Research Article

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Comparative Efficacy of Suction Traps Baited with Different Chemical Lures for Collecting Sandflies (Diptera: Psychodidae) in Northern Ethiopia

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***Abstract*—**Studies of sandfly-parasite interactions and vector bionomics or taxonomy require sampling methods that generate reliable data for surveillance of vector control programs. The current study evaluated effectiveness of suction traps baited with rodent (*Taterarobusta*), sugar-yeast mixture (SYS), human hair in ethanol, green and red light sticks for the collection of sandflies. In this field study, using Latin square experiments with thorough rotation and replication, six different trap-bait combinations were tested for their attractiveness to wild caught sandfly species, by placing unlit CDC traps in an up-draft position. In total, 4,640 sandfly specimens belonging to six species of the genus *Phlebotomus* and nine of the genus *Sergentomyia* were captured. *P. orientalis* and *S. africana* constituted 55.5% and 26.2% of the collected sandflies, respectively. Significantly, a higher number of sandflies were caught in suction traps baited with *T. robusta* followed by SYS. However, baits of human hair in ethanol, green and red light sticks attracted a lesser number of sandflies. Traps with *T. robusta* attracted significantly more number of *P. orientalis* than other baits. Similarly, in the SYS-baited traps more *P. orientalis* were attracted, though the number of, *P. orientalis* collected with green and red chemical light-sticks was very low. These findings present an essential step in the development of a cheap and easily applicable CO2 source that could be used for sandfly surveillance in rural or remote areas

Keywords: *Baits, lure, Phlebotomus orientalis, sandfly, sugar-yeast mixture*

1. Introduction

Phlebotomine sandflies are of considerable public health importance in the tropics and subtropics. The importance of sandflies is attributed mainly to their role as potent vectors of the various forms of leishmaniasis (visceral, dermal and mucocutaneous), bartonellosis and 3-day fever (Killick-Kendrick, 1999; Ready, 2013). Such diseases are transmitted by through the bite of infected female sandflies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World when taking repetitive bloodmeal (Ready, 2013).

Parasites belonging to the *Leishmaniadonovani* complex, *L. donovani* and *L. infantum* (synonym = *L. chagasi*), are the main causative agents of visceral leishmaniasis (VL), also known as kala-azar (WHO, 2010; Alvar *et al.*, 2012). An estimated 500,000 cases occur annually, mostly in India, East Africa and Brazil (Guerin *et al.*, 2002; Lukes *et al.*, 2007). In Ethiopia, VL is considered as an emerging disease with an estimated incidence that ranges from 3,700 to 7,400 cases per year (Alvar*et al.*, 2012).

This systematic disease has been reported from at least 40 areas, with the most important endemic foci being the arid south-west and the north-west lowlands of the countries y bordering Kenya and Sudan, respectively (Lyons *et al.*, 2003; Hailu *et al.*, 2006). In recent years, the disease has spread to the highlands of Libo-Kemkem and in Fogera districts, where it claimed the lives of more than 200 people (Alvar*et al.*, 2007; Bashaye *et al.*, 2009).

Besides, reports have described increasing numbers of VL cases as well as new foci of disease in the semi-arid lowlands of Tigray Regional State, northern Ethiopia (Abbasi *et*

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*al.,*2013). For instance, between 2006 and 2011, 209 VL cases were treated in TahtayAdiyabo district (Desjeux *et al.*, 2013).

In order to understand sandfly bionomics, vector species identification, and to develop methods for sandfly control, it has become necessary to sample sandflies in their habitat. Several sampling methods have been developed for this purpose. The most commonly used techniques for measuring sandfly abundance are CDC's light traps, aspirators and sticky traps (Davies *et al.*, 1995; Alexander, 2000; Alexander and Maroli, 2003). However, the relative efficiency of catching sandflies by different trapping methods varies for both environmental and intrinsic behavior of the vector species (Alexander, 2000).

For instance, light traps that are less labor-intensive, preferentially sample females of certain species that are highly phototropic (Davies *et al.*, 1995; Lane *et al.*, 1988; Alexander, 2000). Baiting traps with different chemical lures might help to minimize the sampling biasness seen on such traps (Kasili *et al.*, 2010; Hoel *et al.*, 2011). Odors from different sources, carbon dioxide and animals are common baits. Previous studies indicate that carbon dioxide can enhance trap collections (Alexander, 2000; Veronesi *et al.*, 2007). *Lutzomyia* spp. sandflies were attracted to the combination of human odors and carbon dioxide in laboratory (Rebollar-Tellez *et al.*, 1999) and field studies (Pinto *et al.*, 2001). Lane *et al.* (1988) and Burkett *et al*. (2007) reported that sticky traps baited with chemical light sticks are effective at collecting sandflies.

