**Survey of *Glossina* and other biting flies in Assosa and Bambasi districts of Benishangul Gumuz Region, Western Ethiopia**

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**Abstract:** A cross-sectional entomological survey was carried out from November 2018 to March 2019 with the objective to estimate seasonal apparent density of *Glossina* and other biting flies in Assosa and Bambasi districts of Benishangul Gumuz region. Seasonal entomological survey result revealed that only one species of *Glossina* (*Glossina morsitans submorsitans*) and three genera of other biting flies (Stomoxys, Tabanus and Haematopota) were captured and identified. The apparent density of *Glossina* was 0.31 fly per trap per day in the late rainy season and 0.13 fly per trap per day in the dry season, with statistically significant difference (P<0.05) while the apparent density of other biting flies were 4.66 fly per trap per day in the late rainy season and 1.5 fly per trap per day in the dry season with statistically significant difference (P<0.05). Seasonal entomological survey findings revealed that the presence of Glossina in any number is an important risk factor for the occurrence of animal trypanososmosis in Assosa and Bambasi districts of Benishangul Gumuz region; hence designing participatory and integrated control measures including regular surveillance, community based vector prevention and control should be implemented to mitigate the problem.[Birhanu Eticha, Mussie H/Melekot, Yechale Teshome. **Survey of *Glossina* and other biting flies in Assosa and Bambasi districts of Benishangul Gumuz Region, Western Ethiopia.** *N Y Sci J* 2022;15(8):1-10] ISSN 1554-0200(print); ISSN 2375-723X (online) <http://www.sciencepub.net/newyork>. 01. doi:[10.7537/marsnys150822.01](http://www.dx.doi.org/10.7537/marsnys150822.01).

**Keywords**: Benishangul Gumuz, *Glossina morsitans submorsitans,* *other biting flies*, Season

# Introduction

Trypanosomes are extracellular protozoan parasites that cause debilitating diseases called trypanosomosis in animals and sleeping sickness in humans and have great socio-economic impact adversely affecting food production and economic growth in many parts of Africa, particularly in Sub-Saharan Africa (SSA) (Shaw *et al*., 2014; Taylor, 2015). The disease in animal is transmitted cyclically by the genus *Glossina*, but it can also be transmitted mechanically by other biting flies among which *Tabanus* and *Stomoxys* are presumed to be the most important, as exemplified by their presence in South and Central America as well as in some areas of Africa free or cleared of *Glossina* such as Ethiopia, Chad, Senegal, Sudan *etc* (Truc *et al.,* 2013).

The distribution of the disease coincides with the habitat of tsetse fly vector and is called the tsetse fly “belt” or it is sometimes referred to as “green desert” because ~10 million km2 of potential fertile land is rendered to be unsuitable for cultivation (Shaw *et al*., 2014). Within this area, the majority of *Glossina* infested countries are underdeveloped, poor, heavily indebted and food-deficit due to lack of productive animals as far as meat/milk production and draft power are concerned, resulting in an annual economic loss of about 5 billion US$ (Giordani *et al*., 2016; Yaro *et al*., 2016).

In Ethiopia, western and southern river basins are the most severely affected areas by trypanosomosis. In the areas specifically in the western part, a wide diversity of *Glossina* and trypanosome spp and strains co-exist (Abebe, 2005). Benishangul Gumuz is one of the five regions of Ethiopia infested with more than one spp of *Glossina*. In the region, four *Glossina* spp namely, *G. tachinoides, G. m. submorsitans, G. pallidipes* and *G. fuscipes* were identified (NTTICC, 2004;Worku *et al*., 2017).

Although, *Glossina* species are widely distributed in Assosa and Bambasi districts of Benishangul Gumuz region, seasonal variation in fly per trap per day (F/T/D) is scarce and not well documented to take preventive and control measures at the right time.

Therefore, to control animal trypanosomosis with the aim to achieve food security and for poverty alleviation, comprehensive quantification of seasonal variation in F/T/D of vectors responsible to transmit the disease should be done. Hence, the objective of this study is to estimate seasonal apparent density of *Glossina* and other biting flies in the late rainy season and during the dry period of the year in the study districts.

# Materials and methods

## Description of the study areas

Benishngul Gumuz region is one of the nine regional states established in 1994 by the new constitution of Ethiopia that created a federal system of governance. The region is located in the western end of the country bounded by Sudan Republic in the west, Amhara region in the north and northeast, Oromia region in the east and southeast and Gambella region in the south and found at a distance of about 687 km away from Addis Ababa. According to the current administrative structure, the region is divided in to 3 administrative zones, 20 districts and 482 *rural kebeles* with a total area of approximately 50,380 km2 (BGRBoC, 2017).

