showed significantly higher abortion of egg/larvae compared to the lowest dose (60Gy) and untreated control. However, the general trend of the results showed a general increase on aborted egg/larvae in females mated with treated males as dose increased. Previous studies indicate that, radiation may be used as genetic control involving the release of irradiated sterile males so that most of the females will lay sterile eggs (Hasan and Khan, 1998) The present laboratory based results show that the irradiation effects on male *G. pallidipes* at different doses (60Gy, 80Gy, 100Gy and 120Gy) mated with females in that the proportion of aborted egg/larvae was dose dependent. These relations showed that sterility with a highest dose (120Gy) may have more effects on the normal reproduction performance of females leading to decreased number of flies over time.

The present investigation indicated that irradiation had effects on male *G. pallidipes* subjected to different doses (60Gy, 80Gy, 100Gy and 120Gy). The results showed that both the number of pupae produced and the number of adults emerged from pupae produced by females mated with sterile males were inversely proportional to dose. This is in agreement with that by Parker and Mehta (2007) in which they indicated that radiation dose absorbed by an insect to induce sterility is the main advantage in sterile insect release program. They further examined that insects those receive too low dose may not be sterile effectively. Other studies also indicate that Irradiation of male insects may result in dominant lethal mutations in the sperm, killing spermatogonial cells, inactivation of sperm and weakening of males (Coleman and Alphey, 2004). The current results show that at low dose (60Gy) irradiated males mated with females have produced pupae and emerged to adult more or less similar to the controls. However, in the highest dose (120Gy), there was a significant reduction in the number of pupae produced by females mated with sterile males and in the number of emerged adults when compared with those subjected to the lowest dose (60Gy) and the untreated controls.

These results may indicate that in order to fully eradicate tsetse fly using SIT, males need to be treated with highest doses to cause significant reduction of the next generation of the target population. The results of the current experiments showed that production of pupae and emergence of adults from pupae produced by females mated with treated males were inversely proportional to doses, whereas the numbers of aborted egg/larvae were positively correlated to dose.

In addition, observation on the reproductive competitiveness of male *G. pallidipes* at different ratio of sterile males and fertile males mated with female with the higher number of sterile male than fertile male (9 sterile to 1 fertile male) per cage showed significant effects on egg/larvae abortion compared to the lowest number of sterile and fertile males (3 sterile to 7 fertile males) and to positive control. The general trend of the result showed that increase sterile males per cage than fertile males led to increased abortion of egg/larvae. Among the different ratios of sterile males and fertile males used, the highest number of sterile to fertile (9 sterile to 1 fertile male) was found to have higher effect over the post treatment observation days. A similar previous study indicated that parous female flies when mated with sterile males show imbalance between their uterus content and development of the follicle next in the normal ovulation sequence (A, B, C, D) i.e. uterus becomes empty due to expulsion of a dead embryo or with a degenerating egg in uterus classified as having mated with a sterile males (Van den Bossche *et al.*, 2000). The present results also showed that females mated with sterile males had more abortion of egg/larvae at higher number of sterile males in the cage than fertile males.

The research also shows that in sterile: fertile ratio (9: 1) has resulted in 90% reduction in pupae production and 95% reduction in emergence to adult. On the other hand, 7 sterile to 3 fertile males ratio lead to 75% reduction in pupae production when compared with that in which the normal males mated with females. These indicate that increasing sterile males in the female cages increases the competitive of sterile males to mate to femals than fertile male under the current lab condition. Previous recommendations on sterile males to fertile males ratio in SIT for tsetse fly eradication requires a ratio of 10 sterile to 1 wild male whereas a ratio of 3 sterile to 1 fertile male is recommended for control programs (not for eradication) (Takken *et al.*, 1986). Curtis (1995), stated that in the 10: 1 ratio on strategy, the overwhelming number of sterile males would limit the reproduction of the natural population in proportion to the ratio of sterile to fertile insect (with a 1:1 ratio and a 9:1 ratios, the reproductive capacity of the natural population is reduced by 50% and 90% respectively).

The current laboratory experiments also indicated that groups containing 9 sterile to 1 fertile males and 7 sterile and 3 fertile males ratio caused reduction on the number of flies by affecting the normal reproductive activity of female leading to the reduction of pupae and that emerged to adults. On the other hand, groups containing greater number of fertile male

ratios had greater number of blocked pupae. Previous workers advise that in order to achieve population eradication using SIT technology in each generation, the ratio of sterile to wild insects should increase and the technique becomes more efficient with lower population densities (inversely-density dependent) (Nagel, 1995).

