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Comparative Gamma Radiation Sensitivity of *Glossina pallidipes* and *G. fuscipes fuscipes* Species in Kaliti Tsetse Fly Mass Rearing and Irradiation Center, Addis Ababa, Ethiopia

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Abstract

The study was conducted in Kaliti Tsetse fly Mass Rearing and Irradiation Center in November 2018 with the aim of determining the radiation dose of Co60 gamma in the *G. pallidipes* and *G. f. fuscipes* species in tsetse fly factory. The effect of gamma cell radiation dose ranged from 100gy-140gy 4-6 days old adult males of *G. pallidipes* and *G. f. fuscipes* was studied. Fecundity of their mates was reduced by 95% following exposure to 120gy of adult male of two species. The number of pupae produced and adults emerged from pupae were lower in groups where sterile males were higher than fertile males and the effect was inversely proportional the insemination ability of the males and sperm motility were not adversely affected by the radiation dose. The study showed that irradiation effects on tsetse flies at different doses (100Gy, 120Gy, and 140Gy) mated fertile females showed that the number of aborted eggs/larvae was directly proportional with dose. In conclusion, in tsetse fly eradication campaign using SIT, the higher dose at least 120Gy irradiated males should be used.

Keywords: Gamma Radiation; *G. F. Fuscipes; G. pallidipes*

Introduction

Tsetse flies are vector for transmission of the parasites causing human and animal trypanosomosis has made them one of the most devastating insect in Africa, 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^2 of sub-Saharan Africa and the trypanosomes they transmit can cause severe illness in livestock and people [1]. According to [2], 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 and 120,000 km, respectively. Tsetse infested areas lie in the lowlands and in the river valleys of Abay (Blue Nile), Baro, Akobo, Didessa, Ghibe and Omo [3].

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) [4].

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 (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. 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^2 Unguja Island [5].

The SIT is no 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 if adequate quality assurance is practiced in the production process and the sterile insects cannot be established in the released areas as with other biological control programs. 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 [6].

The genetic technique is one of several methods that could be useful in providing more effective and acceptable solutions to key

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insect problems. Genetic control is the introduction of genetic factors into pest population's that reduce or eliminate the pest problem by mating. 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 [7] or treatment of the natural population with an agent that will induce sterility in the native insects.

Cytoplasm incompatibility is one of the genetic control techniques in which cytoplasm 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 sterility induces 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 [8]. Therefore, the study was organized to determine radiation dose of Co60 gamma in the *G. pallidipes* Uganda and *G. f. fuscipes* strains and to investigate the dynamics of the follicle development in females after exposure to sterilizing dose of Gamma cell.

Materials and Methods

Experiment flies

All flies used for the experiment were derived from the tsetse fit mass rearing colonies on membrane feeding system, at Kality tsetse fly rearing and irradiation center Addis Ababa Ethiopia. The flies were kept in the same insectarium at temperature of 23-24 degree Celsius and relative humidity of 75-80% for *G. pallidipes* and 22-23˚C for *G f. fuscipes*. Radiation procedure and experimental design a C060 Gamma cell providing a dose rate of 100-140gy were used for all treatments. Males were given an irradiation dose of 100 129 and 140gy on a day 4-6 age following emergence. The experiment was with dose ranging given in procedure steps of 100, 120 and 140gy on day following emergence, male were mated in-group of 100-140gy at mating ratio 1:1 with 2-3 days virgin females flies.

Radiation procedures and experimental design: A60Co Gamma cell provided a dose rate 100-140gy used for all treatment. Males were ready for radiation on day 4-6 following the emergence. Male and female kept together for 3 days in standard colony cage after three days separation pooled in individual cage. Their survival and production was checked daily for 55 days. Larviposition receptacles were checked for expelled eggs and mature larval stages every days starting on days 15 days following emergence.

All pupae were weight on the next day and recorded on larviposition date. The end of experiment period all female were dissected and their reproductive status assed. The motility of sperm was recorded by visual observation of ruptured spermathecae under a compound microscope. Fecundity was expressed as the number of pupae produced per mature female day, taking day 18following emergence as the first larviposition day. After mating, control and treated males were held in individual cage to monitor their survival.