The region is found at latitude of 9-11°N and longitude of 34-35°E and altitude ranges generally between 580-2731 m.a.s.l., the highest peak being at Belaya mountain (2731 m.a.s.l) and the lowest (580 m.a.s.l) in the extreme west lowlands near the Ethio-Sudan boundary. The mean annual temperature of the region ranges from 17-29oc. Rainfall is uni-modal and occurs for 6 or 7 months between April and October. The mean annual rainfall amount is estimated to be 1275 mm. Higher rainfall period is between May and September, the highest being in July or August (NMSA, 2015).

The region possess 777,915 cattle,531,229 shoats, 82,080 equines and 1,249,578 poultry of which cattle population of the region accounts for only 1.3% of the country (CSA, 2016/17).

### Assosa district

Assosa district is located at 9.600-10.450 N latitude and 34.200-34.580E longitude with an altitude that ranges from 580-1544 m.a.s.l. The district has a rainfall that ranges from 850-1200 mm. Its mean annual temperature ranges between 16.750c and 37.90c (NMSA, 2015). The total area of the districts is 2317 Km2 and its livestock population is 27,850 cattle, 25,943 goats, 5,689 sheep, 5,420 donkeys and 53,185 poultry (ADOoA, 2017). The soil types of Assosa distinct are mainly silty loam (70%) and sandy loam (30%). Vegetations such as woodlands and shrublands, bush lands, bamboo woodlands, forest and savannah grassland are mainly found in the district. Maize, sorghum, finger millet, teff, mango and coffee are some of the major crops grown in Assosa district. The livelihood of the society largely depends on mixed crop livestock production. Hoha, Affa, Affa Megele, Affa Belbenare and Bildigilu Gambashire are few of small rivers found in Assosa district (BGRBoA, 2017; ADOoA, 2017).

### Bambasi district

Bambasi district is located at 9.45- 9.750N latitude and 34.35-34.880 E longitude, with minimum and maximum altitude of 1350 and 1770 m.a.s.l., respectively. The total area of the district is 2100 km2 and has average minimum and maximum annual rainfall of 900 mm and 1200 mm, respectively; while the average minimum and maximum temperature is 230c and 320c, respectively (NMSA, 2015). The total livestock population of the district is 38,964 cattle, 11,990 goats, 3,452 sheep, 1,995 donkeys and 38,442 poultry (BDOoA, 2017).

Clay loam, sandy loam and red soils are the main soil types of the district. Woodlands and shrub lands, bush land, bamboo woodlands, forest and savannah grasslands are the main vegetation types found in the district. Maize, sorghum, finger millet, teff and mango are the major crops grown in the district and similar to Assosa district, the livelihood of the society largely depends on mixed livestock and crop production. Some of the rivers found in the district include Affa, Selga, Jema, Qontsa, Sonka and Shebora (BGRBoA, 2017; BDOoA, 2017). Bamboo woodland, one of the most important resources of the region extensively occurs below 1600 m.a.s.l, the largest part being found in Assosa zone mainly in the two study districts.

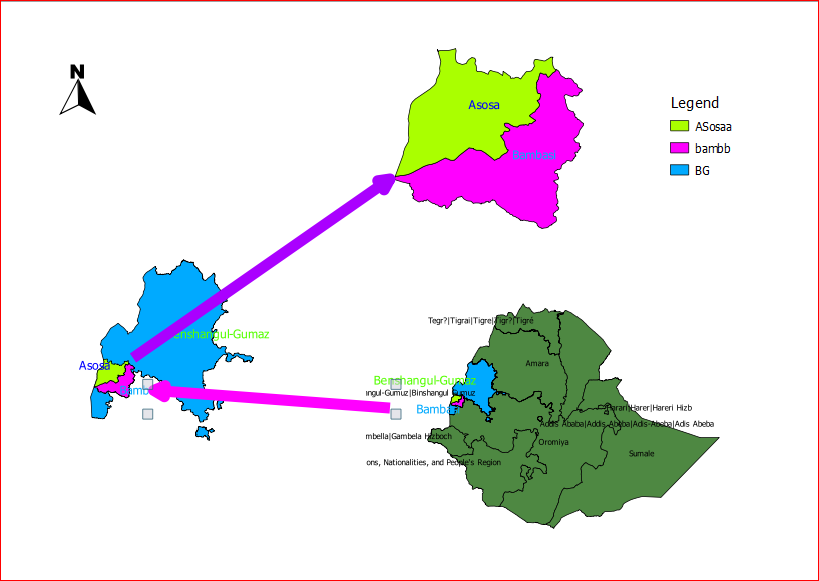


Fig.1. Map of the study areas

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## Study design

Cross-sectional entomological survey was conducted from November 2018 to March 2019 to estimate seasonal apparent density of *Glossina* and other biting flies in the late rainy season and during the dry period in the study districts.

