Journal of Experimental Sciences 2024, 15: 13-18
doi: 10.25081/jes.2024.v15.8925
https://updatepublishing.com/journal/index.php/jes

Research Article

ISSN: 2218-1768

Impact of Indoor Residual Spray (IRS) and Insecticide Treated Nets (ITNs) for sand flies (Diptera: Psychodidae) elimination in some remote communities

Amaechi Austin[1]*, Iwunze John[1], Njoku Francis[1], Nwoke Murph[1], Ebeh-Njoku Augusta[1], Aderibigbe Atinuke[2], Orimogunje Ifedayo[3]

[1]Medical Entomology Unit, Department of Zoology Imo State University, PMB 2000 Owerri, Nigeria, [2]Chemistry Department University of Lagos, Nigeria, [3]Microbiology Department Federal University of Technology Akure, Nigeria

Received: April 22, 2024
Revised: July 08, 2024
Accepted: July 10, 2024
Published: September 19, 2024

***Corresponding author:**
Amaechi Austin
E-mail: amaechiaustine@ymail.com

ABSTRACT

Insecticide Treated Nets (ITNs) and Indoor Residual sprays (IRS) in synergy have been an effective way to drive vector transmissions to low levels. A comparison-based survey for the field efficacy of (IRS+ITN) versus single interventions, IRS or ITN alone compared with control was conducted in the Mbaise Area of Imo State Nigeria. Sticky trap (ST) and Human bait (HB) techniques were used for sandflies collection. Results from the two techniques showed that after intervention, the lowest sandflies proportion were from households that intervened with combined measures (IRS+ITN) when compared with single intervention households (IRS) and (ITN) or control. The percentage reduction (%RI) at the sites that intervened with IRS+ITN as compared to either control site or single interventions was found to be the highest. The study strongly advocates the use of integration vector management for sand fly reduction and subsequent disease transmission especially in our rural areas.

KEYWORDS: Indoor Residual Spray (IRS), Insecticide Treated Nets (ITNs), Integrated Vector Management (IVM), Coetaneous Leishmaniasis elimination, Sand flies

INTRODUCTION

Phlebotomus sand flies (Diptera: Psychodidae) occur through the tropics and subtropical countries as well as in temperate zones. They are vectors of leishmaniasis, a vector borne disease endangering 350 million people in 88 countries, most of them in the poorer regions of the globe (Orshal *et al.*, 2010). There are about 700 known species but only 30% transmit leishmaniasis to humans (Moncaz *et al.*, 2012). They are known to be vectors of other human pathogens such as *Bartonella* sp (carrion's disease) and several viral agents causing sand fly fever, summer meningitis, vesicular stomatitis and Chandipura virus elephantiasis (Depaquit *et al.*, 2010).

Leishmaniasis is found in every continent except Antarctica and Australia. Its clinical types include cutaneous, diffuse cutaneous, mucocutaneous and visceral leishmaniasis. Cutaneous leishmaniasis (CL) or oriental/tropical sore occurs in various parts of the world mainly in tropical and subtropical regions. In the African continent, CL is evenly distributed from the Northern to the Southern areas and it is the common form in Nigeria (FMOH, 2012).

For controlling other vector-borne diseases, intervention tools such as the introduction of indoor residual spray (IRS) and scaling up of long-lasting insecticide treated Nets (ITNs) have remained highlighted (Muniaraj *et al.*, 2010). Where IRS was used indiscriminate exploitation of used chemicals (Singh *et al.*, 2012) together with logistic problems associated with sprayers and spray pumps (Huda *et al.*, 2011) had contributed to lowered efficacy of IRS as well as increased proportions of flies followed by the decreased susceptibility (Rama *et al.*, 2017). Thus, it is configured to be weak when used alone.

