INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN CHEMISTRY AND PHARMACEUTICAL SCIENCES
(p-ISSN: 2348-5213: e-ISSN: 2348-5221)
www.ijcrcps.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijcrcps **Coden: IJCROO(USA)** **Volume 12, Issue 8- 2025**

Research Article

DOI: http://dx.doi.org/10.22192/ijcrcps.2025.12.08.003

Comparing the efficiency of different odor baits for tsetse flies survey in Deme River Valley in South West Ethiopia: - an implication for trypanosomosis control

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Abstract

Tsetse fly transmitted African Trypanosomosis is closely linked to the Public health problem as that was reported in Deme valley and also associated to deep rooted poverty in the endemic areas. So this research was conducted aiming to compare the efficiency different odor baits for surveying tsetse flies in the Deme river valley, Gamo Zone, Ethiopia. 180traps were set up in three *Kebeles* near the valley, using both baited and un-baited traps. The bait locations were rotated each month to minimize the bias. The daily mean of tsetse flies caught was used to assess the effectiveness of various odor baited traps. The comparison of the efficiency of baited traps was done using the ANOVA Test. Of the 1208 tsetse flies captured, 751 (62.2%; 95% CI: 59.4-64.9) were *G. pallidipes* and 457 (37.8%; 95% CI: 35.1-40.6) were *G. fuscipes*. The most successful odor bait for *G. pallidipes* was a mixture of cow urine and acetone, with the mean catch of 3.4 flies/trap/day (F = 26.8; P<0.0001), followed by urine (2.1 flies/trap/day) (F = 20.5; P <0.0001) and acetone (1.3 flies/trap/day) (F = 14.7; P<0.0001), compared to 0.43 flies/trap/day for the control. Traps baited with acetone, cow urine, and a combination of cow urine with acetone increased the catch rate by 3.2x, 5.1x and 8.3x respectively. Traps baited with all odor baits did not increase the number of catches for *G. fuscipes*.

Keywords: *Glossina pallidipes, Glossina fuscipes*, Odor baited traps, Deme River Valley, Ethiopia

Introduction

Tsetse flies, a cyclical vector of African trypanosomosis have infected 36 countries and a total of 10 million square kilometers of fertile and arable land in 36 African countries. For centuries, they have had a great impact on human and animal health in Africa. Throughout these areas the disease transmitted by the tsetse fly, is seriously affecting the livestock production [1]. They are largely responsible for an uneven distribution of cattle in Africa, leading to overgrazing and severe environmental degradation in some areas and preventing the introduction of productive farming and livestock systems in other areas. Direct losses in meat production and milk yield and the costs of programs to control trypanosomosis are estimated to be very high [2]. Avoiding the problem related with trypanosomosis in Africa improve overall agricultural production and gradually increase the benefits to US $4.5 billion per year [3]. According to the World Health Organization, over 55 million people living in rural areas of sub-Saharan Africa are at risk of contracting tsetse transmitted human African sleeping sickness [4]. About 30,000 new cases of sleeping sickness were reported annually but this was extremely under-reported because of poor surveillance. About 180,000 to 200,000 km^2 of agriculturally suitable land, and about 14 million of cattle and small ruminants, nearly 7 million equines and 1.8 million camels are at the risk of trypanosomosis in Ethiopia[5].

Human African trypanosomiasis was first identified in Ethiopia in 1967 around the Baro and Akobo Rivers The causative agent identified was *Trypanosoma brucei rhodesiense[6]*. A 2022 outbreak of African human trypanosomiasis in the Deme River valley also confirmed *T. brucei rhedosiense* as the etiological agent[7][8]. Therefore, entomological surveillance was important for evaluating the abundance of the prevalent species of tsetse flies, their behavioral difference to host odors.

There are five species of tsetse flies in Ethiopia, namely *Glossina morsitans submorsitans, Glossina pallidipes, Glossina fuscipes fuscipes, Glossina tachinoides* and *Glossina longipennis*[9]. Although there are multiple species of tsetse flies that can transmit multiple species of trypanosomes, *Glossina pallidipes* is the most economically important species followed by *G. fuscipes* in East Africa particularly in Ethiopia. As they inhabit the grazing areas (*G. pallidipes*) and watering points (*G. fuscipes*) they are the main vector of Human and Animal African Tryanosomiasis[10].