Statistical analysis

As the study was descriptive, the statistical analysis was exclusively descriptive and limited to the point and interval estimates along with frequency summary.

Table 1: The fecundity of two tsetse fly species irradiated with different doses.

Species	Dose in Gy	Female	Female survival (%)	Abortion (%)	Fecundity
G.pallidipes	100	25	88	20	0.008
	120	25	84	16	0.006
	140	25	72	10	0.001
G.f. fuscipes	100	25	84	22	0.003
	120	25	76	18	0.001
	140	25	52	14	0

Results

Reproduction of untreated female mated with male irradiated as adult. Reproductive female performance for the two species are presented in Table 1. For both the control and experimental groups, minimum survival of the female at the end of the experimental period was above 88%, 72%for *G. pallidipes* and 84%, 76% and 52% for *G. f. fuscipes* respectively. The radiation treatment did not affect the insemination ability of the male of the two species. The average life span of untreated and treated males of two species is depend upon radiation dose. The average life spans of untreated males survive significantly longer as compared to treated males. High radiation dose were required to decrease the life span.

Discussion

The expected proportional of dominant lethal indicated in the sperm of male *G. pallidipes* and *G. f fuscipes* by gamma radiation increased with increasing doses. A dose of 100, 120 and 140gy administered to 4-6 days old males following the emergence respectively induced 95% sterility in the two species.

The induction of dominant lethal mutation in 100-140gy treated males' sperm was expressed in the female mated by a high rate of extruded dead embryos. In addition examination of reproductive status of female of the two species almost absence of immature larvae in the uterus. These finding show as the sterilizing gamma dose inhibits development in the tow species during early or advanced stage s of embryogenesis and are in accordance with the effect of radiation.

The results in the present work revealed that females mated with the higher doses (120Gy and above) treated males showed significantly higher abortion of egg/larvae compared to the lowest dose treated (100Gy) 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 increases. Radiation may be used as genetic control involving the release of irradiated sterile males so that most of the females will lay sterile eggs [9]. The results showed that the irradiation effects on the given male *Glossina* species at different doses (100-140Gy) mated with females in that the proportion of aborted egg/larvae was dose dependent. These relations showed that sterility with a higher dose (120Gy and above) 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 the male *Glossina* species subjected to different doses (100-140Gy).

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 [10] in which they indicated that radiation dose absorbed by an insect to induce sterility is the main advantage in sterile insect release program. Studies also indicated that irradiation of male insects may result in dominant lethal mutations in the sperm, killing spermatogonial cells, inactivation of sperm and weakening spermatogonial cells, inactivation of sperm and weakening of males [11].

In this study, females mated with males irradiated at low dose (100Gy) have produced pupae and emerged to adult more or less similar to the controls. However, in the higher doses (120Gy and above), 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 (100Gy) and the untreated controls.

These results may indicate that in order to fully eradicate tsetse fly using SIT, males need to be treated with higher doses (120Gy and above) 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 number of aborted eggs/larvae was positively correlated to dose [12,13].

In this study, higher doses resulted in significant reduction of female fecundity. Mews et al. [14] and Jordan [15] also indicated females mated with irradiated male were had very low fecundity.

In the current study, all are important that the sterilization using appropriate dose, the appropriate ratio of sterile to fertile male and the time at which sterility was done efficiently are relevant. This is in agreement to that by Vreysen et al. [16], 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.

Conclusion

The study showed that irradiation effects on tsetse flies at different doses (100Gy, 120Gy, and 140Gy) mated fertile females showed that the number of aborted eggs/larvae was directly proportional with dose. Females mated with males irradiated at higher dose were more efficient, resulted in high abortion of eggs/larvae, reduction of pupae, and reduced the number of emerged adult from pupae than the lower irradiation doses. In addition, the number of pupae produced and adults emerged from pupae were lower in groups where sterile males were higher than fertile males and the effect was inversely proportional. Therefore, to ensure the sterility of *G. pallidipes* Ethiopia, *G. pallidipes* and *G. fuscipes* in a Co Gamma cell 200 60 the decay time must be calculated each month of irradiation. In eradicate tsetse fly eradication campaign using SIT, the higher dose at least 120Gy irradiated males should be used.

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