Similarly for containing vector density ITNs also provide an alternative protective way for defending vector populations

(Kleinschmidt *et al.*, 2009). The biological activity of these nets lasts as long as the nets themselves (3 to 4 years for polyester nets, 4-5 years for polyethylene nets). The long-lasting insecticidal net should maintain bioefficacy for >95% knockdown and/or >80% mortality against target mosquito species for at least 20 serial washings in the laboratory and 3 years of continuous use in the field conditions (WHO, 2005). Among the nets recommended, permanent® 3.0 net stands out and has been in use in Nigeria and studies on the wash resistance and bioefficacy against local malaria vectors have shown good results (Adeogun *et al.*, 2012). However, a single application of ITN doesn't guarantee complete protection against vectors due to limitations (improper implementation, impact of insecticide treatment, provision of protection only on people shaping inside the nets, etc.,) (Lindsay *et al.*, 1989). These points to the futility of intervention for controlling vector populations. Despite limitations with single interventions with either IRS or ITN alone, conventional tools producing unsatisfactory results for control of vector population are still in practice. In this line, the lack of testimonials on the comparative assessment of IRS and ITN alone or in combination for sand fly vector reduction in endemic areas led to the study. Judging from the successful mosquito control approach (Hamel *et al.*, 2011), added benefits or IRS and ITN were tailored to be exploited for controlling sand fly vector population in these areas of study. We reasoned that ITNs and IRS in synergy when combined may be an effective way to drive cutaneous leishmaniasis transmission to very low levels. This served as a hypothesis for the present study. There are reports on sand flies and different forms of leishmaniasis in parts of Africa. In Nigeria, attention has been paid to sand fly species and leishmaniasis. The few reports on the vectors are from Northern Nigeria (Asimeng, 1990; Agwale *et al.*, 1995) and diseases (Isa *et al.*, 2017). Only the works of Mbagwu and Udoye (2014) and Ikpeama and Obiajuru (2018) exist for Imo State. Ajero *et al.* (unpublished) is on the species composition and observable skin manifestations linked to sandfly bite. Unfortunately, none was tailored towards control of the vector species.

MATERIALS AND METHODS

Study Area and Study Period

The Mabise Area of Imo State consists of 3 LGAs; Ahiazu Mbaise, Aboh Mbaise and Ezinihitte Mbaise (coordinated around the latitude 5° 28′ - 1° 34′ N and longitude 7° 19′ - 28° 01′ E of the globe). The vegetation is typical rainforest characterized by tall trees and soil that is rich in humans. Mean rainfall ranged from 19,000 to 20,000 mm. The climate is characterized by two seasons (wet and dry season) from April to October and November to March respectively. The months may vary slightly due to climate change. On the basis of report observed from LGA health officers, four villages i.e. Obohia, Udo and Oboama were selected for IRS, ITN, and IRS+IN treatment respectively and Umuokrika was allocated as a control site that remained untouched from any treatment. Each village contained at least 20 households that were randomly chosen. Overall 40HHs were targeted for study. The study was from July 2018 to February 2019 including a baseline interview on socioeconomic and sandfly density observation at the study sites conducted just a week prior (i.e. June 2018) to the implementation in interventions and observatory phase (July 2018 - February 2019). The observatory phase centered on the efficacy of intervention over the vector density at intervals following the bi-monthly assessment of entomological surveys together with household interviews conducted at the end of the study.

Ethical Approval

This work was performed under the project titled Integrated Vector Management for the control of Leishmaniasis Vectors - A pilot study approved by the Scientific Advisory Committee and Institutional Ethical Committee of Zoology Board of Postgraduate Studies Imo State University Owerri. Ministry of Health and participants gave informed consents.

Baseline Interview Survey

At the beginning of the survey, house-to-house surveys were carried out at the 4 villages by the trained interviewers' team using pre-tested, structured questionnaires. The questions were designed to obtain data on the socio-economic status of the households and the protective measures taken against insects and bites.

Sandfly Collection Techniques

Two types of collection were adopted simultaneously in the villages; Sticky trap (for estimating reductive abundance) and Human bait (for estimating bite rates) as described below.

a. Sticky Trap (ST)

The sticky paper traps were made from a sheet of paper 20x20 cm, coated with castor oil and with lights. The traps were placed in and near houses, pens, poultry yards and other livestock homes. On each catch night, 15 to 20 sticky papers and high traps were set in each of the selected catch points. The heights were placed about 3cm from human dwellings which were hooked at 1.5 cm above the ground. The specimens collected were first immersed in 90% ethanol to remove oil and transferred to 70% ethanol. They were labeled, kept humid and cool in a cold box preserved in the refrigerator.

b. Human bait (HB)

This was done by 2 trained volunteer entomological scouts to estimate the biting rate. The catching points were selected in the villages mapped for the study. The volunteers were trained on how to bait and catch sandflies on landing to feed on the host. Catches were made between 07.00 am and 08.00 am twice a month. The flies were caught as they landed on the host by inverting specimen tubes over them.

