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Review Article

Sustainability Assessment of the Integrated Biological Methods for Tsetse Fly (*Glossina spp*) Control in Sub-Saharan Africa

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ABSTRACT

Tsetse flies (Diptera: Glossinidae) are the major important vectors of the trypanosomes, which causes nagana or African Animal Trypanosomiasis (AAT) and Human African Trypanosomiasis (HAT) or sleeping sickness. The disease affects most rural communities in Sub-Saharan Africa (SSA) where it affects both human and animal health, as well as agricultural production. Due to the higher costs of disease treatment, the risk of drug resistance, the residual effects of insecticides on the environment and the lack of effective vaccines, tsetse fly control remains the most efficient and sustainable method to control trypanosomosis. Among the existing control methods available, the use of the Sterile Insect Technique (SIT) in the frame of area-wide integrated pest management technique (AW-IPM) has been successful in most areas. Also, the integration of SIT with Entomopathogenic Fungi (EPF) and auto-sterilization by using Insect Growth Regulators (IGR) seems to be the most efficient and sustainable method to suppress the tsetse flies' population. In this essay, I examine the current methods that involve integrated biological control of tsetse flies and discuss the efficacy and strategies for their use in order to suppress the tsetse population.

Keywords: Tsetse fly; Trypanosomosis; Parasitoid; Biological control; Vector

INTRODUCTION

Tsetse flies (Diptera: Glossinidae) are the major important vectors of the trypanosomes, which causes nagana or African Animal Trypanosomosis (AAT) and Human African Trypanosomosis (HAT) or sleeping sickness, that affects most rural communities in Sub-Saharan Africa (SSA). The mammalian host gets the infection through the bite of an infected tsetse fly, and the intensity of the disease varies between species and geographical locations. According to the World Health Organization (WHO), there are approximately 10,000-45,000 cases of HAT each year, whereby 60 million people are at higher risk in 36 countries, covering almost 10 million km^2 in Sub-Saharan Africa (Figure 1). On another hand, approximately 3 million cattle die every year and about 50 million cattle and tens of millions of small ruminants are at risk of HAT in SSA [1].

The impacts of African trypanosomosis can be viewed in a broad perspective, where it affects both human and animal health as well as agricultural production. The occurrence of the disease in infested areas has major impacts in socio-economy of the rural community and national income at large. The deaths of animals due to trypanosomosis reduces the amount of manure and draught power required for farming which results in the decrease in agricultural production.

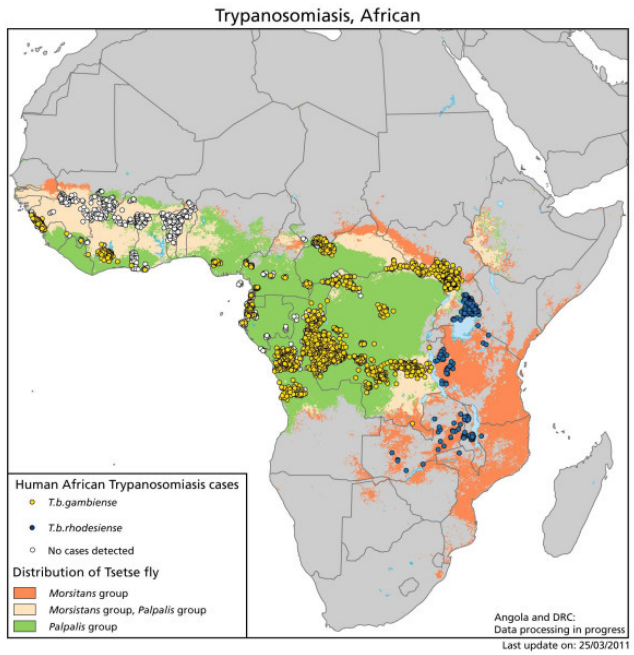

Figure 1: Map showing distribution of *Glossina spp* and spread of Trypanosomosis cases in SSA.

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It is approximated that each year the costs for control of trypanosomosis, and the direct losses in milk yield and meat production are between US $600 million and $1.2 billion. Also, it is approximated that the total agricultural productivity decreases for about US$4 billion each year due to the decrease in draught power [2]. Successfully eradication of tsetse flies in SSA will allow the expansion of marginal lands which are currently unsuitable for animal and agricultural production. So far, there is a crude relationship that the increase in livestock numbers by 50% may increase the agricultural output by 10% in the infested areas [3].