Identification of tsetse flies was essential to know the species of tsetse flies present in an area considered for AW–IPM. Different tsetse species has different habitat preferences and behavioral differences that needed to be taken into account when planning their control or eradication. Trypanosomiasis is controlled by administration of trypanocidal drugs or by breaking the transmission cycle by control of the vector tsetse fly[11]. But trypanocidal drugs are expensive and their effective administration requires a setup which is often unavailable in Africa. No vaccine is yet available as the trypanosome parasite changes surface coat antigen[12]. Various techniques have been used to control or eradicate tsetse fly. In the last 25 years, there have been clearance of host game animals and bush habitat, but these methods are now environmentally undesirable[19]. Control of tsetse by release of sterile males has been tried, but this technique requires sophisticated equipment which is difficult to sustain in most African countries including Ethiopia[14]. This technique is also not particularly appropriated for tsetse because the slow rate of reproduction of the flies makes it hard to rear the huge numbers required to be released in the field. At the present time tsetse fly control is being carried out by using insecticides and insecticide free traps[15]. The insecticide free control method is better than that of insecticide method as affects only the targeted population. Traps have their own efficiency to attract tsetse fly visually. Certain odors motivated activity in tsetse and influenced the direction of flight to the device that can kill or catch flies. The direction of flight of tsetse taking off in the presence of certain wind-borne odors shows a significant shift to windward. The efficiency of traps could be improved2-11 times with different olfactory baits like acetone, more than three weeks fermented cow urine and their blends[16]. Riverine species of tsetse can be effectively trapped with devices that employ only visual cues[17]. However, savannah species depend more on olfactory cues, and use of trapping techniques with these species has required innovation of effective odor attractants[18]. In order to have an efficient tsetse fly control strategy, the scarce and an expensive tsetse control resources allocation and taking immediate control action must be in accordance with trypanosomosis risk which is related to tsetse flies' density. The animals and vector contact always happens when a vector tries to feed on bloods of preferred animals[19]. In Ethiopia, few studies have attempted to determine the efficiency of odor baits in for surveying different species of tsetse flies while no studies were performed in the current study area.

Therefore, the current study evaluated the tsetse fly tsetse species composition andthe efficiency of different odor baits for tsetse fly sampling or controlling.

Materials and Methods

Study area description

The study was conducted in South Ethiopia in the Deme River Valley, Gamo Zone. The Deme River basin contains several small and large streams. The largest river is the Deme River, which feeds the Kulano River. At Dana Kebele, the Gogara River meets the Deme River, and the combined rivers flow into the Omo River Valley. This ecology creates an optimal environment for the multiplication of tsetse flies. The study area has a sub-humid climate with moderately high temperatures and an average yearly rainfall ranging from 900mm to 1800mm [8]. The dry season stretches from December to March, while the wet season is from April to November. The highest recorded temperature was 32.8°C. The cooler months are June, July, and August, with the lowest temperature being 17.5°C. The three Kebeles for our study are in rural areas near to the savanna grasslands and riverine forests, which provide homes for various wild animals. Domestic animals such as cattle, goats, and sheep are common in the study area. Thus, they might encounter tsetse flies in the savanna grassland and forest while they are grazing. *Glossina pallidipes* and *G. f. fuscipes* have been identified in the Deme River Valley [3,8]. AAT has been identified as a major challenge in the valley, and several programs have been initiated to control tsetse fly populations. The programs involved were removing shrubs that were believed to be tsetse fly habitats. In 1997, the Southern Tsetse Eradication Project (STEP) initiative launched a tsetse control plan that introduced insecticide-impregnated targets, ground spray, aerosol, and cattle pour on using synthetic pyrethroids [9]. Every two to three months, 1% deltamethrin was applied to the backs of cattle. Sterile males (SIT) of two species of tsetse fly (*G. pallidipes* and *G. f. fuscipes*) were released by aircraft every week for seven years since 2011. During 2011-2014, pour-on and other insecticide-based methods for controlling tsetse fly populations were halted due to concerns that insecticide treated animals might kill the sterile male tsetse flies released into the field. This interruption led to a significant increase in trypanosome infection in cattle, and farmers in the area have expressed dissatisfaction with the renewed tsetse fly suppression activities following the rise in tsetse flies. HAT was documented in the same valley in 2022 [4].