Insecticide Treated Nets (ITNs)

Before the start of all trials, meetings were held in the study villages and inhabitants allocated for interventions of ITN and IRS+ITN respectively. They were educated on the proper and regular use of nets. Perma Net® 3.0 manufactured by Vestergaard Frandsen® Switzerland was donated to the researcher by the Carter Center/Global 2000 Owerri. The nets were distributed at the intervention sites of ITN and IRS+ITN during July. The numbers distributed are summarized in Table 1. Perma net® 3.0 was distributed in the villages for IRS (Obohia) and IRS+ITN (Oboama) intervention respectively to cover the entire sleeping spaces of the population in these villages. The population-treated net distribution ratio was stabilized as 3:1 keeping in view the comfort of the size net (Mondal *et al.*, 2010). A team was recruited to monitor the proper use and maintenance of mosquito nets in untreated net villages.

Indoor Residual Spray (IRS)

Pyrethrum insecticide (Baygon; pymethrin dosage 0.02-0.1 mg) was used. A hand compressor pump was used as it provides better coverage with minimum wastage of insecticide (Kumar *et al.*, 2013). For this study, every corner of each households of village Obohia and Oboama allocated with IRS and IRS+ITN respectively were treated with IRS with Baygon. The IRS campaign was again repeated at the study sites in November.

Post Intervention Sandfly Density and assessment of feeding status

Adult sandflies were caught from 6 randomly chosen households (one room per house) from the allocated study sites for interventions including the control. This activity was done a week before the intervention (for baseline survey) and twice monthly up to 8 months following the interventions. The same houses were used for sandfly collection with ST and HB throughout the study duration. Visual observation was made on the feeding status of collected sandflies from randomly selected 6 households from each study site (Dinesh *et al.*, 2008). Identification (males and females) and feeding rates (fed, unfed and gravid) were made by abdominal appearance.

Assessment of Reduction Percentage Due to Intervention (%RI) in Vector Density

This was assessed by measuring the magnitude of reduction in sandfly density at selected intervention households and comparing those with pre and post-treatment densities with the control houses (Mulla *et al.*, 1999).

Perceived Intervention Assessment Acceptance, Efficacy and Side Effects

Questionnaires were used to obtain information on benefits and side effects during the use of ITNS and ITN together with the IRS.

Data Analysis

Data obtained were analyzed using SPSS (IBM statistics) version 21 software and transformed to obtain homogeneity of variance. 95% CI was taken for the significance level for Reduction in sandflies due to intervention (%RI). ANOVA was used for comparison between different interventions (IRS, ITN and IRS+ITN) to see the effect of interventions.

RESULTS

Vector surveillance for intervention was assessed at the households and control. Socio-demographic data showed that the majority of houses at the study sites; control, ITN, IRS and IRS+ITN were thatched, mud and plastered type which served as the best habitat for sandflies (Table 1). Of 400 selected populations, females (43%, 36% and 37%) were lower than males (57%, 63% and 63%) in the sites to be intervened. The literacy rate was lower (i.e. 46%, 46%) in the villages to be intervened with ITN and IRS and IRS + ITN (50%). The ratio of domestic animal assets was 41, 33, 65 and 51 respectively for Umuokirika, Udo, Oboama and Obohia respectively. Domestic animals include goats, sheep, pigs, etc. Contrary to the insect control options, overall 56.4%, 28.4%, 33.3% for Umuokrika, Udo, Oboama and Obohia respectively were satisfied with the traditional means for controlling insects while (31.8%, 50.6%, 44.1% and 55.6%) preferred the modern means. However, 46%

