ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

INSECTICIDE SUSCEPTIBILITY LEVELS OF *PHLEBOTOMUS ORIENTALIS* AND *P. BERGEROTI* (DIPTERA: PSYCHODIDAE) FROM EASTERN AND NORTHERN ETHIOPIA.

By

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DEDICATION

This work is dedicated to my father Tsegaye Beyene

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ABSTRACT

The status of insecticides resistance was investigated in wild population of *P. orientalis* from Melka-Worer (Eastern Ethiopia) and Libo-Kemkem (Northern Ethiopia) and laboratory breed *P. bergeroti* (F_1) from collection in Melka-Worer between December 2007 and June 2008. Sand flies were exposed to six insecticides approved by WHO for indoor residual spray (IRS) and insecticides treated nets (ITNs). Standard WHO diagnostic bioassay kits for susceptibility studies with WHO discriminating doses of DDT (4%), malathion (5%) and permethrin (0.75%), lambda-cyhalothrin (0.05%), deltamethrin (0.05%), alpha-cypermethrin (0.1%) impregnated papers were used. Knock down every 5 minute up to 20 minute and every 10 minute up to one hour and mortality of sand flies 24 hour after one hour of exposure was recorded. In population of *P. bergeroti* from Melka-Worer mortality was over 98.8% with all insecticides tested (DDT, permethrin, deltamethrin, lambda-cyhalothrin and malathion). The lowest 50% and 95% knock down time (KT_{50} and KT_{95}) recorded were 18.2 (15.8-20.8) minutes and 31.1 (27.3-37.9) minutes respectively for DDT followed by permethrin. However, deltamethrin revealed the weakest knock down effect with KT_{50} 30.6 (27.6-33.9) minutes and KT_{95} 46.3 (41.7-53.6) minutes. *Phlebotomus orientalis* in Melka-Worer showed more than 98.8% mortality to DDT, permethrin, lambda-cyhalothrin, alpha-cypermethrin, and deltamethrin. But it showed 93.75% mortality to malathion. The lowest KT_{50} was found to be 8.2 (4.1-10.7) minutes for permethrin and the lowest KT_{95} 21.3 (19.0-27.4) minutes for lambda-cyhalothrin. DDT showed the lowest knock down effect at both values. The Libo-Kemkem population of *P. orientalis* showed over 98.8% mortality to all insecticides. Both permethrin at KT_{50} and alpha-cypermethrin at KT_{95} showed highest knock down effect 11.7 and 21.1 minutes, respectively. But DDT showed the lowest knock down effect at both level. Based on WHO criteria where susceptibility is defined by mortality rate greater than 98% 24 hour after exposure, no evidence for resistance were found. This implies the use of these insecticides during epidemic or high prevalence of disease would be effective to control sand flies in the study areas though the low level of resistance in *P. orientalis* to malathion in Melka-Worer needs to be confirmed.

1. INTRODUCTION

1.1. Leishmaniasis global overview

Phlebotomine sand flies (Diptera: Psychodidae) are vectors of various forms of leishmaniasis, bartonellosis, numerous arboviruses, including the medically important sand fly fever group (WHO, 2006).

Protozoan parasites of the genus *Leishmania* (Kinetoplastida: Trypanosomatidae) are the etiological agents of leishmaniasis that are important public health problems in many countries (WHO, 1990). More than 20 species of *Leishmania* are known to be pathogenic to humans (TDR, 2005). Only the female sand fly transmits the protozoa, ingest *Leishmania* amastigote contained in the blood it sucks from its human or mammalian host in order to obtain the protein necessary to develop its eggs. When the now infected female sand fly feeds on a fresh source of blood, it injects its new victim with the promastigote (WHO, 2007b).

Leishmaniasis currently threatens 350 million people in 88 countries around the world. More than 12 million people are known to be infected with leishmaniasis and two million new cases of the disease occur annually (WHO, 2007a). The disease contributes significantly to the propagation of poverty, because treatment is expensive and hence either unaffordable or it imposes a substantial economic burden. Among the two million new cases each year in the 88 countries where the disease is endemic, it is estimated that 80% earn less than $2 a day (Davies *et al*., 2003).