Study design and period

An entomological study was conducted from September 2024 to February 2025 to collect tsetse flies using baited NGU traps. The sampling occurred over 48 hours each month, with the attractant locations being rotated monthly. Ten NGU traps were placed in selected locations monthly in each Kebele. Three of the ten traps were baited with acetone, three with cow urine that had been fermented for three weeks, and three more with a combination of cow urine and acetone. One trap was installed without any odor bait. The feet of the traps' central and lateral poles were greased to deter predators. Fly sampling took place for six months, and there were 180 trapping events conducted in the three Kebeles, with 60 in each. We performed monthly randomization and rotation of odor baits to minimize variation due to trap location. This was achieved by creating ten lottery cards with codes representing odor baits. Upon arriving at a trap location, a card was randomly drawn, and the corresponding odor was used. Each position had an equal chance of receiving any odor in the initial round. However, in subsequent rounds, the odor bait used in the previous month was excluded from the draw for the same position to avoid the repetition of the same odor in each location. The distance between the traps and Kebeles was calculated using the Global Positioning System (GPS) and ranged from 200 to 250 meters.

Tsetse fly sampling

Ten Kebeles near the Deme River Valley are infested with tsetse flies, and of these, three Kebeles (Gale, Sikole, and Dana) were randomly selected for the study. NGU traps were placed in the tree sheds 20 cm above the ground to capture the tsetse flies. Acetone was released from glass bottles vial with aperture size of 6mm in diameter rate of 500 mg/h, and three weeks old cow urine was released from plastic bottles vial with aperture size 45mm diameter at rate of 1000mg/hour[20]. The trapping sites were kept open to allow the flies to see the traps. When there was no open location to deploy the traps, the vegetation obstructing trap visibility was cut within a 3–6 meter radius around the trap, depending on the height of the vegetation. The traps were set up in various locations, including riverbanks, forests, and grasslands. The typical habitats include communal grazing areas with wooded grassland and riverine forest. Each trap in the field was labeled with the date, collection site, and bait type and then transported to the nearby animal health center. The flies in each odor baits were counted, sexed, and identified for species.

To assess the relative abundance of tsetse flies at each trapping site, the apparent density or daily mean catch was estimated as the total number of tsetse flies caught per trap per day.

The index factor computed for the tested attractants was defined as the factor by which the mean number of catches using the various attractants was compared relative to the control (unbaited traps).

Tsetse identification

The tsetse control personnel training manual key for Glossina species identification was used to distinguish between different species of tsetse flies [10]. Adult tsetse flies possess unique morphological traits that differentiate them from other insects. These features include a distinct proboscis, folded wings at rest with hatchet cells, and branched arista hairs on their antennae. Glossina species can be identified based on crucial characteristics such as the color of their hind and front legs' tarsal segments and the color and form of the dorsal surfaces of their abdomen, with or without banding. It's possible to distinguish male flies from females by examining their abdomen. The male fly has a hypopygium on the posterior end of the ventral abdomen, which is absent from the underside of a female tsetse [10].

Outcome variables

The number of tsetse catch in association different baits were the primary outcome variable. The species of tsetse fly was secondary outcome variable.

Data analysis

Apparent density of each odor baitwas calculated by dividing total fly catch per trap number per number of days. The index factor was calculated by comparing or dividing the mean catches of baited traps to the mean catches of control traps. T-test was used to compare the mean catches among the three study sites and baits used. Conventional data on vector distribution, apparent densities from the three sites was subjected one way ANOVA to statistical test using SPSS version 20.0 software at 95% confidence interval and 5% level of precision.

Results

Tsetse flies risk abundance in study areas

One hundred and eighty (180) NGU traps (60 traps in each kebele) were placed in three randomly selected kebeles, and 1208 tsetse flies were caught across all locations, yielding 3.36 flies per trap per day. The catch included 382 flies with mean of 1.77 FTD in Gale, 823 flies with mean of 3.81 FTD in Sikole, and 972 flies with mean of 4.50 FTD in Dana Kebele (Table 3 and figure 6). Tsetse density was highest in Dana, followed by Sikole, and lowest in Gale kebele, with a statistically significant difference in the mean catch among the study Kebele P-value = 0.000 (Table 1).