Control of trypanosomosis in SSA has been undertaken since the beginning of the 20th century, when initiatives were on removing the tsetse preferred habitats. This method involved the slaughter of game animals and the destruction of areas of woodland in the infested areas. Although this method was quite successful, it was discouraged due to being environmentally unfriendly. Since then, different strategies to eradicate the disease prevalence were carried out by developing vector control measures, developing vaccines and the use of trypanocidal drugs were carried out, but none has been documented to control the disease at a sustainable level. Generally, vector control measures developed ranged from the use of chemicals insecticides, and mechanical and biological control as well as the combination of either of these methods. Chemical control involves synthetic pyrethroids, and aerial/ground spraying of DDT, dieldrin, endosulfan and other effective insecticides in different countries in SSA, and has shown a decrease in the number of tsetse. However, the chemical control method was limited due to the environmental pollution, labour intensive and high costs of the application, and possibilities of re-invasion of the controlled areas. Similarly, chemical control of animals against trypanosomosis with trypanocidal drugs has been limited due to the high costs of drugs and the challenge of continuous drug-resistance in most SSA countries [4].

In recent years, the strategies for tsetse fly control have been changing, whereby governments no longer give much support for insecticides spraying campaigns. Instead, much emphasis is given on the development and application of sustainable methods to control the vector with less environmental effects. Also, due to the higher costs of disease treatment, the risk of drug resistance, residual effects of insecticides to the environment and lack of effective vaccines, biological control remains the most efficient and sustainable method to control trypanosomosis [1].

The present control method involves the use of Sterile Insect Technique (SIT) in the frame of Area-wide Integrated Pest Management (AW-IPM) and has been successful in most areas, though it is constrained by mass production of sterile males and its application in large areas. Similarly, biological control of tsetse by using symbionts and pathogens such as Entomopathogenic Fungi (EPF), bacteria symbionts and salivary hypertrophy virus which modulate the fecundity of the infected tsetse has shown promising outcomes [1]. On the other hand, the use of trypanotolerant breeds of cattle has decreased the prevalence of the disease in most areas, but the breed is constrained by low production and productivity. Other successful control methods used under small-scale operations involves odour-baited tsetse traps or screens impregnated with insecticides and colours and auto-sterilization of tsetse by using growth regulators and chemosterilants in trapping devices [3,4].

In this essay, I examine the current methods that involve integrated biological control of tsetse flies, and discuss the efficacy and strategies for their use in order to suppress tsetse population.

DESCRIPTION OF THE VECTOR (*GLOSSINA*) AND THE PARASITE (*TRYPANOSOMA SPP*)

Tsetse flies (genus *Glossina*), are strictly hematophagous insects of the family glossinidae and the order diptera. They are mainly found in Sub-Saharan Africa, infesting an area approaching 10 million km^2, and 38 countries (Figure 1) [5]. Tsetse flies are classified into 33 taxa, of which 30 taxa have well-defined habitats in SSA. Within the defined taxa, there are 22 species, of which 6 specie groups are further sub-divided into 14 subspecies. Furthermore, tsetse flies have been divided into three major categories based on their adaptation and ecological distribution in SSA. These include *Glossina morsitans* (savanna subgenus), *Glossina fusca* (forest subgenus) and *Glossina palpalis* (riverine subgenus) [6].

Both male and female tsetse are exclusively feeding on blood and normally takes a fresh blood meal after every 3 to 4 days from vast host option ranging from humans, domestic and wild mammals and reptiles depending on the availability. During blood feeding tsetse ingests the trypanosomes from the infected animal host that will undergo several cycles of development within the vector and then transmitted to another host during feeding. However, among the dipterans tsetse has the longest life expectancy of up to 7 to 9 months; with an average of one to two months depending on species, nutrient availability and environmental condition. During the lifetime, a male tsetse can mate several times while a female mate only once. After a single mating, the sperms are stored in an organ called spermathecae in the female reproductive tract and will last for their lifetime.