However, such baits have not been sufficiently tested on Ethiopian sandfly species. Therefore, the current study was designed to evaluate the relative attractiveness of suction traps baited with rodent (*Taterarobusta*), sugar-yeast mixture (SYS), human hair in ethanol, green and red light sticks for the collection of sandflies.

2. Materials and Methods

2.1. Study Area

The experiment was carried out in a the small farming community of Ademeyti ((14°21'31.53'' N; 37°41'37.89'' E; 1,060 m above sea level), located approximately 16 km west of Sheraro town, Tigray Regional State, northern Ethiopia. The ecology and physical environment of the study area have previously been described in detail in Gebresilassie *et al.* (2015a).

2.2. Lures

The lures studied were carbon dioxide, human hair in ethanol, rodent (*T. robusta*), green and red chemical light sticks. Carbon dioxide was delivered via placing yeast-produced carbon dioxide prepared by mixing dry yeast (15 g), sugar (150 g) and tap water (2 L). The human hair sample was clipped or shaved off from a volunteer individual daily and placed into a 10 ml absolute ethanol in the vial. Then, this vial was sealed with a cap containing a rubber septum. The septum was pierced from the inside by a 22 gauge pointed Luer needle to allow for evaporation rates of approximately 0.5 ml/trap night (range of 0.2-2.0 ml) as indicated in Hoel *et al.* (2011). Similarly, an incandescent CDC light trap was run along the other treatments.

Three of the traps were baited with CO2 in the form of yeast-produced carbon dioxide in an insulated 2-liter polystyrene canister. Rubber tubing (0.5 m long, 7 mm internal diameter) were attached to the canister opening. The other end of the tube was secured to the fan guard of the CDC traps. Likewise, the vials containing human hair in ethanol solution were hung at the outlet base of unlit CDC trap using string of ~0.32 cm diameter in such a way that the needles were positioned on air inside the trap outlet.

2.3. Rodent Trapping

A rodent species, *T. robusta* were trapped from its natural habitat in the study area. The rodents were trapped from their natural habitat found in the study area using Sharman trap measuring 15.2x17.7x28 cm. Traps baited with peanut butter were set near rodent runs and burrow openings, inside and outside homesteads, and along fences. Then, the traps were checked for catches in the morning.

2.4. Experimental Set up

The study was carried out during March 2013, when sandflies are considerably more abundant in the study area (Gebresilassie *et al.*, 2015a). Traps were placed 0.5 m above the ground level and at least 20 m apart to reduce the possibility of mutual interference (Faiman *et al.*, 2009). The position of the control traps and attractant-baited traps was changed each night in Latin-square design to avoid spatial bias. The experiment was repeated three times, constituting 18 trapping nights. Experimental sessions started 1 hour before sunset and terminated 1 hour after sunrise, the following morning. After each trap night, collected sandflies were preserved in glass vials containing 70% alcohol and later were taken to the laboratory for latter processing.

2.5. Mounting and Identification of Sandflies

All the sampled sandflies were dissected and separately slide-mounted on glass slides in Hoyer medium for later identification (Faiman *et al*., 2011). Species were identified based on the morphology of the external genitalia of males and the pharynx and spermathecae of females, using several keys (Quate, 1964; Abonnenc and Minter, 1965).

2.6. Data Analysis

Data were entered into MS Excel sheet and there then after, imported into SPSS version 20.0 software package (Chicago, IL, USA) for analysis. Descriptive statistics was used to describe proportion of sandfly species. Sandfly trap data were normalized using log10 (n+1) and subjected to one-way analysis of variance (ANOVA) to compare the average yields of the different trap-attractant combinations. Means were separated with Tukey's multiple range test and unless otherwise stated, P<0.05. Though log10 (n + 1) values were used for the analyses, actual values are reported in the text, figures and tables.

3. Results

3.1. Species Composition and Relative Abundance of Sandflies

A total of 4,640 sandfly specimens, belonging to 6 species of the genus *Phlebotomus* and 9 of the genus *Sergentomyia* were captured during 18 nights (Table 1).