1.2. Leishmaniasis and its vectors

The leishmaniases are divided in to three general clinical patterns according to the form of the disease: visceral**,** cutaneous and mucocutaneous. The cutaneous leishmaniasis (CL) is caused primarily by *L. tropica*, *L. major*, *L. aethiopica* in the Old World and by *L. Mexicana* or *L. braziliensis* in the New World countries (Killick-Kendrick, 1999). The disease results in formation of skin ulcers at the site of the sand fly bite, usually on exposed parts of the body, the face, neck, arms and legs (WHO, 2007b). In

mucocutaneous leishmaniasis (MCL), caused by *L. braziliensis* and *L. aethiopiaca*, lesions can lead to partial or total destruction of the mucous membranes of the nose, mouth, throat cavities and surrounding tissues. These disabling and degrading forms of leishmaniasis can result in victims being humiliated and cast out from society (WHO, 2007b). Visceral leishmaniasis (VL), also known as kala-azar is caused primarily by *L. donovani*, *L. chagasi*, or *L. infantum*. The disease is characterized by irregular bouts of fever, substantial weight loss, anemia, swelling of the spleen and liver. It results in death if left untreated. In eastern Africa countries, VL causes at least 4000 deaths annually (Reithinger *et al*., 2007). Nowadays, the burden of leishmaniasis is increasing mainly as a result of HIV/AIDS co-infection (TDR, 2005).

At least 29 species of *Phlebotomus* from the Old World and 44 *Lutzomyia* species or subspecies from the New World are proven and probable vectors of leishmaniasis. Principally sand flies are found in warm parts of the world including southern Europe, Asia, Africa and Central and South America (Killick-Kendrick, 1999).

In Africa, leishmaniasis is endemic to countries mostly in the North, Central, East and the Horn of Africa. The disease is also endemic in West Africa (Boakye *et al*., 2005).The most important vectors of VL caused by *L. donovani* in Africa are *P. orientalis*, *P. martini* and *P. celiae*. Among these, two species, *P. martini* and *P. orientalis* are the principal vector of kala-azar in East Africa (Gebre-Michael *et al*., 2004b). For CL caused by different species of *Leishmania*, *P. longipes*, *P. pedifer*, *P. guggisbergi*, *P. rossi*, *P. duboscqi*, *P. papatasi*, *P. sergenti* and *P. saevus* are recorded as important vectors (Killick-Kendrick, 1999; Gebre-Michael *et al*., 2004b).

1.3. Leishmaniasis in Ethiopia

In Ethiopia, two main forms of leishmaniasis; visceral and cutaneous are known to exist. The former is caused by *L. donovani* and is of significant public health importance in the lowlands and mid altitudes of the country (below 2000 meters above sea level (m.a.s.l)). The known important endemic areas of VL are in the south and southwest (Segen, Woito, the lower Omo and Gelana River valleys) and in north and northwest Ethiopia (the

Humera and Metema lowlands, the Belessa high land valley and the Libo-Kemkem highland district) (Hailu *et al*., 2006; Alvar *et al*., 2007). Other low lying areas such as the Awash valley, the Ogaden area are highly suspected (Hailu *et al*., 2006). In the Awash valley moderate to high leishmania skin test positivity have been reported (Ali, 1997; Ali *et al*., 2002; Ali *et al*., 2004).

Nowadays, visceral leishmaniasis has emerged as a major opportunistic infection associated with HIV that demands urgent attention. HIV-VL co-infection has been reported in 35 countries, among which Ethiopia carries the greatest burden (Horst *et al*., 2008). HIV infection can lead to reactivation of latent *Leishmania* infection or to symptomatic VL at initial stage of infection. Even when co-infected patients receive proper treatment, it relapses repeatedly and the outcome frequently is fatal (MOH, 2006).

On the other hand, zoonotic cutaneous leishmaniasis (ZCL), caused by *L. aethiopica*, is a major public health problem affecting thousands of people in the highlands of Ethiopia. *Phlebotomus longipes* and *P. pedifer* are the vectors and hyraxes are reservoir host of *L. aethiopica* in Ethiopia (Ashford *et al*., 1973a, Hailu *et al*., 2006). Little is known about the epidemiology of CL caused by *L. major* and *L. tropica* which appear to be of minor importance in Ethiopia (Hailu *et al*., 2006). No estimates are given to show the general burden of the leishmaniasis in Ethiopia in respect of the incidence and population at risk of infection, although it is of significant public health problem.