The present study showed that sterile males following 1, 3, and 10 days of emergence survived up to 8 days without food under lab condition indicated that males irradiated three days after emergence from pupae showed more survived from those males irradiated at one and ten days of emergence from pupae. Previous studies show that flies are capable of surviving for more than seven weeks and indicated a population half-life of 7days for the first six weeks. This assures us that released sterile males live long enough to have opportunity to mate with wild females and to locate hosts in the wild (Msangi *et al.*, 1999). The present experiments also indicated that males irradiated three days after emergence survived similar duration to the control after treatment. This may indicate that irradiated males may need to rest up to 7 or 10 days before released to the field.

In the current study, the importance of sterilization using appropriate dose, the appropriate ratio of sterile to fertile male and the time at which sterility was done efficiently all are relevant this is in agreement to that by Vreysen *et al.*, (2000), who stated that SIT relies on the production of large numbers of the target insect in specialized production centers, the sterilization of the males pupae or adult fly (or sometimes both sexes), and the sustained and systematic release of the sterile males over the target area in numbers large enough in relation to the wild male population to out compete them for wild females.

## **7. CONCLUSIONS AND RECOMMENDATIONS**

### **7.1 CONCLUSIONS**

The present laboratory studies showed that irradiation effects on male *G. pallidipes* at different doses (60Gy, 80Gy, 100Gy and 120Gy) mated with females showed that the number of aborted egg/larvae was directly proportional with dose. Results indicated that to eradicate tsetse fly using SIT the highest dose (120Gy) irradiated males mated with females made efficient causing abortion of egg/larvae, reduction of pupae and reduced the number of emerged adult from pupae than the lowest doses.

Similarly this study showed that the number of aborted eggs/larvae was higher in groups containing greater numbers of sterile males than fertile males. On the other hand, the number of pupae produced and adults emerged from pupae was lower in groups where sterile males were more than fertile males mated with females. The effect was inversely proportional.

The present laboratory assessments showed that cage with higher sterile males than the fertile males resulted in larger number of egg/larvae abortion and increased reduction on pupae produced and number of pupae emerged to adult.

On the other hand groups containing greater number of fertile male ratios than sterile male had greater number of blocked pupae. Generally, this experiment showed that, the number of pupae produced by females was directly proportional to the number of fertile males mated with female.

The present experiments also indicated that males irradiated three days after emergence was more survived than one day after emergence and ten days after emergence after treatment. This may indicate that irradiated males may need to rest up to 7 or 10 days before released to the field.

## **7. 2 RECOMMENDATIONS**

- ❖ In this study male *G. pallidipes* was irradiated with a dose of 120Gy mated with female caused reduction in number of pupae produced and emerged to adult. Further studies with extended period would help verify these results.
- ❖ The lowest dose of irradiation with 60Gy males mated with females lead to the production of higher number of pupae and higher number of pupae that emerged to adult. This dose however needs to be verified under field condition at small scale before a final conclusion is made.
- ❖ In this investigation groups containing 9 sterile to 1 fertile male and 7 sterile to 3 fertile males mated to females were able to eradicate and control of tsetse flies respectively. This again needs further investigation in the field.
- ❖ However, further research is needed that combines sterile to fertile males ratio with other factors such as feeding, temperature and humidity that determines the releasing of sterile male flies.
- ❖ To ensure the sterility of *G. pallidipes* in a <sup>60</sup>Co Gamma cell 2000 the decay time must be calculated each time of irradiation.
- ❖ To know the efficiency of sterility of male to eradicate tsetse fly under field condition further field research is needed before mass field release.

## **8. REFERENCE**

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## 9. APPENDICES

Appendix 1. Experimental flies in cages.



Appendix 2. Experimental setup in Insectory to study reproductive performance of sterile male *G. pallidipes* at different dose of irradiation.



Appendix 3. Experimental setup in insectary to study reproductive competitiveness of sterile and fertile males *G. pallidipes*



Appendix 4. Experimental setup in insectory to study survival of sterile male *G. pallidipes* versus age of emergence



Appendix 5. Analysis of Variance (ANOVA) at different doses for abortion of egg/larvae over 20, 25 and 30 days of observation after treatment.

		Sum of square	df	Mean square	F	Sig.
Number of aborted egg/larvae at day 20	Between Groups	19.600	4	4.900	12.250	.001
	Within Groups	4.000	10	.400		
	total	23.600	14			
Number of aborted egg/larvae at day 25	Between Groups	49.067	4	12.267	26.286	.000
	Within Groups	4.667	10	.467		
	total	53.733	14			
Number of aborted egg/larvae at day 30	Between Groups	41.600	4	10.400	19.500	.000
	Within Groups	5.333	10	.533		
	total	46.933	14			

Appendix 6. Analysis of Variance (ANOVA) at different doses for production of pupae over 18, 22, 26, 30 and blockage of pupae after 30 day of observation after treatment.