Table1: Sandfly species captured by suction traps-baited with incandescent light, green and red light-sticks, sugar-yeast mixture (SYS), human hair, and a rodent species of *T. robusta* in Ademeyti and their relative abundance (%).

Sandflyspp.	Female	Male	Total	(%)
P. orientalis	1831	745	2576	55.52
P. rodhani	59	33	92	1.98
P. lesleyae	13	6	19	0.41
P. bergeroti	1	0	1	0.02
P. martini	0	1	1	0.02
S. Africana	243	972	1215	26.19
S. schwetzi	344	51	395	8.51
S.clydei	81	41	122	2.63
S. bedfordi group	47	59	106	2.28
S. antennata	16	67	83	1.79
S. dubia	17	0	17	0.37
S. calcarata	5	0	5	0.11
S. squamipleuris	0	5	5	0.11
S. adleri	2	0	2	0.04
Total	2641	1913	4640	100

Females and males comprised 57.31 % and 42.69 % of catches during the trapping period, respectively. Out of the collected total, *Phlebotomusorientalis* was found to be the most abundant (55.52%) species while *Sergentomyiaafricana* accounted for 26.19% of the sandflies that were identified. Other species were *P. rodhaini* (1.98%), *P. lesleyae* (0.41%), *P. martini* (0.02%), *P. bergeroti* (0.02%), *P. heischi* (0.02%), *S. schwetzi* (8.51%), *S. clydei* (2.63%), *S. bedfordi* group (2.28%), *S. antennata* (1.79%), *S. dubia* (0.37%), *S. squamipleuris* (0.11%), *S. calcarata* (0.11%), and *S. adleri* (0.04%).

3.2. Comparative Efficacy of Different Baits

There was a significant difference among the different bait-trap combinations (P<0.05) (Table 2). The suction traps baited with *T. robusta* species collected significantly higher mean number of sandflies (179.75) than all other traps combined (P=0.00). While the updraft CDC traps baited with SYS was the second most effective attractant for collecting sandflies (72.83). Baits of red light-sticks and human hair attracted fewer numbers of sandflies compared to other baits tested in the field. The total numbers of captured sandflies were 2157, 874, 634, 382, 308, and 285 in the traps baited with *T. robusta*, SYS, Incandescent light, green light stick, human hair, and red light stick, respectively.

Table 2: Mean numbers (± SE) of Sandflies Captured in Suction Traps Baitedwith various Attractants

Bait types	**Total sandflies captured**	**Mean ± SEM**
Taterarobusta.	2157.00	179.75 ± 25.59^{a}
YSS	874.00	72.83 ± 13.78^{b}
Incandescent light	634.00	52.83 ± 7.31^{bc}
Green LS	382.00	31.83 ± 7.36^{cd}
Human hair	308.00	25.67 ± 2.97^{d}
Red LS	285.00	23.75 ± 3.64^{d}

Means followed by the same letter on the same line are not significantly different (P< 0.05; Tukey's mean separation applied to log (n + 1)-transformed data.
(Ethiopian names should be written in full)

3.3. Evaluation of Sandfly Species' Response to Different Baits

There was a significant difference (P<0.05) in the sandflyspecies' response to traps with different baits (Table 3).

Table 3: Mean numbers (± SE) Catch of Different Sandfly species by Updraft CDC Traps Baited with Different Attractants.

	Sandfly species Attracted							
Bait types	***P. orientalis***	***P. rodhani***	***P. lesleyae***	***S. Africana***	***S. schwetzi***	***S. clydei***	***S. bedfordi group***	***S. antennata***
Taterarobusta	132.75±24.07a	2.08±0.05a	0.42±0.15a	30.08±4.39a	2.83±0.80b	6.50±0.96a	1.58±0.43a	3.00±0.39a
Sugar YeastSolution	44.00±10.66b	3.67±1.82a	0.33±0.19a	8.00±1.69cd	14.67±6.26a	0.58±0.23b	0.33±0.19a	0.58±0.19cd
Incandescent Light	9.17±1.46cd	0.25±0.18b	0.42±0.26a	26.75±5.21ab	10.33±2.98a	0.92±0.26b	2.33±0.0.81a	3.00±0.87ab
Human Hair	17.83±2.65bc	1.08±0.34ab	0.08±0.08a	3. 5.08±1.54d	1.83±0.23b	0.83±0.30b	0.25±0.18a	0.08±0.83d
Green LS	5.42±1.78d	0.33±0.26b	0.25±0.14a	18.25±3.65abc	0.50±0.67ab	0.67±0.28b	3.75±2.01a	1.83±0.44abc
Red LS	5.50±1.27d	0.25±0.13b	0.08±0.08a	13.08±3.66bc	2.75±1.59ab	0.67±0.19b	0.58±0.29a	1.00±0.30bcd