1.4. Vectors of leishmaniasis in Ethiopia

At least 18 *Phlebotomus* species that belongs to six subgenera (*Larroussius*, *Phlebotomus*, *Paraphlebotomus*, *Synphlebotmus*, *Anaphlebotomus*, and *Adlerius*) are known to exist in Ethiopia (Gebre-Michael and Lane, 1996a, b; Gebre-Michael and Balkew, 2003). Of these, at least seven species are confirmed or highly suspected vectors of some *Leishmania* species in Ethiopia. These include *P. orientals*, *P. longipes*, *P. pedifer*, *P. martini*, *P. celiae*, *P. sergenti* and *P. duboscqi* (Hailu *et al*., 2006).

Phlebotomus orientalis of the subgenus *Larroussius* is the most likely vector of VL in most endemic areas such as the lower Omo valley, north and NW Ethiopia, although it has not yet been conclusively proven (Ashford *et al*., 1973b; Gemetchu *et al*., 1975; Hailu *et al*., 1995; Gebre-Michael *et al*., 2007). In lowlands of the northeastern Rift Valley of Ethiopia (Awash Valley), VL occurs sporadically and *P. orientalis* is the most abundant and widely distributed species (Gemetchu and Fuller, 1976; Gebre-Michael *et al*., 2004a; Hailu *et al*., 2006). *Phlebotomus orientalis* seem to have a very widespread distribution in the country than the known distribution of VL. It is also abundantly found in Metema and Humera low lands in North west (Gemetchu *et al*., 1975; Gebre-Michael *et al*., 2004a) Belessa Valley (Ashford *et al*., 1973b) and in the recent epidemic area near Addis Zemen (Gebre-Michael *et al*., 2007) where it has been implicated as the most probable vector (Hailu *et al*., 2006). *Phelebotomus orientalis* is the most widespread and proven vector of the disease in neighboring Sudan (Ashford *et al*., 1992). In both Ethiopia and Sudan, *P. orientalis* commonly occurs in *Accacia* forest and black-cotton clay soil habitats (Fuller *et al*., 1979; Hailu *et al*., 2006).

On the other hand, in the Aba Roba VL focus (Segen valley) of southern Ethiopia *P. martini* and *P. celiae* which belong to the subgenus *Synphlebotomus* are principal and secondary vector, respectively of VL (Gebre-Michael and Lane, 1996a). These two species which are associated with eroded termite hills have wider distribution in southern Ethiopia and are also suspected vectors of the disease in Gelana and Woito (Gebre-Michael and Lane, 1996a).

Phlebotomus longipes and *P. pedifer* of the subgenus *Larroussius* are the two species involved in the transmission of CL caused by *L. aethiopica* in Ethiopia. The former has a much wider distribution in Ethiopia and is responsible for the disease in much of the endemic highlands of Ethiopia, while the latter having a rather restricted distribution in some highland areas in southern regions such as the Ochollo and Kindo Kidaye wereda (near Welayeta Sodo) foci (Ashford *et al*., 1973a, Gemetchu *et al*., 1990; Gebre-Michael, Person. Commn.).

Other species such as *P*. (*Phlebotomus*) *duboscqi*, *P*. (*Paraphlebotomus*) *saevus* and *P*. (*Pa*.) *sergenti*, have been found infected with CL causing parasites in various regions, but human infection with these parasites appear to be very rare in Ethiopia (Hailu *et al*., 2006). The former species was found infected with *L*. *major* in the Segen valley (Gebre-Michael *et al*., 1993) and the latter two species were found infected with *L*. *tropica* and *L*. *aethiopica* in the Awash Valley (Gebre-Michael *et al*., 2004a).

1.5. Sand fly control methods

Vector control has a proven record in prevention and control of vector-borne diseases. Thus, it has an important role to reduce transmission and make prevention of infection a high priority. The available drugs for treatment of existing human cases of leishmaniasis are generally expensive and toxic and there is yet no effective vaccine on the market against this disease (TDR, 2005; WHO, 2007a). Therefore, interruption of the cycle by vector control may offer a cheaper and more practical solution to treatment.