The mode of reproduction in tsetse termed as adenotrophic viviparity, associated with slow reproductive rates, whereby a single fertilized egg is hatched and reared within the uterus until it reaches the third instar larvae stage. On average, a female tsetse delivered a matured larva after every ten days that pupates and burrow into the soil immediately after delivery, and develops into an adult after 25-55 days. Also, on average, a female tsetse produces between 3 to 6 matured larvae during her lifetime depending on nutrient availability and environmental condition. However, due to the unique way of reproduction in tsetse flies, where there is no eggs and free larvae stage in nature, and the occurrences of pupal stage under the soil, the adult stage remain the most accessible stage for various control methods [7,8].

Generally, the transmission of AAT and HAT involves the interaction between four organisms; the wild animal reservoir, domestic animal, the insect vector (tsetse fly), the pathogen parasite (Trypanosoma spp) and the human host. However, the duration of the life cycle of trypanosomes (Figure 2) within the vector and the host's body depends on the species and temperature condition. So far, due to the vector roles of tsetse flies, they facilitate the linkage between these organisms by carrying the pathogen from one host to another. And therefore, this implies that successful eradication of tsetse will significantly reduce the cases of trypanosomosis in both humans and animals [9,10].

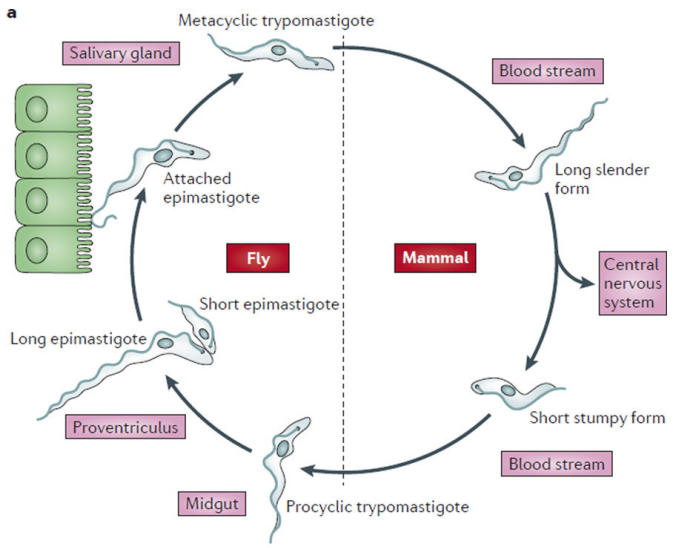

Figure 2: The life cycle of *Trypanosoma brucei* within the host and vector.

In reality, only few species of tsetse fly are playing a vector role of trypanosomes that cause HAT and AAT. These include G. *palipalis palipalis*, G. *palidipes*, G. *morsitans morsitans*, G. *morsitans centralis*, G. *fuscipes fuscipes*, and G. *tachinoides*. However, among the group only two species play a significant vector role in trypanosomosis in most SSA countries (Figure 1). G. palpalis is the major vector of *Trypanosoma brucei* gambiense that transmit HAT, and represents about 98% of declared cases in the Western and Central African countries. Similarly, G. *morsitans* is the major vector of *Trypanosoma brucei* brucei, *Trypanosoma brucei* rhodesience and Trypanosoma congolense that cause both AAT and HAT [11].

INTEGRATED CONTROL OF TSETSE FLIES, WITH EMPHASIS ON BIOLOGICAL CONTROL

The term Biological Control (BC) is defined as 'the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be [12]. In terms of applied biological control, we can also define BC as an ecological control method whereby a man tries to use the naturally occurring antagonists to lower the pests or parasites populations to acceptable and non-harmful level. Generally, the BC of insects involves the direct attack by symbionts, pathogens, predators (e.g. spiders), parasites and parasitoids (insect parasites of insects). Normally, BC method has no direct impact to the pathogenic parasites but indirectly regulates their parasitic stages in the insect vector and the host animal [13].

On another hand, there are genetic control methods which involve Sterile Insect Technique (SIT) and selection for host resistance. Selection for host resistance involves the breeds of cattle that are resistant to trypanosomiasis. The trypanotolerant cattle breeds may survive in trypanosomosis endemic areas due to the high natural immunity against the disease. On another hand, some of the BC methods such as auto-sterilisation regulates the parasitic stages of the parasites by the combination of growth hormones and chemical substances with less environmental effects [13]. Furthermore, it is recommended that the effective BC method should be effective, cheap, fast and easily applicable in all conditions [14].

However, the use of predators and parasitoids as BC methods was not successfully in tsetse fly control after the release in 1920's, and also there is only a few literature reports on these techniques [15]. In this essay, I have not discussed the use of predators and parasitoids as BC.