## Sampling methods

Assosa and Bambasi districts were selected purposively for this study because of wide spread occurrence of *Glossina* spp. Nine *rural kebeles* (5 from Assosa and 4 from Bambasi) districts were selected by simple random method.

## Data collection

### Entomological survey

A cross-sectional entomological survey was conducted twice during the study period (in the late rainy season (November) and during dry period (March) by deploying a total of 150 monoconical and bicononical traps (78 in the late rainy season) and (72 during the dry period) of the study at an interval of about 100-200m in savannah grassland/woodland, along the river banks and at grazing field of cattle to see the F/T/D or mean differences between the two trap types. Each trap was odor baited with acetone and 3 weeks old cow urine. The underneath of each trap pole was smeared with grease in order to prevent the entry of insect predators like ants towards the collecting cage that can damage flies. The cages were emptied 48 hours later and after capturing, *Glossina* were counted, identified and separated into their respective spp. The spp of *Glossina* was identified based on characteristic morphology (Leak *et al*., 1993). Other biting flies were also separated based on their morphological characteristics such as size, color, proboscis and antenna at the genus level (Walle and Shearer, 1997).

## Data management and analysis

Raw data collected were entered into a Microsoft Excel spreadsheet. The data were summarized and presented in tables and analyzed by using STATA version 13.0 for Windows (Stata Corp. College Station, TX). The density of fly population was calculated by dividing the number of flies caught by the number of traps deployed and number of days of deployment and expressed as F/T/D (Leak *et al*., 1988) and differences in F/T/D or mean catches of flies in relation to variables such as season, vegetation and trap types were determined using Kruskual-Wallis test. Throughout the analysis, the test result was considered as significant when the calculated P-value was ≤ 0.05 at 95% confidence interval and 5% absolute precision (Thrusfield, 2005).

1. **Results and discussion**

## Entomological Survey

During entomological survey, only one spp of *Glossina (G. m. submorsitans)* and three genera of other biting flies (*Stomoxys, Tabanus* and *Haematopota*) were caught. A total of 815 and 241 flies were caught in the late rainy season and during the dry period of the survey, respectively, of which *Glossina* accounted for 49 of the flies in the late rainy season and 18 of the total flies during dry period of the survey as shown in Fig. 2.

Fig.2. Relative abundance of *Glossina* and other biting flies in Assosa and Bambasi districts

The present finding was in line with (Aki and Godesso, 2016; Ayana and Zerihun, 2016; Golassa and Mekonnen, 2017) who reported only one spp of *Glossina* (*G. m. submorsitans)* and three genera of other biting flies(*Stomoxys, Tabanus* and *Haematopota*)in the western parts of Ethiopia namely, Odabildiglu, Assosa and Bambasi districts of Benishangul Gumuz region, respectively. It was also in agreement with earlier research work of (Duguma *et al*., 2015) who reported that only one *Glossina* spp (*G. m. submorsitans*) was observed in Assosa district of Benishangul Gumuz region in their study on spatial distribution of *Glossina* and *Trypanosoma* spp in southwestern Ethiopia.

The presence of only *G. m. submorsitans* alone in the current survey might be due to the fact that Assosa and Bambasi districts are characterized by unspoiled savannah environment, as this spp is particularly sensitive to encroachment. Moreover, the environment in the current survey areas might not be conducive to other spp of *Glossina* present in different parts of the country in general and in the region in particular.

*Glossina* spp accounted for 6.01% (in the late rainy season) and 7.47% (in the dry season) of the survey as shown in Figs. 3 and 4, respectively. The proportion of *Stomoxys* was much higher than other biting flies in both the late rainy season (89.20%) and dry period (89.21) of the survey.