1.5.1. Biological control

Biological control of vectors through the use of living organisms has now become component of vectors control. Use of entomopathogens, *Bacillus thuringiensis israeliensis* (Bti.) and *B*. *sphaericus* (Bs) are widely used for the control of mosquitos' larvae and black flies larvae and are known to be lethal to sand flies (Killick-Kendrick, 1999). Against sand flies application of *Bacillus sphaericus* on larval breeding medium in the laboratory condition resulted in a significant larval mortality of *P*. *papatasi* and *P*. *duboscqi* (Pener and Wilamovski, 1996; Robert *et al*., 1997). Pener and Wilamovski (1996) also reported that when *B*. *sphaericus* left in the rearing medium for 18 days there was a significant increase in larval mortality, indicating persistence and possibly recycling of Bs. Efficacy of this pathogen was also studied under field condition. Application of Bs around animal burrows and eroded termite mounds, which are larval breeding sites of *P*. *duboscqi* resulted in significant larval mortality (Robert *et al*., 1997).

Adulticidal effect of Bti was evaluated by Yuval and Warburg (1988). They allowed sand flies to feed for 48 hours on a 30% sucrose solution laced with Bti toxin. Concentration

of 4.4 x 10^{-4}, 4.4 x 10^{-3}, and 4.4 x $l0^{-2}$ mg/ml Bti killed 100% of adult *P*. *papatasi*, *P*. *argentipes* and *Lu*. *longipalpis* after 72 hours presentation of the baited sugar.

Reithinger *et al*. (1997) evaluated entomopathogenic fungi *Beauveria bassiana* as control agent against *Lutzomyia* species in Colombia under natural condition. A trial of *B*. *bassiana* was unsuccessful for sand fly control in natural condition. But Warburg (1991) demonstrated that *B*. *bassiana* smeared on filter paper, caused 100% mortality of adult *P*. *papatasi* and *Lu*. *longipalpis*.

Biological control method might be successful in experimental situation which allows both pathogens and the target species in close contact. However, in real natural conditions this may be difficult. This is due to the poor present knowledge of breeding sites as well as diverse breeding habitat of sand flies (Kishore *et al*., 2006; Killick-Kendrick, 1999). Therefore, knowledge of habitat of immature stages is essential to integrate biological control method with other control techniques.

1.5.2. Environmental management

Vector control may be accomplished by environmental management, which consists of permanent or temporary modification of the environment without causing adverse effect on the quality of environment. The principle behind the environmental control is to manage the environment to make it unsuitable for breeding of sand flies (Kishore *et al*., 2006).

Forest clearance operation in a forest village in French Guyana resulted in sharp decline of *Lu*. *umbratilis*. As a result incidence of CL decreased from 22 cases in 1982 before intervention to 0 after 18 month among the village population and reservoir host, living in the forest strip rapidly disappeared from the area (Esterre *et al*., 1986). Killick- Kendrick (1999) also reviewed the success of this method in Bolivia where, a program of deforestation resulted in the disappearance of two sylvatic sand flies: *Lu*. *umbratilis* and *Lu*. *yuilli*. In the USSR plowing up of the burrows of great gerbil (*Rhombomys opimus*,

the reservoir host of CL caused by L. major), the living and breeding place of the vector *P. papatasi* was employed to control zoonotic CL (Killick-Kendrick, 1999).

In some instances, modification of habitats rather than complete destruction of the environment may be effective. In Uttar Pradesh district a brick and cement-plastered skirting at the junction of floor and walls inside the rooms throughout corners helped in reduction of population of *P. papatasi* by 76 %. Plastering of houses with mixture of mud and lime (to fill all cracks and crevices) resulted in a sudden drop in the sand fly density (Kumar *et al*., 1995)

In general, environmental management may have long term effect and relatively low long run cost, but application of this method should require knowledge of breeding and resting sites of vectors followed by ecological studies to take maximum advantage of natural processes to avoid unnecessary environmental changes.

1.5.3. Personal protection

Vector-borne diseases and associated discomfort caused by biting arthropods can be largely prevented with proper use of personal protective measures. Personal protective measure covers all methods, in which a barrier is established between vectors and humans, and it includes repellent and insecticides impregnated materials.

1.5.3.1. Repellents

The use of repellents is only a measure of individual protection which protects man from blood-sucking arthropods. Insect repellents can be recommended to people who have to stay outdoors during some part of the night especially when entering an endemic area to live a few days (Alexander and Maroli, 2003).