THE USE OF SYMBIONTS AND PATHOGEN

In nature tsetse harbours numerous pathogens such as bacteria, fungi and viruses. Different studies have been conducted to modify the bacterial symbionts, Entomopathogenic Fungi (EPF) and viruses under laboratory conditions and applied as biological control of tsetse fly. EPF are able to infect tsetse fly easily through the cuticle while the bacteria and viruses have to be ingested and penetrate the midgut to cause infection. However, the use of bacteria and viruses in the field is constrained by the difficulties in application and bringing the pathogens in contact with the adult tsetse fly [14,15].

THE USE OF TSETSE FLY SYMBIONTS

The survival of tsetse fly requires means to supplement a single source of food (vertebrate blood) in order to get all essential nutrients required by the body. Based on this fact, tsetse flies have developed a symbiotic association with bacterial microbes in order to supplement the required nutrients for their survival. The symbiotic relationship starts during the development of larvae in the uterus, whereby the larva is nourished with milk as well as three distinct endosymbiotic bacteria are supplied from its mother. The three symbiotic bacteria identified in different tsetse fly species include Wigglesworthia, Sodalis and Wolbachia. The Wolbachia are transmitted to the larvae transovarially while the Sodalis and Wigglesworthia are acquired by the larvae *via* the intrauterine route from the mother's milk glands. By doing so, there is a strict vertical transmission of the symbiotic bacteria from one generation to another. In this way, BC control aimed at suppression of these symbiotic bacteria which are essential for tsetse fly survival and reproduction. Suppression of the symbiotic bacteria in tsetse fly can be attained by using antibiotics supplements in tsetse fly diets in order to kill the bacteria symbionts. The use of antibiotic supplements has proven to kill Wigglesworthia glossinidia and result to reduced fecundity among the target population [16].

THE USE OF ENTOMOPATHOGENIC FUNGI (EPF)

Generally, there is a close association between different species of fungi and tsetse fly in nature. Although the association and level of pathogenicity between EPF and tsetse vary between different species of fungi. For instance, the obligate bacterial symbionts and salivary hypertrophy viruses are able to modulate the fecundity of the infected tsetse fly. On another way, most entomopathogenic fungi have lethal effects where they infect tsetse fly through the cuticle and can be transferred from one individual to another through simple contact. The most important feature of EPF is that they should be able to penetrate and proliferates inside the insect and deprive the nutrients in the hymolymph in the digestive system. At the same time, the EPF should release toxins which will kill an insect. Example of the EPF in this group includes the Beauveria and Metarhizium species [14].

There are few comprehensive ecological studies reported about the use EPF as a BC measures. In Table 1 below I have highlighted some of the important fungal pathogen species reported from

Glossina *spp* under laboratory and field conditions [14]. Biological control by EPF was implemented since the first half of 20th century, whereby the use of *Absidia repens* and *Penicillium lilacinum* fungus has reduced hatchability of pupariaa of G. congolensis in central Africa Republic by 45%-50%. Similarly, the application of phytomycetes fungus reported to cause mortality of G. *morsitans* in Tanzania in 1930's. In the list of fungus provided in Table 1, all EPF species are facultative fungus, which means that they are not host specific for completion of their life cycle.

Table 1: Multivariable logistics regression of factors associated with prevention practice of C0VID-19 among childbearing age (n=493) in Debre Tabor Town, Northwest Ethiopia, 2020.