Table 1. Statistics of tsetse fly across different locations

Groups	Kebele	Number of traps(N)	Tsetse fly caught	Mean catch	Mean difference	T	*P-value*
1	Gale	60	212	1.77	2.04	9.08	0.000
	Sikole	60	457	3.81			
2	Gale	60	212	1.77	2.72	10.11	0.000
	Dana	60	539	4.50			
3	Sikole	60	457	3.81	0.68	2.275	0.000
	Dana	60	539	4.50			

Two species of tsetse flies, Glossina fuscipes and Glossina pallidipes, were collected in the study area. The mean catch of Glossina pallidipes (2.09) was considerably higher than that of Glossina fuscipes (1.27). The difference of mean catch between the two species was statistically significant at *P-value* < 0.05.

Odor Bait efficiency in the experiments

As table 2 indicated, traps baited with acetone alone, cow urine alone, cow urine and acetone, and traps without odor baits were caught the daily average of 1.3, 2.1, 3.4, and 0.4G. pallidipes species of tsetse flies, respectively. The mean catch of acetone alone, cow urine alone, cow urine and acetone, and traps without odor baits for G. fuscipes was 1.13, 1.38, 1.30 and 1.3respectively. The overall capture was strongly related with odor baits. The blend of cow urine and acetone collected the most flies, followed by cow urine alone, acetone alone, and traps with no attractants in decreasing order. The difference in attractive efficiency for catch across odor baits was extremely significant with P-value < 0.05 (Table 2).

Table 2: Odor baits efficiency between two species

Odor baits	G. pallidipes					G. fuscipes				
	Number of traps	Number of fly	Mean catch	F	P-value	Number of traps	Number of fly	mean catch	F	P-value
Acetone	54	145	1.3	48.50	0.000	54	119	1.1	0.862	0.461
Cow urine	54	224	2.1			54	153	1.42		
Cow urine with acetone	54	367	3.4			54	140	1.30		
Control	18	15	0.4			18	45	1.25		
Total	180	751	2.1			180	457	1.26		

By ranking the means, the mean catches of cow urine with acetone were determined to be significantly higher for *G. pallidipes* than any other baited or non-baited attractant. However, no statistically significant attraction or repellency of odor baits reported for *G. fuscipes* (Table 2 and Figure 6 below).

An index of increased catch due to different attractants was calculated as the ratio of the mean catch to the comparable response for the unbaited trap.

Table 3:An index of increase for each odor baits

Odor baits	*G. pallidipes*		*G. fuscipes*	
	Mean	Index factor	Mean	Index factor
Control	0.4	1	1.3	1
Acetone	1.3	3.2	1.1	0.85
Cow urine	2.1	5.1	1.4	1.08
Blend of acetone & cow urine	3.4	8.3	1.8	1.4

So for *G.pallidipes* male and females,the mean catches from every attractants were significantly different from each other and catches from blends of acetone and cow urine was significantly higher than the rest of odor baits. But for *G.fuscipes* males and females the mean catch from every odor baits were more not significantly different.