Traps baited with *T. robusta*, rodent species attracted considerably higher number of *P. orientalis* than other baits. Similarly, in the SYS-baited traps greater numbers of *P. orientalis* were attracted. However, *P. orientalis* was collected in very low numbers with green and red chemical light-sticks. Among *Sergentomyia*spp., *S. africana* comprising 26% of the entire catch, was attracted to *T. robusta*-baited traps, the incandescent light traps and the green light-stick traps with no significance difference (P>0.05). Nevertheless, in the human hair and SYS-baited traps *S. africana* was were captured in less number as compared to *T. robusta*¬-baited and the incandescent light traps, respectively.

3.4. Sex Ratio

On the basis of sex, significant difference (P<0.05) between sexes was observed on the total number of collected species. Accordingly, irrespective of the traps, females were higher than males with a ratio of 1.34:1. However, considering the different traps, only in *T. robusta* and SYS-baited traps were, the ratios biased in favor of females (Fig. 1.) while in other four baited traps, males were relatively abundant.

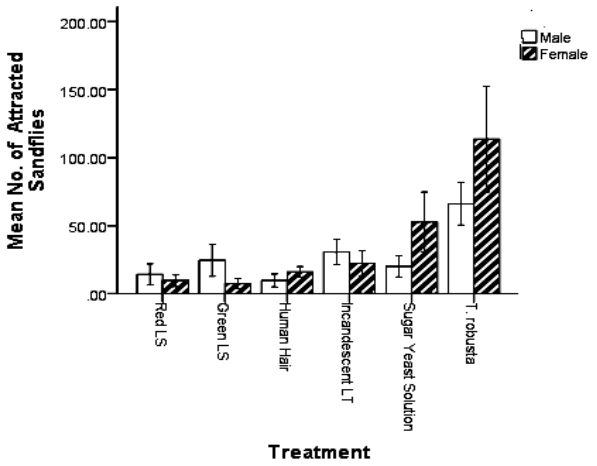

Figure1: Mean (± SE) number of male and female sandflies collected by traps-baited with incandescent light, green and red light-sticks, sugar-yeast mixture (SYS), human hair, and a rodent species of *T. robusta*

4. Discussion

Reliable data from vector collection surveys are crucial for research and for the evaluation of vector control programs. Several studies in the past have tried to assess the different methods available to collect *Phlebotomus* species in various habitats (Killick-Kendrick, 1987, Davies *et al.*, 1995; Alexander, 2000). In the current study, six different means of

sandfly attractants were evaluated in order to measure their efficacy to monitor sandfly populations in the remote areas of TahtayAdiyabo district. During 18 days of trapping, 4,640 sandflyies specimens, belonging to 6 species of the genus *Phlebotomus* and 9 of the genus *Sergentomyia* were collected using updraft suction traps baited with *T. robusta*, SYS, Incandescent light, green light stick, human hair, and red light stick, respectively.

Out of the six species of *Phlebotomus* trapped, *P. orientalis* was the most abundant species, accounting for more than 55.2%. This species is the proven vector of *L. donovani* in northern Ethiopia (Gebresilassie *et al.,* 2015b*). S. africana* was found to be the predominant *Sergentomyia* species (26.1%) while other sandfly species combined together constitute 19% of the total catches. In the overall, the species composition of sandflies encountered in the present study is congruent concord with previous reports in northwest Ethiopia (Gemetchu, 1983; Gebre-Michael *et al.,* 2010, Yared *et al.,* 2016) as well as in the same district (Kirstein *et al.,* 2013; Gebresilassie *et al.,* 2015b). However, collection of large number of *P. orientalis* in this study moderately differed from previous observations, where *Sergentomyia* spp. were the predominate catches in light traps (Kirstein *et al.,* 2013). Such differences are partly attributed to the use of different baits, which largely modulate the visual and chemical receiving behavior of *Phlebotomus* spp. (Lane *et al.,* 1988; Alexander, 2000).