S/N	Fungus species	Glossina spp.	Country
1	*Cicadomyces sp.*	G. *tachinoides*, G. *palpalis*,	Congo and Uganda
2	*Cicadomyces sp.*	G. *morsitans*	Germany (laboratory)
3	*Candida sp.*	G. *morsitans*	Portugal (laboratory)
4	*Cryptococcus*	G. *morsitans*	Portugal (laboratory)
5	*Torulopsis sp.*	G. *morsitans*	Portugal (laboratory)
6	*Rhodotorula sp.*	G. *morsitans*	Portugal (laboratory)
7	*Beauveria bassiana*	G. *pallidipes*, G. *fuscipes*	Kenya
9	*Absidia repens*	G. *congolensis*	Central Africa Rep.
10	*Penicillium lilacinum*	G. *congolensis*	Central Africa Rep.
11	*Penicillium sp.*	G. *pallidipes*	Kenya
12	*Aspergillus niger*	G. *pallidipes*	Kenya
13	*A. flavus sp.*	G. *pallidipes*	Kenya
14	*A. ochraceus*	*Glossina sp.*	Chad
15	*Aspergillus sp.*	G. *pallidipes*	Kenya
16	*Fusarium sp.*	G. *pallidipes*	Kenya
17	*Fusarium semi-tectum var. Majus*	*Glossina sp.*	South Africa
18	*Mucor sp.*	G. *pallidipes*	Kenya
19	*Rhizopus sp.*	G. *pallidipes*	Kenya
20	*Trichoderma sp.*	G. *pallidipes*	Kenya
21	*Phycomycetes*	G. *palpalis and* G. *morsitan*	Tanzania
22	*Phycomycetes*	G. *brevipalpalis*	Somalia
23	*Ascomycetes*	G. *fuscipes*	Uganda
24	*Fungi Imperfecti*	G. *fuscipes*	Uganda
25	*Unidentified sp.*	G. *palpalis*	Ghana, Nigeria and DR Congo

STERILE INSECT TECHNIQUE (SIT)

Sterile Insect Technique (SIT) is among the genetic control methods that are carried out on the area-wide basis. SIT in tsetse control involves the production of a large number of sterile males in the specialized production centres, followed by systematic release into the target population in a relative proportion to the indigenous target population so as to out-compete for wild females. In the production centres, males are sterilized by low doses of radiation at the appropriate stage in order to reduce their reproduction fitness prior to release to the target area. Successful mating of a sterile male tsetse with the native female tsetse results to infertility throughout their lifetime. This will result in decreased tsetse population densities and an increased ratio of sterile males to wild males within each generation [17]. Furthermore, it is recommended that the ratio of released sterile male to wild males should be at least 2:1, while in some circumstances should be increased to 15:1 where there are higher population densities [17].

Initial studies on SIT conducted in the 1960's in Zimbabwe and 1970's in Burkina Faso and Chad indicated that the method was effective in suppression of savannah and riverine species of tsetse fly. Similarly, initial studies conducted in Tanzania reported that the release of sterile to wild male at an average ratio of 1.12:1 was effective against G. m. morsitans in a 195 km^2 savannah area [8]. Since then SIT was used in different countries and has contributed in the suppression of tsetse fly population. However, successful application of SIT in tsetse eradication requires much attention on the quality of sterilised males. After the release, the sterilised males should be able to intermingle and compete in mating at the same rate with the wild tsetse population. In the studies conducted in Burkina Faso and the Island of Unguja indicated that the sterilized males of G. *p. gambiensis* and *Glossina austeni* respectively, were able to congregate in the same ecological niche with the wild population and was very successful [8].

AUTO-STERILIZATION OF TSETSE BY USING INSECT GROWTH REGULATORS (IGR)

Auto-sterilization is an integrated BC method which involves regulation of the parasitic stages of the parasites by chemical substances with less environmental effects as compared to chemical insecticides. Auto-sterilisation in tsetse control involves the sterilisation of tsetse fly by using insect juvenile hormones and growth regulators impregnated in traps. The use of hormones in BC has good persistence and possibilities of transmission from male to female tsetse during mating. However, this method does not kill the insect, but instead they affect their capacity to reproduce, and thus the eradication of tripanosomosis requires long time [14].

Oloo et al., reported that the use of Insect Growth Regulators (IGR) namely permethrin and triflumuron impregnated in traps, were successfully used in the suppression of G. *fuscipes fuscipes* in Buvuma Island in Lake Victoria, Uganda. The application of triflumuron at a dose of 0.5 micrograms per tsetse arrested four reproductive cycles (45 days). The effects of triflumuron reported include abortion of eggs and fully grown larvae which fail to develop to viable puparia.

SELECTION FOR HOST RESISTANCE

Selection for host resistance involves the use of trypanotolerant breeds of cattle which show slightly lower mortality as compared to trypanosusceptible breeds when exposed to trypanosomosis endemic areas. According to FAO trypanotolerant breeds has low mortality and reductions in calving rate (up to 10% and 1 to 12%) as compared to (10%-20% and 11%-20%) for trypanosusceptible breeds. Based on this fact trypanotolerant breeds are being promoted in different African countries especially in the highly endemic areas. However, most trypanotolerant breeds are constrained by slow growth, small body size, lower milk yield and productivity. Also, the resistance to trypanosomosis decrease when the cattle are

exposed to stress factors such as ploughing and insufficient feeds during the drought period [17].