Table 1: Demographic, socio-economic and protective measures against sandflies

Classification of data	Villages and intervention types				
	Umuokirika (control)	Udo (ITN)	Oboama (IRS+ITN)	Obohia (IRS)	Total
No of participants					
Males	63 (57.31)	50 (61.7)	70 (63.1)	57 (63.3)	240
Females	47 (47.7)	31 (38.3)	41 (36.9)	33 (36.7)	152
Education profiles					
Illiterates	59 (53.1)	47 (58.0)	56 (50.5)	49 (54.4)	211
Literates	51 (46.4)	34 (42.8)	55 (49.5)	41 (45.6)	181
Domestic Animals assets					
Total no	41	33	65	51	190
Total with shed	26	17	23	32	98
Control of insects/measures					
Modern methods (Bed nets, IRS, repellent)	35 (31.8)	41 (50.6)	49 (44.1)	50 (55.6)	175
Traditional methods (Smooking, oil spilling)	62 (56.4)	23 (28.4)	37 (33.3)	30 (33.3)	152
Both methods	7 (8.6)	10 (12.3)	18 (16.2)	13 (11.8)	46
None	5 (5.6)	7 (6.3)	0 (0.0)	5 (5.6)	19

preferred both methods while 19% rejected either of any means for controlling biting-insects nuisances. Also, 5.6%, 12.3%, 16.21% and 11.8% in Umuokirika, Udo, Oboama and Obohia accepted both types of control while 8.6%, 6.3% and 5.6% rejected either method. Among the study sites, availability and use of ITN before the intervention was 31.8%, 50.6%, 44.1% and 55.6% respectively (Table 1). The lowest acceptability (5.6%) and highest rejection of either modern or traditional qualified Umuokirika as the control intervention site. The highest acceptability of both methods (16.2%) of either method and the lowest rejection (0.00%) served best for the selection of Oboama as the intervention site for ITN+IRS. The results (Table 2) showed that overall, 73.3% and 26.7% of catches were from sticky traps and human bait respectively. More sandflies were caught by ST than HB. For ST techniques, significantly higher proportions of females were caught than males (73.9% versus 26.1%). Of these females, 36.5%, 14.7% and 22.7% were fed, unfed and gravid respectively. Similarly for HB, more females than males were caught (84.4% versus 15.6%). A greater proportion was fed (18.1%) while unfed and gravid were 14.3% and 22.1%. After intervention, the least sandfly proportion was from households with combined measures (IRS+ITN) when compared with both single intervention households (IRS) and (ITN) or control sites. The percentage reduction (%RI) at the sites with IRS+ITN showed no re-emergence as compared to either the control site or the single intervention

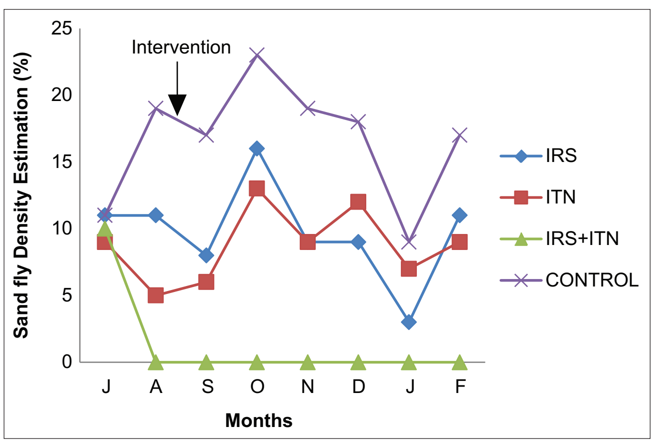

Figure 1: Sand fly density during pre and post intervention phases

shown (Figure 1). Assessment of collateral effectiveness of interventions showed that all respondents with ITNS confirmed proper usage and continued good physical conditions of the ITNS. The unpleasant smell was reported particularly among IRS and IRS+ITN users 87% and 73% respectively. Much less frequent were skin initiation (11% and 09%), and sneezing (0.9% and 07%) in households with either IRS or ITN or both respectively. The reported added benefits reduction in the nuisance of insects was highest in villages where ITNS were involved (i.e. 91% against sandflies and mosquitoes). Reductions or disappearances of insects of other classes and order were also reported. Overall, villages that used ITN were more satisfied than villages that used IRS as an intervention with 65% acceptability (Table 3).