In the Sudan synthetic repellents: diethyl-toluamide (DEET), ethoxy-diethyl benzamide, chloro-diethylbenzamide, butyryl-tetrahydroquinoline provided complete protection against the bite of *P. papatasi* at least for four hours (Schmidt and Schmidt, 1969a). They also pointed out that excessive perspiration and washing, contributed to a high rate of

repellent loss; thus, repeated applications were necessary. N, N-diethyl phenylacetamide (DEPA), DEET and dimethyl phthalate (DMP) as repellency against *P. papatasi* was evaluated by Kalyanasundaram *et al*. (1994). At the same application rate, DEPA and DEET on test animal found to give equivalent protection times of about 4.5 h at 0.1ng/cm^2, whereas the protection time of DMP lasted only about two-thirds as long. Nopikex, a repellent soap formulation containing 20% DEET and 0.5% permethrin were commonly used in Colombia where, it provided 100% protection up to 4 hours against the bites of *Lu. longipalpis* (Alexander *et al*., 1995a).

Although synthetic insect repellents applied to the skin are effective, their disadvantages include high cost, harmful if used continuously, skin irritation, absorption into the systemic circulation, unpleasant odor and potentially poor conformity (Alexander *et al*., 1995a; Alexander and Maroli, 2003; Asilian *et al*., 2003).

Natural repellents have been much studied against mosquitoes compared to sand flies. Neem oil, eucalyptus, citronella grass, galingale, clove thyme and others are commonly used natural repellents (Xue *et al*., 2006). However, little has been studied on the effect of these plants against sand flies. Accordingly in Venezuela Rojas and Scorza (1991) found essential lemon oil (*Citrus medica*) against *Lu. youngi* provided better protection (70%) than DEET and citronella which gave 63% and 33% protection, respectively. Application of 2 % neem oil on exposed face and legs provided 97.6% protection for 10 hours against a bite of *P. papatasi* under field condition in Rajastan, India (Dhiman and Sharma, 1994), and 100% protection against a bite of *P. argentipes* throughout the night in Bihar, India (Sharma and Dhiman, 1993). In Ethiopia repellency effect of neem oil and chinaberry (*Melia azedarach*) were evaluated against *P. orientalis* in the laboratory by Kebede (2006). He found that 2% and 5% oil provided about 96 % protection up to mean time of 7-8 hour. But 2% chinaberry provided 95% protection for about 7 hour.

1.5.3.2. Insecticide treated nets (ITNs)

In areas where sand flies are endophagic and most active when people are asleep, bed nets provide considerable protection. Owing to the small size of sand flies, untreated bed

nets are effective only if they have small mesh size, which might not be acceptable in warmer climates due to poor air circulation. However, untreated bed nets were reported effective in the control of VL in Nepal where, people using untreated nets were 70% less likely to develop VL than those using no nets (Bern *et al*., 2000).

The protection provided by wide-mesh size (156 per inch^2, 100denier) which is large enough for sand flies to pass through) is enhanced by treating them with pyrethroids (Alten *et al*., 2003). Due to rapid knock down effects, high insecticidal potency at low dosages, low volatility, relative safety for human contact and domestic handling, WHO recommended the following pyrethroids for impregnation of net: permethrin, deltamethrin, lambda-cyhalothrin, alpha-cypermethrin, cyfluthrin, and etofenprox (Zaim *et al*., 2000; WHO, 2005; Courtenay *et al*., 2007).

In Sudan λ -cyhalothrin impregnated bednets reduced sand fly (*Phlebotomus orientalis*) biting rates by 100% (Elnaiem *et al*., 1999b). In studied area *P. orientalis* died after 1 hour post-exposure for 30 second to cage netting treated with λ -cyhalothrin. Alten *et al*. (2003) in Turkey and Yaghoobi-Ershadi *et al*. (2006) in Iran found significant reduction in CL incidence in these areas due to effectiveness of deltamethrin impregnated bednets in reducing man biting rate of *P. papatasi* and *P. sergenti*. The bioassays test result in Iran showed percentage mortality of wild-caught *P. papatasi* after 3 minutes contact and 24 hour observations on deltamethrin treated ITN was found 100% until the end of week 12 (Yaghoobi-Ershadi *et al*., 2006).

To determine effect of washing on efficacy of ITN, Bongiorno *et al*. (2005) undertaken a test against laboratory colony of *P. papatasi* in Rome. They found that cyfluthrin impregnated nets at 50 mg /m^2 caused 100% knock down and mortality rate. After washing, cyfluthrin treated net provided 66.7% and 48.9% mortality within 24 hours after two and four washes, respectively. From the study result, it was concluded that the efficacy of ITNs is affected by washing. Hence, re-impregnation of nets is required.