DISCUSSION

The use of EPF has a greater opportunity in BC due to easy applicabilities by simple contact, as compared to bacteria and viruses which have to be ingested by tsetse and penetrate through mid-gut in order to be effective [14]. This method is environmentally friendly and there is no study report for tsetse-resistant against EPF. The EPF has been applied in the field and has shown potential in the suppression of G. *fuscipes fuscipes* in areas around Lake Victoria, Kenya [15].

On another hand, the SIT is environmentally friendly and species-specific technique, with no adverse effects on non-targeted organisms, and also, there is no evidence of the development of resistance reported. The SIT can be used in the frame of area-wide integrated pest management (AW-IPM), integrated with biological techniques such as EPF and produce the best results since both are environmentally techniques. The AW-IPM in tsetse fly control was successful in the eradication of G. austeni from Unguja Island of Zanzibar, which was finalised by the release of sterile male flies, and the island is now declared as tsetse-free.

However, the SIT requires efficient release and monitoring programmes applied on an AW-IPM. Also, the use of SIT in tsetse fly control is most effective under low population density and requires sufficient knowledge on the ecology and biology of the targeted specie. In addition, the effectiveness of SIT requires that the tsetse fly specie to be responsive to mass-rearing on the production station. However, due to low reproductive potential of tsetse, it becomes challenging in rearing large numbers and requires efficient release and monitoring strategies.

For successful application of SIT, it requires much consideration on the quality of the released sterilised males, they should be able to intermingle very fast and compete in mating at the same rate with the wild tsetse population [8]. Also, it is recommended that the ration of released sterile male to wild males should be at least 2:1 and in some circumstances should be increased to 15:1 in higher population densities. This imply that SIT is most cost-effective when the target population size is low, which is in contrast with the application of insecticides. Therefore, complementary use of both SIT and conventional method would give a maximum efficiency in eradication of tsetse fly by dividing the control program into two phases. First, tsetse eradiation in large population can be controlled by conventional method and then followed by application of SIT after the decrease of the population density [17].

Similarly, Abd-Alla et al., suggested that it is possible to integrate the SIT with symbionts through paratransgenic processes, that will produce lines of tsetse that are unable to transmit trypanosomes in the future. This could be achieved by establishing an *in vitro* culture condition and introduction of the modified Sodalis symbionts into tsetse fly population.

Furthermore, SIT can be improved by the production of sterile hybrids obtained when crossing two closely related species or subspecies of tsetse to produces semi-sterile females and completely sterile males [11]. Here, it is expected that the cross-bred will have a higher ability to compete in mating with the wild population and hence suppress the target population.

On another way, the use Auto-sterilization of tsetse by using Insect Growth Regulators (IGR) in tsetse fly control is compatible with the SIT. The approach is environmentally friendly as compared to the use of insecticides impregnated traps because it involves the target species only. In addition, the best results can be achieved by sterilising both sexes as compared to the release of males only [18].

When SIT is incorporated into AW-IPM, it is very important to avoid the use of insecticides and remove the impregnated traps before the release of sterilised tsetse so as to avoid mortality [18]. However, based on the cost-benefit analysis it can be concluded that SIT is economically superior to conventional methods when we consider a longer time frame. The technique is also attractive to developing countries due to possibilities to overcome the high costs of pesticides importation and also, it creates labour opportunities in the production centres. In addition, the SIT provides environmental and economic benefits equally for small-scale farmers, and large commercial farmers and the whole community at large [19].

CONCLUSION

The use of SIT and integration with EPF and IGR in bait technologies (traps and targets) are potential methods of AW-IPM and the most sustainable approach to control tsetse fly, due to the following reasons: (1) no evidence of development of resistance reported, (2) there is no effects to non-target biodiversity, (3) there is less pollution to the environment and food chain and (4) there is no health hazard reported during the application. In addition, it is very important to plan for the application of the control programme into separate phases to avoid mortality of the sterilized males. Furthermore, exploring genetic basis and selection of tripanotolerant breeds of cattle could become possible to incorporate the trait into other breeds and hence generating transgenic cattle. However, this will depend on the acceptability of the innovation.

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