DISCUSSION

Both ST and HB techniques complement each other and it is noteworthy to use them together. The overall pattern of distribution of sandflies in the study showed that they have representatives throughout the year. The extent of the increase depends on the local climate with significant seasonal changes in temperature and precipitation (Nwoke, 1986). Previous control of sandflies with IRS has been exploited extensively (Kumar *et al.*, 2015; Coleman *et al.*, 2015). In these areas, sandflies rather than being killed had their behaviour manipulated from endophilic to exophilic nature (Kumar *et al.*, 2015). Also, these IRS (especially DDT) fail to hold efficacy for a long duration, leading to increased lethal time, logistic problems (Rama *et al.*, 2017) configures decreasing susceptibility or increasing resistance to sandflies (Rama *et al.*, 2017).

ITN also plays a significant role in providing a protective way against malaria and mosquitoes (Kleinschmidt *et al.*, 2009) but not complete control against mosquitoes due to certain limitations (Lindsay *et al.*, 1989). Chemical constituent depletion over time has made neither IRS nor ITN provide satisfactory results for controlling vector nuisances. Thus the combined strategy of IRS and ITN proved to be successful and provided an effective means for vector control (Kleinschmidt *et al.*, 2009; Hamel *et al.*, 2011). This method has not been exploited for leishmaniasis vector control in Nigeria. Over

Table 2: Entomological classification of data according to collection techniques

Collection techniques	Villages and intervention types			
	Umuokirika (control)	Udo (ITN)	Oboama (IRS+ITN)	Obohia (IRS)
Sticking trap (N=211; 73.3%)				
Males (N=55; 26.1%)	07 (12.7)	17 (30.9)	25 (45.5)	06 (10.9)
Females (N=156; 73.9%)				
Fed (N=77; 36.5%)	21 (27.3)	17 (22.1)	16 (20.8)	23 (29.9)
Unfed (N=31; 14.7%)	09 (29.0)	10 (22.3)	05 (16.1)	07 (22.6)
Gravid (N=48; 22.7%)	15 (31.5)	13 (27.1)	06 (12.5)	14 (29.2)
Human bait (N=77; 26.7%)				
Males (N=12; 15.6%)	00 (0)	03 (25.0)	02 (16.7)	07 (58.3)
Females (N=65; 84.4%)				
Fed (N=37; 48.1%)	12 (22.4)	07 (18.9)	05 (13.5)	13 (35.1)
Unfed (N=11; 14.3%)	03 (27.3)	04 (36.4)	00 (0.0)	04 (36.4)
Gravid (N=17; 22.1%)	05 (29.4)	06 (35.3)	03 (17.6)	03 (17.6)

Table 3: Assessment of intervention users perceptions of interventions

Variables	Interventions (%)		
	IRS (*N*=100)	ITN (*N*=100)	IRS+ITN (*N*=100)
Sleep inside nets	NA	77	73
Feel abnormal or uncomfortable inside net	NA	00	02
Suffer the following			
Unpleasant smell	87	65	89
Dizziness	00	01	00
Running nose	03	00	05
Fever	00	00	01
Sore eyes	00	00	00
Headache	00	03	00
Skin irritation	11	00	09
Coughing	00	00	00
Vomiting	00	00	01
Sneezing	09	00	07
Sleeplessness	00	00	00
Presumed benefits of interventions			
Reduction of sandfly/mosquitoes	41	74	91
Reduction in nuisance of other insects	03	88	81
Will continue to sleep under nets	NA	65	73
Satisfied with intervention measures	65	77	73

NA=Not applicable

here, sandflies awareness and resistance to IRS is yet to be documented as in other vectors of public health significance. Biting activities attributable to high level human activities which lead to the high concentration of flies seeking a blood meal is a vivid reflection of the gap about sandflies in public health priorities. The study found that the least collections and feeding status of sandflies (fed, unfed or gravid) were recovered during the post intervention phase from sites intervened with IRS+ITN as against either sites without any intervention (or control) or single control (IRS or ITN). This feeding has probably shown strong evidence that the efficacy of IRS and ITN when applied alone gets depleted over a period of time with resultant unsatisfactory results on sandflies densities. On the contrary, combined strategy provides additional protection.

The results further showed that regardless of the high rate of ITN complaints (in the form of smell, sneezing, skin irritation, etc.,), the majority of the population perceived additional benefits of IRS+ITN to include mosquito, head lice, cockroaches, houseflies, etc., reduction. They also perceived the combined strategy benefits to be so useful and eco-friendly. This attitude probably helped in reducing and maintaining the reduced population of targeted insects. All users at intervention sites with IRS+ITN are assured of continued usage in the future. This points to the efficacy and acceptability of intervention with a positive reduction in vector density and may lead to control of leishmaniasis epidemiology following vector control in the future.