A significant difference in the total number of sandfly specimens captured among traps baited with different attractants was observed. Prominently, suction traps baited with *T. robusta* species captured considerably large mean number of sandflies, followed by sugar yeast solution. Suction traps baited with *T. robusta* and SYS favored females of *Phlebotomus* spp., the more important faction of the population required for determining infection rates with Leishmania. Specifically, *P. orientalis* females were as numerous in *T. robusta*and SYS-baited traps as in the incandescent light traps.

This increased number of sandfly attractions to these baits mainly associated with the emanation of CO2 and other volatile chemicals from the body of baited animal and chemical reaction of fermenting sugar yeast solution, respectively. Various Different researchers noted that baiting CDC traps with different animal species and CO2 source is known to enhance trap efficacy for sandfly (Alexander, 2000; Veronesi*et al.,* 2007; Kasili*et al.,* 2009). In Kenya, CDC traps baited with goats attracted significant number of *P. duboscqi* than unbaited CDC traps (Kasili *et al.,* 2009).

However, in the same greenhouse experiment CDC traps baited with *T. robusta* and *Arvicanthis niloticus* were less attractive to *P. duboscqi*. Such variations could be partly explained by the varying degree of host preference between *P. orientalis* and *P. duboscqi*. Hassan *et al.* (2009) also determined that the dog baited suction trap significantly attracted a higher number of *P. orientalis* compared to CDC light traps.

Carbon dioxide is a known host-seeking cue for many female sandflies and is often used to improve sandfly trap yields (Alexander 2000). Traps baited with CO2 alone (without light) are known to attract significantly more *P. papatasi* compared with to non-baited CO2 traps (Beavers *et al.,* 2004). Previous studies in Italy and Egypt also revealed that the use of CO2 increased the distance, and therefore, the numbers of sandflies attracted to a trap (Veronesi *et al.,* 2007; Bernier *et al.,* 2008). In addition, both laboratory and semi-field experiments demonstrated that traps baited with yeast-produced CO2 caught significantly more number of *Anopheles gambiaes.s.* than unbaited CDC traps (Smallegange *et al.,* 2010). Hence, the current results on the efficacy of animal and yeast-produced CO2 baits for sandfly collection are is in agreement with the previous studies.

Although green and red color chemical light-sticks used as visual baits in the current study, they did not yield considerable number of *P. orientalis* catches. Similarly, no significant difference between the two tried colors was found at by collecting *P. orientalis.* However, chemical light-sticks, especially the green one were strong attractant of different *Sergentomyia* spp., which was equally as attractant as rodent and incandescent light baited traps as. The observed variation in colored light stick-baited trap captures for *P. orientalis* and *Sergentomyia* spp. suggests that there is individual variation in detecting different wave-lengths of light traps. Such individual species wavelength preference was reported for various species of mosquitoes in Kenya (Tchouassi *et al.,* 2012) and sandflies in Iraq (Burkett *et al.,* 2007).

Rodent baited and the incandescent light traps recorded an overall higher capture of *Sergentomyia* spp. compared to traps baited with green and red light-sticks, SYS and human hair. However, such enhanced attraction efficiency of baited traps depends on individual sandfly species response to these baits. For instance, *S. schwetzi* (man-bitter) was significantly attracted to SYS-baited traps than other baited traps.

The sex ratio of *P. orientalis* differed between traps; rodent and SYS-baited traps collected more females. While, other baited traps were mostly dominated by male. Similar results were reported for traps baited with animal and CO2, where the majority of sandfly catches were females (Killick-Kendrick *et*

al., 1985, Alexander 2000). These results show that *P. orientalis* females appear to prefer CO2 and other volatile chemicals in locating their potential hosts for blood feeding activity.

5. Conclusions

In conclusion, the results of the current study indicated that animal and SYS-baited traps as an effective means for monitoring sandflies in remote areas, which is the characteristic of many leishmaniasis foci. In view of this, these baits are worth using as sandfly surveillance tools and may be useful in an integrated sandfly control program. Prominently, fermenting baking yeast-sugar solution producing CO2 and other attractants may be an alternative for industrial CO2 supplied from expensive cylinders or dry ice sources in remote locations. These results present an essential step in the development of a cheap and easily applicable CO2 source that could be used for sandfly surveillance in rural or remote areas.

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Conflict of Interest

The authors declare that they have no competing interests

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