Fig. 3. Proportion of *Glossina* and other biting flies in late rainy season in Assosa and Bambasi districts

Fig. 4. Proportion of *Glossina* and other biting flies in dry season in Assosa and Bambasi districts

This research work is supported by Dagnachew (2004) and Haile *et al*. (2016) where higher proportion of other biting flies particularly of *Stomoxys* was registered in Abbay basin areas of northwest Ethiopia and in Yayo district, Illubabor zone of Oromia region, respectively.

In the current survey, relatively higher (0.31) F/T/D of *Glossina* was recorded (95% CI =0.16-0.44) in the late rainy season when compared to the dry season (0.13) of the study (95% CI=0.04-0.21)with statistically significant difference (P < 0.05).

The current finding was comparable with the finding of (Eticha *et al*., 2017) who reported 0.34F/T/D in Odabildiglu district of Benishangul Gumuz region. However, it was lower than the finding of (Dagnachew, 2004) who reported 1.08 F/T/D in late rainy season and 0.68 F/T/D in the dry season. It was also lower than prior findings of (Aki *et al*., 2016) who reported an overall *Glossina* F/T/D of 3.72 in Kamashi district of Benishngul Gumuz region. It was also much lower than earlier report of (Itefa and Lelisa, 2017) who found 11.98 F/T/D in Dale Wabera district of Oromia region.

The variation in the present and previous findings might be attributed to encroachment to the breeding sites of flies for farming, house construction for settlement and climate fluctuation/change.

The apparent densities of the different flies were significantly higher in the late rainy season when compared to the dry season. This research result was supported by Dagnachew (2004) who reported higher F/T/D of *Glossina* and other biting flies in the late rainy season when compared to the dry season. High population density of various flies following the rain was reported by Dia *et al*. (1997) in Mauritania and McElligott *et al*. (1998) from a subarctic Labrador peat land.

An increase in the number of *Glossina* and other biting flies in the late rainy season in relation to the dry season in the present survey might be attributed to the presence of favorable environmental condition such as enough moisture, vegetation growth and suitable habitat. The relatively high number of *Glossina* and other biting flies in the late rainy season when compared to the dry season in this study suggests that the late rainy season can be considered as the period of potentially higher risk for biological and mechanical transmission of trypanosomes by these flies.

The apparent density of *Glossina* spp was 0.21 F/T/D in Bambasi and 0.37 F/T/D in Assosa districts in the late rainy season while it was found to be 0.06 F/T/D in Bambasi and 0.14 F/T/D in Assosa district in the dry season. Similarly, the apparent densities of other biting flies were 9.8 F/T/D in Bambasi and 2.17 F/T/D in Assosa in the late rainy season and 1.95 F/T/D in Bambasi and 1.23 F/T/D in Assosa district during the dry season as shown in Tables 1 and 2.

This wider variation in the apparent density of different flies between the study districts might be attributed to the difference in agro ecology. In addition, according to our field observation, both Sonka and Shebora *rural kebeles* of Bambasi district have big rivers such as Sonka and Shebora which flow throughout the year that might create favorable environmental condition for breeding of other biting flies particularly of *Stomosys*. Similary, Megele 38 and Abrehamo *rural kebeles* of Assosa district have higher vegetation (savannah grass/wood lands and bush) cover when compared to other *rural kebeles* that might favor breeding and survival of *Glossina* spp (*G. m. submorsitans)*.

*Glossina spp* sexing during the survey indicated that the predominant sex was female 37(75.51%) in the late rainy season and 14(77.78%) during the dry period of the study. The overall *Glossina* spp caught per 48 hours, their F/T/D as well as the overall F/T/D of other biting flies in the late rainy season and during the dry period of the survey was shown in Tables 1 and 2, respectively.

Similar research result was reported by Itefa and Lelisa (2017) where higher proportion (75%) of female *Glossina* was reported. The current finding was slightly lower in the late rainy season; but it was higher during dry period of the study when compared to earlier finding of (Dagnachew, 2004) who reported 88.78% and 53.33% female *Glossina* proportion in the late rainy season and during the dry period, respectively. Although the present finding was slightly higher in both seasons, it agrees well with the finding of (Eticha and Begawi, 2017) that reported 71.5% female *Glossina* proportion. It also agrees well with (Leak, 1999) who indicated that in unbiased sample, female *Glossina* would comprise between 70-80% of the mean population. This higher proportion of female *Glossina* could be attributed to the fact that female *Glossina* has longer lifespan when compared to male (Leak, 1999) and selective attractiveness of traps to female (Rowlands *et al*., 1993; Leak, 1999).