Conclusively, the present study has established that a combined strategy has the potential to overcome the limitations of single interventions. This combined method is important during the attack phase of the elimination initiative to reduce disease transmission in leishmaniasis endemic areas and emerging areas. It may also be important during the maintenance phase in hot spots of leishmaniasis transmission.

REFERENCES

Adeogun, A. O., Olojede, J. B., Oduola, A. O., & Awolola, T. S. (2012). Village-Scale Evaluation of PermaNet 3.0: an Enhanced Efficacy Combination Long-Lasting Insecticidal Net Against Resistant Populations of *Anopheles gambiae* s.s. *Malaria Chemotherapy, Control & Elimination*, 2012, 235543.

Agwale, S. M., Pam, D. D., Donji, B., & Dunh-Linska, D. D. (1995). Preliminary Survey of phlebotomine sandflies (Diptera: Psychodidae) in Northern Nigeria. *Memorias do Instituto Oswaldo Cruz,* 90(5), 551-558. https://doi.org/10.1590/S0074-02761995000500001

Asimeng, E. J. (1990). The distribution of phlebotomine sandflies (Diptera: Psychodidae) in northern Nigeria. *Tropical Medicine & Parasitology,* 41(2), 193-197.

Coleman, M., Foster, G. M., Deb, R., Pratap Singh, R., Ismail, H. M., Shivam, P., Ghosh, A. K., Dunkley, S., Kumar, V., Coleman, M., Hemingway, J., Paine, M. J. I., & Das, P. (2015). DDT-based indoor residual spraying suboptimal for visceral leishmaniasis elimination in India. *Proceedings of the National Academy of Sciences of the United States of America, 112*(28), 8573-8578. https://doi.org/10.1073/pnas.1507782112

Depaquit, J., Grandadam, M., Fouque, F., Andry, P. E., & Peyrefitte, C. (2010). Arthropod-borne viruses transmitted by Phlebotomine sandflies in Europe: a review. *European Communicable Disease Bulletin, 15*(10), 19507. https://doi.org/10.2807/ese.15.10.19507-en

Dinesh, D. S., DAS, P., Picado, A., Davies, C., Speybroeck, N., Boelaert, M., & Coosemans, M. (2008). The efficacy of indoor CDC light traps for collecting the sandfly *Phlebotomus argentipes*, vector of Leishmania donovani. *Medical and Veterinary Entomology, 22*(2), 120-123. https://doi.org/10.1111/j.1365-2915.2008.00724.x

FMOH. (2012). *Nigeria Master Plan for Neglected Tropical Disease (2013-2017)*. Federal Ministry of Health, Abuja.

Hamel, M. J., Otieno, P., Bayoh, N., Kariuki, S., Were, V., Marwanga, D., Laserson, K. F., Williamson, J., Slutsker, L., & Gimnig, J. (2011). The combination of indoor residual spraying and insecticide-treated nets provides added protection against malaria compared with insecticide-treated nets alone. *The American Journal of Tropical Medicine and Hygiene, 85*(6), 1080-1086. https://doi.org/10.4269/ajtmh.2011.10-0684

Huda, M. M., Mondal, D., Kumar, V., Das, P., Sharma, S. N., Das, M. L., Roy, L., Gurung, C. K., Banjara, M. R., Akhter, S., Maheswary, N. P., Kroeger, A., & Chowdhury, R. (2011). Toolkit for monitoring and evaluation of indoor residual spraying for visceral leishmaniasis control in the Indian subcontinent: application and results. *Journal of Tropical Medicine, 2011*, 876742. https://doi.org/10.1155/2011/876742

Ikpeama, C. A., & Obiajuru, I. O. C. (2018). Bionomics of Sandflies (Diptera; Psycodidae) in Some Remote Communities in Ezinihitte Mbaise, South Eastern, Nigeria. *Biological Sciences, 2*(1), 19-28.

Isa, A., Umar, Y. A., & Appah, J. (2017). Species Composition of Phlebotomine Sandfly (Diptera: Psychodidae)Vectors of Leishmaniasis in Katsina State, Northern Nigeria. *International Journal of Scientific & Engineering Research, 8*(11), 1786-1793.