		Sum of square	df	Mean square	F	Sig.
Pupae observation after 18 day	Between Groups	19.067	4	4.767	14.300	.000
	Within Groups	3.333	10			
	total	22.400	14	.333		
Pupae observation after 22 day	Between Groups	58.400	4	14.600	9.125	.002
	Within Groups	16.000	10			
	total	74.400	14	1.600		
Pupae observation after 26 day	Between Groups	62.000	4	15.500	13.676	.000
	Within Groups	11.333	10	1.133		
	total	73.333	14			

Pupae observation after 30 day	Between Groups	71.333	4	17.833	17.833	.000
	Within Groups	10.000	10	1.000		
	total	81.33	14			
Blockage of pupae dissected female	Between Groups	28.933	4	7.233	12.056	.001
	Within Groups	6.000	10	0.600		
	total	34.933	14			

Appendix 7. Analysis of Variance (ANOVA) at different doses for emergence of adult over 25, 27 and 30 days of observation after treatment.

		Sum of square	df	Mean square	F	Sig.
Emerged adult after 25 day	Between Groups	51.600	4	12.900	32.250	.000
	Within Groups	4.000	10	.400		
	total	55.600	14			
Emerged adult after 27 day	Between Groups	138.667	4	34.667	104.000	.000
	Within Groups	3.333	10	.333		
	total	142.000	14			
Emerged adult after 30 day	Between Groups	24.000	4	6.000	18.000	.000
	Within Groups	3.333	10	.333		
	total	27.333	14			

Appendix 8. Analysis of Variance (ANOVA) at different ratio of sterile to fertile males for abortion of egg/larvae over 20, 25 and 30 days of observation after treatment.

		Sum of square	df	Mean square	F	Sig.
Number of aborted egg/larvae at day 20	Between Groups	30.500	5	6.100	12.200	.000
	Within Groups	6.000	12	.500		
	total	36.500	17			
Number of aborted egg/larvae at day 25	Between Groups	52.667	5	10.533	17.236	.000
	Within Groups	7.333	12	.611		
	total	60.000	17			

Number of aborted egg/larvae at day 30	Between Groups	77.333	5	15.467	39.771	.000
	Within Groups	4.677	12	.389		
	total	82.000	17			

Appendix 9. Analysis of Variance (ANOVA) on different ratio of sterile to fertile males for production of pupae over 18, 22, 26, 30 days and blockage of pupae after 30 days of observation after treatment.

		Sum of square	df	Mean square	F	Sig.
Pupae observation after 18 day	Between Groups	13.167	5	2.633	5.925	.006
	Within Groups	5.333	12	.444		
	total	15.500	17			
Pupae observation after 22 day	Between Groups	176.444	5	35.289	90.743	.000
	Within Groups	4.667	12	.389		
	total	181.111	17			
Pupae observation after 26 day	Between Groups	86.278	5	17.256	14.118	.000
	Within Groups	14.667	12	1.222		
	total	100.944	17			
Pupae observation after 30 day	Between Groups	23.611	5	4.722	14.167	.000
	Within Groups	4.000	12	.333		
	total	27.611	17			
Blockage of pupae dissected female	Between Groups	35.611	5	7.122	12.820	.000
	Within Groups	6.667	12	.556		
	total	42.278	17			

Appendix 10. Analysis of Variance (ANOVA) at different ratio of sterile to fertile males for emergence of pupae after 25, 27 and 30 days of observation.

	Sum of square	df	Mean square	F	Sig.
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Emerged adult after 25 day	Between Groups	12.444	5	2.489	8.960	.001
	Within Groups	3.333	12	.278		
	total	15.778	17			
Emerged adult after 27 day	Between Groups	158.278	5	31.656	63..311	.000
	Within Groups	6.000	12	.500		
	total	164.278	17			
Emerged adult after 30 day	Between Groups	188.278	5	37.656	39.871	.000
	Within Groups	11.333	12	.944		
	total	199.611	17			

Appendix 11. Analysis of Variance (ANOVA) on survival of sterile male after 2, 5 and 8 days of observation.

		Sum of square	df	Mean square	F	Sig.
Survival after 2 day stop feeding	Between Groups	18.917	3	6.306	9.458	.005
	Within Groups	5.333	8	.667		
	total	24.250	11			
Survival after 5 day stop feeding	Between Groups	.917	3	.306	.204	.891
	Within Groups	12.000	8	1.500		
	total	12.917	11			
Survival after 8 day stop feeding	Between Groups	36.667	3	12.222	16.296	.001
	Within Groups	6.000	8	.750		
	total	42.667	11			