Different ecological habitats in which flies are numerous or scarce investigated so far showed that each fly genera has adapted to a certain locality for breeding, feeding, resting and host seeking. Dia *et al.* (1998) reported that high number of *Tabanus* was collected from an area with ample water and traps placed in the pasture near this watery area captured 80% of the fly population.

Table 1: Apparent density of *Glossina* and other biting flies in the late rainy season in Assosa and Bambasi districts

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***Rural kebele*** | **Altitude range of traps deployed** | | **Traps deployed** | **Total flies caught** | ***Glossina*** | | | | | **Other biting flies** | |
| **No** | **Spp** | **M** | **F** | **F/T/D** | **No** | **F/T/D** |
| Keshimando no 2 | | 1369-1383m | 7 | 15 | 1 |  | 0 | 1 | 0.07 | 14 | 1 |
| N/keshimando | | 1378-1406m | 7 | 34 | 6 |  | 2 | 4 | 0.43 | 28 | 2.7 |
| Sonka | | 1391-1426m | 7 | 316 | 2 | Gm | 0 | 2 | 0.14 | 314 | 22.4 |
| Shebora | | 1415-1433m | 7 | 196 | 3 |  | 1 | 2 | 0.21 | 193 | 13.8 |
| **Subtotal (Bambasi)** | |  | **28** | **562** | **12** |  | **3** | **9** | **0.21** | **549** | **9.8** |
| Amba 11 | | 1439-1471m | 10 | 35 | 5 | Gm | 1 | 4 | 0.25 | 30 | 1.5 |
| Komeshiga 27 | | 1375-1391m | 10 | 46 | 6 | 1 | 5 | 0.30 | 40 | 2 |
| Komeshiga 28 | | 1346-1378m | 10 | 51 | 3 | 0 | 3 | 0.15 | 40 | 2.4 |
| Abrehamo | | 1246-1303m | 10 | 52 | 11 | 4 | 7 | 0.55 | 49 | 2.1 |
| Megele 38 | | 1463-1492m | 10 | 70 | 12 | 3 | 9 | 0.60 | 58 | 2.9 |
| **Subtotal (Assosa)** | |  | **50** | **254** | **37** |  | **9** | **28** | **0.37** | **217** | **2.17** |
| **Overall total** |  | | **78** | **815** | **49** |  | **12** | **37** | **0.31** | **766** | **4.66** |

Gm= *G. m. submorsitans*, F/T/D= Fly per trap per day, M= Male, F= Female, m= Meter

Table 2: Apparent density of *Glossina* and other biting flies during the dry period in Assosa and Bambasi districts

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| ***Rural kebele*** | | **Altitude range of traps deployed** | **Traps deployed** | | **Total flies caught** | ***Glossina*** | | | | | **Other biting flies** | |
| **No** | **Spp** | **M** | **F** | **F/T/D** | **No** | **F/T/D** |
| Keshimando no 2 | | 1369-1376m | 8 | | 10 | 1 |  | 0 | 1 | 0.06 | 9 | 0.56 |
| N/keshimando | | 1373-1382m | 8 | | 16 | 3 |  | 1 | 2 | 0.19 | 13 | 0.81 |
| Sonka | | 1384-1393m | 8 | | 39 | 0 | Gm | 0 | 0 | 0 | 39 | 2.4 |
| Shebora | | 1391-1395m | 8 | | 64 | 0 |  | 0 | 0 | 0 | 64 | 4 |
| **Subtotal (Bambasi)** | |  | **32** | | **129** | **4** |  | **1** | **3** | **0.06** | **125** | **1.95** |
| Amba 11 | | 1413-1468m | 8 | | 15 | 1 | Gm | 0 | 1 | 0.06 | 14 | 0.88 |
| Komeshiga 27 | | 1377-1413m | 8 | | 21 | 2 | 1 | 1 | 0.13 | 19 | 1.2 |
| Komeshiga 28 | | 1361-1387m | 8 | | 23 | 2 | 0 | 2 | 0.13 | 21 | 1.3 |
| Abrehamo | | 1154-1229m | 8 | | 23 | 4 | 1 | 3 | 0.25 | 19 | 1.2 |
| Megele 38 | | 1455-1487m | 8 | | 30 | 5 | 1 | 4 | 0.31 | 25 | 1.6 |
| **Subtotal (Assosa)** |  | | **40** | | **112** | **14** |  | **3** | **11** | **0.14** | **98** | **1.23** |
| **Overall** |  | | | **72** | **241** | **18** |  | **4** | **14** | **0.13** | **223** | **1.5** |