Kleinschmidt, I., Schwabe, C., Shiva, M., Segura, J. L., Sima, V., Mabunda,S. J., & Coleman, M. (2009). Combining indoor residual spraying and insecticide-treated net interventions. *The American Journal of Tropical Medicine and Hygiene, 81*(3), 519-524.

Kumar, V., Kesari, S., Chowdhury, R., Kumar, S., Sinha, G., Hussain, S., Huda, M. M., Kroeger, A., & Das, P. (2013). User friendliness, efficiency & spray quality of stirrup pumps versus hand compression pumps for indoor residual spraying. *The Indian Journal of Medical Research, 138*(2), 239-243.

Kumar, V., Shankar, L., Kesari, S., Bhunia, G. S., Dinesh, D. S., Mandal, R., & Das, P. (2015). Insecticide susceptibility of Phlebotomus argentipes & assessment of vector control in two districts of West Bengal, India. *The Indian Journal of Medical Research, 142*(2), 211-215. https://doi.org/10.4103/0971-5916.164260

Lindsay, S. W., Shenton, F. C., Snow, R. W., & Greenwood, B. M. (1989). Responses of Anopheles gambiae complex mosquitoes to the use of untreated bednets in The Gambia. *Medical and Veterinary Entomology, 3*(3), 253-262. https://doi.org/10.1111/j.1365-2915.1989.tb00225.x

Moncaz, A., Faiman, R., Kirstein, O., & Warburg, A. (2012). Breeding sites of *Phlcbotomus sergenti*, the sand fly vector of cutaneous Leishmaniesis in the Judean Desert. *Plos Neglected Tropical Diseases, 6*(7), e1725. https://doi.org/10.1371/journal.pntd.0001725

Mondal, D., Chowdhury, R., Huda, M. M., Maheswary, N. P., Akther, S., Petzold, M., Kumar, V., Das, M. L., Gurung, C. K., Ghosh, D., & Kroeger, A. (2010). Insecticide-treated bed nets in rural Bangladesh: their potential role in the visceral leishmaniasis elimination programme. *Tropical Medicine & International Health, 15*(11), 1382-1389. https://doi.org/10.1111/j.1365-3156.2010.02635.x

Mulla, M. S., Su, T., Thavara, U., Tawatsin, A., Ngamsuk, W., & Pan-Urai, P. (1999). Efficacy of new formulations of the microbial larvicide *Bacillus sphaericus* against polluted water mosquitoes in Thailand. *Journal of Vector, 24*(1), 99-110.

Muniaraj, M. (2014). The lost hope of elimination of Kala-azar (Visceral Leishmaniasis) by and cyclic occurrence of its outbreak in India, blame falls on vector control practices or co-infection with human immunodeficiency virus or therapeutic modalities? *Tropical Parasitology, 4*(1), 10-19. https://doi.org/10.4103/2229-5070.129143

Nwoke, B. E. B. (1986). *Studies on the field epidemiology of human Onchoerciesis in Jos, Plateau, Nigeria*. Doctoral Dissertation, University of Jos.

Orshan, L., Szekely, D., Khalfa, Z., & Bitton, S. (2010). Distribution and seasonality of Phlebotomus sand flies in cutaneous leishmaniasis foci, Judean Desert, Israel. *Journal of Medical Entomology, 47*(3), 319-328. https://doi.org/10.1603/me09096

Rama, A., Kesari, S., Das, P., & Kumar, V. (2017). Studying DDT Susceptibility at Discriminating Time Intervals Focusing on Maximum Limit of Exposure Time Survived by DDT Resistant Phlebotomus argentipes (Diptera: Psychodidae): an Investigative Report. Japanese *Journal of Infectious Diseases, 70*(4), 437-441. https://doi.org/10.7883/yoken.JJID.2015.604

Singh, R. K., Mittal, P. K., & Dhiman, R. C. (2012). Insecticide susceptibility status of *Phlebotomus argentipes*, a vector of visceral leishmaniasis in different foci in three states of India. *Journal of Vector Borne Diseases, 49*(4), 254-257.

WHO. (2005). *Guidelines for laboratory and field testing*. *of long-lasting insecticidal mosquito nets*. Geneva, World Health Organization.