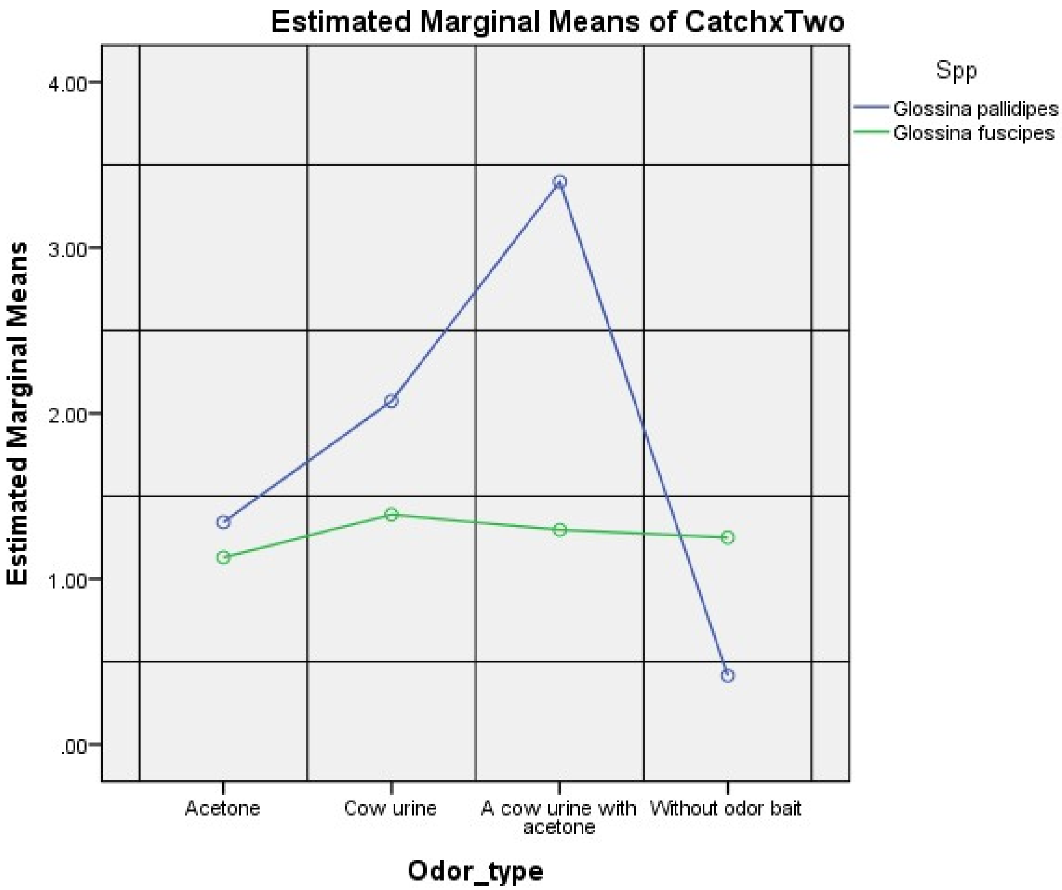

Figure 1: Estimated marginal mean of *G. pallidipes* and *G. fuscipes*

Discussion

The entomological survey revealed that tsetse fly species in the study area are *G.pallidipes* and *G.fuscipes*. Both of the identified fly species in the present study are among the five Glossina species recorded in Ethiopia particularly in SNNPR state [9]. Similar result was stated in Guraferda and Sheko districts [21]*Glossina pallidipes* followed by *Glossina fuscipes* as the only tsetse fly species caught in the study area along with other biting flies. In the study area, there is a suitable micro-habitat for both riverine (*G.fuscipes*) and savannah (*G.pallidipes*) species along the rivers in Deme valley. That was why it has been possible to identify these two species in the study valley.

The recent finding revealed that from a total 180 NGU traps (60 traps in each kebele) deployed in the randomly selected three positions the apparent density of 3.36 flies /trap/day was recorded. Similar result (3.4) was recorded in eastern part of Dangur district [22]. Again the same result(3.41) was found in Bilo Naph district[23]. But this finding was not in lined with (2.86 FTD) that reported by Edget [24] in the controlled areas of the same district. The reason for the difference in the apparent density between the two studies in the sane district may be due to difference in tsetse suppression intensity and variation in season in the area at the time of studies. The apparent density of *Glossina* species ranges from 1.77, 3.81 and 4.49 in Gale, Sikole and Dana respectively. Such wide variations could have been resulted from differences in density of vegetation cover as a result of the expansion of settlements and farmland in the area. It may also be explained by the migration of the game as a result of climate and habitat changes due to settlements. The result indicated that *G.pallidipes* was more abundance (62.2%) than *G. fuscipes*

(37.8%) in the study areas. The reason for the highest proportion of *G.pallidipes* than *G.fuscipes* may be due to either NGU trap used in this study was highly preferred by *G.pallidipes* than *G.fuscipes* as a result of their difference in visual orientation, horizontal orientation for *G.pallidipes* and vertical orientation for *G.fuscipes* or relatively low density of *G.fuscipes* and due to poor response of *G.fuscipes* for odor attractants[21]. Equivalent result was reported in Guraferda and Sheko districts, in which *G.pallidipes* was the dominant species followed by G.fuscipes. Another research in Omo-Ghibe tsetse belt, South Ethiopia by [25], stated same result 'the tsetse flies caught were identified to be *Glossina pallidipes* and *G. f. fuscipes*; however, the former was predominant and widely distributed'.