ITN provide benefits not only to those who actually sleep under ITNs but also to non-net users in the same room. Courtenay *et al*. (2007) found 55.9% reduction on the human landing rate of *Lu*. *longipalpis* on unprotected persons exterior to ITNs in the same room (from 48.6% in untreated rooms to 21.5% in ITN houses) and increased 24-h mortality to 67.7% compared to 0.4% in untreated houses.

Compared with IRS, ITNs have the following advantages: less insecticide is used and it requires no specialized equipment that the household exerts control over its application. (Alexander *et al*., 1995c; Kishore *et al*., 2006)

Although the bed nets are very valuable, they have limitations. In some communities people do not sleep on beds for economic or cultural reasons (Mutinga *et al*., 1992). Users also dislike bednets where they felt 'trapped' or 'imprisoned' in the net. Another limitation of ITN is that much of man biting activity of sand flies takes place in the early evening before bed time of most people (Alexander and Maroli, 2003).

1.5.3.3. Insecticides treated curtains

In areas where sand flies bite indoors but are significantly active from dusk onwards, insecticide treatment of curtains can provide effective protection for family members who are at risk of sand fly bites, even before they have gone to bed. In Burkina Faso Majori *et al*. (1989) and in Italy Maroli and Majori (1991) found permethrin impregnated curtains reduced the density of indoor sand flies by 99% and 90%, respectively, than untreated curtains. In the Sudan Elnaiem *et al*. (1999a) found that biting rate of *P*. *papatasi* in rooms without curtains was significantly higher compared with rooms provided with un-impregnated or permethrin impregnated curtains. Since sand flies are preadapted to rest in a very confined space during the day, even small holes in the walls or roofs of human dwelling would allow them to enter. Therefore a well structured house is required to implement this method Alexander *et al*. (1995c).

1.5.4. Chemical control

Despite various attempts at alternatives, the principal method for the control of sand flies remains obviously chemical insecticides. This is due to their more efficient, economic and rapid action (Zaim and Jambulingam, 2007). These insecticides include the organochlorines, the organophosphates, the carbamates and the pyrethroids.

1.5.4.1. Indoor residual spraying (IRS) of human and animal dwelling

Vector control, through spraying of houses with insecticide is the most widely used and effective method for controlling sand flies that are endophilic in controlling indoor transmission of VL and CL (WHO, 2006). DDT still remains the insecticide of choice because of its low cost, high efficacy and long residual action when used for IRS (Najera and Zaim, 2003; Kishore *et al*., 2006). WHO recommended 12 insecticides for IRS use, among which DDT is the one with longest residual efficacy. As a result WHO proposed and supports the continued use of DDT for indoor residual spray to control disease vectors (WHO, 2004b).

In many countries like Brazil, Algeria, India, China, Italy and USSR (where the vectors are endophilic) after house spraying with DDT, the prevalence of leishmaniasis fell dramatically (Killick-Kendrick, 1999; Alexander and Maroli, 2003). In Uttar Pradesh India, application of two rounds of DDT on *P. argentipes* population resulted in complete reduction of *P. argentipes* during the peak season but large number were trapped in unsprayed village (Kaul *et al*., 1994).

Recently the use of synthetic pyrethroids has been increased. In the New World countries: Peruvian Andes (Davies *et al*., 2000), Venezuela (Feliciangeli *et al*., 2003) and Brazil (Kelly *et al*., 1997) application of lambda-cyhalothrin inside walls and ceilings of human habitation and animal shelters significantly reduced the indoor abundance of *Lutzomyia* species (*Lutzomyia peruensis*, *Lu. ovallesi*, and *Lu. longipalpis*) in the target localities. Moreover, application of deltamethrin on the internal walls, roofs and buildings was effective to reduce the number of sand flies captured inside houses in state of Espirito

Santo, Brazil (Falcao *et al*., 1991). These observations agree with the result of Le Pont *et al*. (1989) in Bolivia, where one spray application per year was enough to control *Lu. longipalpis* from dwellings and chicken houses up to 10 months.

IRS to be effective the following features are required: **i.** the vector should be endophilic and/or endophagic, **ii.** the vector should be susceptible to pick up the lethal dose, **iii**. the wall or roof should be suitable for spraying, **iv**. suitable public health infrastructure like adequate supplies of insecticide, spraying equipment and trained personnel should be available and **v**. cooperation of house owners (Alexander *et al*., 1995b; Najera and Zaim, 2003; Alexander and Maroli, 2003**)**.