Gm= *G. m. submorsitans*, F/T/D= Flyper trap per day, M= Male, F= Female, m= Meter

Slightly higher F/T/D of *Glossina* was registered in savannah vegetation type (0.39) in the late rainy season while during the dry period of the study it was found to be slightly higher in riverine vegetation/river bank (0.15). In both seasons, the lowest F/T/D of *Glossina* was recorded at the grazing land of cattle which was 0.17 and 0.08 in the late rainy season and during the dry period of the study, respectively with no significant difference (P>0.05). This finding revealed that *Glossina* spp move to river boarder in search of suitable environmental conditions in the dry season. According to Leak (1999), vegetation is vital for providing suitable environmental conditions; where the savannah, forest and riverine *Glossina* spp concentrate in the wooden savannah, in the bush vegetation and near the edge of the river, where the vegetation is dense, respectively.

With regard to other biting flies, unlike to *Glossina*, relatively higher apparent density was recorded in riverine vegetation in the late rainy season as well as during the dry period of the study, while the lowest F/T/D was reported at the grazing land of cattle in both seasons with no significant variation (P>0.05). Apparent density of *Glossina* and other biting flies in the three vegetation types in late rainy season and during dry period of the study was shown in Table 3.

Table 3: Apparent density of *Glossina* and other biting flies in the three vegetation types in the two seasons in Assosa and Bambasi districts

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Season** | **Vegetation type** | **Number of traps** | **Flies and their apparent density** | | | |
| ***Glossina*** | ***Stomoxys*** | ***Tabanus*** | ***Haematopota*** |
| Late rainy | Savannah | 33 | 0.39 | 5.1 | 0.11 | 0.14 |
| Riverine | 27 | 0.31 | 5.4 | 0.15 | 0.02 |
| Grazing land | 18 | 0.17 | 2.7 | 0.06 | 0.08 |
| Dry season | Savannah | 27 | 0.13 | 1.7 | 0.04 | 0 |
| Riverine | 27 | 0.15 | 2.1 | 0.07 | 0.04 |
| Grazing land | 18 | 0.08 | 0.3 | 0 | 0 |

The mean catches of *Glossina* spp with monoconical traps were 0.71(95% CI= 0.47-0.82) and with that of biconical traps were 0.43 (95% CI= 0.23-0.65), respectively in the late rainy season, with no significant variation (P>0.05). In the dry season the mean catches of *Glossina* were 0.35 (95% CI=0.22-0.51) with monoconical traps and 0.07(95% CI= 0.01-0.24) with biconical traps, respectively with no significant difference (P>0.05). The mean fly catches of other biting flies were slightly higher (P>0.05) with monoconical traps when compared to biconical traps in the two seasons. The mean fly catches with the two trap types in the two seasons in Assossa and Banbasi districts were indicated in Table 4.

This finding was in line with (Dagnachew, 2004) in which higher mean fly catches were registered by using monoconical traps when compared to either biconical or NGU traps. The difference in efficiency of mean fly catches of the two trap types in the current survey might be attributed to the difference in trap designs where biconical traps were found to be less effective for morsitans group (NTTICC, 2014).

Table 4: The mean fly catches with two trap types in the two seasons in Assossa and Banbasi districts

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Season** | **Trap types** | **Number of traps** | **Mean fly catches/trap** | | | |
| ***Glossina*** | ***Stomoxys*** | ***Tabanus*** | ***Haemaopota*** |
| Late rainy season | Monoconical | 55 | 0.71 | 10.29 | 0.27 | 0.35 |
| Biconical | 23 | 0.43 | 7.0 | 0.04 | 0.17 |
| Dry season | Monoconical | 45 | 0.35 | 3.91 | 0.13 | 0.04 |
| Biconical | 27 | 0.07 | 1.44 | 0 | 0 |

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# In conclusion, the current survey revealed the presence of only one spp of *Glossina* (*G. m. submorsitans*) and three genera of other biting flies (*Stomoxys*, *Tabanus* and *Haematopota*) that serve as a potential vectors for the transmission of animal trypanosomosis. The apparent density of *Glossina* spp was significantly higher in the late rainy season when compared to the dry season. In this surevy, season was found to be an important risk factor for apparent density of *Glossina.* Therefore, designing control strategies including regular surveillance, community based vector prevention and control measures should be implemented to mitigate the problem and taking other seasons into consideration, further study on apparent density of *Glossina* and other biting flies should be conducted in the study districts.

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