The efficiency of the traps to *G.pallidipes*(index factor of 3.2-8.3) was high in presence of odor attractants and low in absence of any odor. However, no significant efficiency was observed in the presence of odor for *G.fuscipes*. This agrees with the compiled reports of [26] at Sor Hydroelectric Station Ethiopia. The highest proportion of total flies was caught by the blend of cow urine and acetone followed by cow urine alone and the least flies were caught by traps deployed without any attractants. The result agreed with that report by [27] in Nech Sar National Park Southern Ethiopia in which blends of acetone, cow urine and octenol , and blends of cow urine & octenol increased the catch significantly. In the present finding cow urine alone contributed higher proportion of total catch than acetone alone.This result coincided with the finding by[28]. Again this study agreed with works of [26] at Sor Hydroelectric Station, Ethiopia in which traps baited with cow urine alone and the combination of cow urine and acetone or octenol increased the catch index (2.64–12.54)x. Among the single attractants used cow urine was highly efficient in tsetse fly catching than both acetone alone and control which is similar with that reported by [29]; cow urine was the only one of the attractants that showed a significant increase in most instances.

According to IAEA in Ethiopia, taken individually, cow urine was the dominant odor attractant for *G. pallidipes*, being responsible for nearly 60% of the catches [28]. Generally the catch indices of traps baited with acetone alone, aged cow urine alone and the blends of the acetone and cow urine ranged from 3.2 – 8.3x and it was highly in agreement with (2.64 – 12.54x) that reported by [26] at Sor Hydroelectric power station, Ethiopia. These index factors were lower than that reported by[30] in which the catch was increased 20x. The difference of the index factors between the recent finding and that reported by Hall DR and Valey GA might be as a result of variation of relative abundance of tsetse flies, difference of the combination of odor baits and seasonal variation of the study period. In this trial achieved for Glossina fuscipes, 500mg/hr acetone and 1000mg/hr cow urine were used alone and in combination, the catch number of samples from baited and unbaited (control) traps differed very slightly. There was no significant repellence or attraction effects as the efficiencies were at roughly the unbaited level. This result was in agreement with the result reported by Terfa, Olani and Tamiru [24] at Sor Hydroelectric Power Station, Ethiopia. However, as reported by[31], there was a significant increase catch of blue-black polyethylene bipyramid traps using local zebu urine (x 1.4) in three of the four trails with the greatest effect (x 4.2) obtained for male G. f. fuscipes when the densities of flies were low (less than five male per trap per day) in Central African Republic. The difference between these works may be related to a particular environment of the trails and difference in trap types used.

Conclusion

The overall apparent density of tsetse fly in the study area was 3.36 FTD. To control tsetse flies thereby trypanosomosis, surveillance and monitoring was mandatory. Controlling and monitoring tsetse it requires effective, cost efficient environmentally friendly and affordable tools. Among these tools odor bait was the one that enhanced the efficiency of tsetse catch in the field. From four types of odor baits compared for their efficiency in catching tsetse flies, the blends of acetone and three weeks aged cow urine was the most efficient followed by cow urine alone. The odor baits were not efficient in catching *G. fuscipes*. The study area, Deme River Valley was infested with two Glossina species; *G. pallidipes* and *G. fuscipes* with predominance of the former

Therefore the following recommendation should be forwarded;

- It would be necessary to apply large scale tsetse control method using insecticide free, cost effective tools in the Deme River valley, by considering the habitat of the two tsetse fly species
- Tsetse control personnel have to use the combination of a fermented cow urine and acetone in order to enhance the efficiency tsetse catch.

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DOI: 10.22192/ijcrcps.2025.12.08.003	

How to cite this article:
Tarekegn Desta Madebo. (2025). Comparing the efficiency of different odor baits for tsetse flies survey in Deme River Valley in South West Ethiopia: - an implication for trypanosomosis control. Int. J. Curr. Res. Chem. Pharm. Sci. 12(8): 21-31.
DOI: http://dx.doi.org/10.22192/ijcrcps.2025.12.08.003