1.5.4.2. Insecticides application on outdoor resting and breeding site

In areas where transmission takes place mainly outside the villages and the vector sand flies breed and rest outside, treatment of insecticides to these sites away from houses may provide an alternative to IRS (WHO, 1990). Outdoor resting and breeding sites of sand flies include trunks, ground litter and foliage of forests, animal burrows, termite mounds, tree holes, rock fissures, caves and cracks in the ground (Service, 2000). In China, application of alphamethrin in the wild cave resulted in a considerable reduction in density of *P. chinensis* and human cases of VL (Jin *et al*., 2004). In Kenya, Robert and Perich (1995) sprayed termite mounds and animal burrows, which are resting and larval habitats of *P. martini* with cyfluthrin. This provided control of existing and emerging sand flies from treated sites up to 12 week. Chloropicrin application of the great gerbils burrow around human settlement in former USSR reduced incidence of leishmaniasis from 70 to 0.5% (Vioukov, 1987; cited in Sivagnaname and Amalraj, 1997). Perich *et al*. (1995) demonstrated that single ground level barrier spray application of cyfluthrin on 100m wide swath of vegetation provided significant suppression of sand fly population and prevented sand flies from reaching the designated protected area for nearly 3 months in Guatemala.

Application of insecticides on outdoor resting sites especially space spraying of insecticides has the following disadvantages: it may be dangerous to non-targeted

organisms, it requires special and expensive equipment and trained personnel and its persistence very often depends on the meteorological conditions (Najera and Zaim, 2003). The effect is also of short-term duration. Moreover, the area to be sprayed might be quite extensive, and these insecticides lost might be very high.

1.5.4.3. Insecticides susceptibility status of sand flies

Although insecticides remain the primary tools in all control programs of sand flies, the reported data on susceptibility and/or resistance of sand flies to insecticides is rather scarce (Alexander and Maroli, 2003). These data are of great value in selecting most effective insecticides and in providing baseline data for further resistance monitoring. Moreover, upon detection of low to intermediate resistance levels, a decision must be made whether to continue the use of the insecticide until operational failure or to change to an alternative insecticides or other control method. This assists to use insecticides optimally in the context of the integrated vector management approach. The status of different sand flies to various insecticides evaluated in different countries as reviewed by Alexander and Maroli (2003) is shown in Appendix 4.

The first report of insecticides (DDT) resistance occurred in the populations of *P. papatasi* during a kala-azar outbreak in North Bihar State, India in 1978 (Kaul *et al*., 1978). However, in the same area, they found that *P. argentipes* was susceptible to DDT and dieldrin. Later, Rahman *et al*. (1982), Mukhopadhyay *et al*. (1987) and Saxena (1987) found similar resistance patterns among *P. papatasi* to DDT in areas around Delhi and Bihar State (India).

The susceptibility status of *P. papatasi*, a well known vector of zoonotic CL has been studied in different countries. For example Dergacheva and Strelkova (1986) in Mubarek region of Uzbek, Pener and Wilamovski (1987) in the Jordan Valley of Israel reported 100% mortality rate of *P. papatasi* after 1 hour exposure to DDT. In the Jordan Valley of Israel *P. papatasi* also showed 100% susceptibility to permethrin but it showed low level of resistance to methoxychlor. Later Wilamovski and Pener (2003) compared susceptibility level of the same species to the pyrethroids: permethrin and cypermethrin

and the organophosphates: diazinon and chlorpyrifos against *P. papatasi*. It was found that the latter two insecticides were more effective than the former two insecticides. In Iran Yaghoobi-Ershadi *et al*. (2006) reported mortality rates of *P. papatasi* to 0.025% deltamethrin after 2, 4, 7.5, 15, 30 and 60 minutes of exposure time followed by 24 h recovery time were 100%. Thus this insecticide was effective in Iran even at the lowest dose recommended to control sand flies. The discriminating dose is the lowest concentration of a given insecticide that systematically results in 100% mortality in a given susceptible species (WHO, 2006). Thus, for the early detection of resistance, the use of a single diagnostic dose is proposed by WHO (1986).

In Egypt, Aboul-Ela *et al*. (1993) and Fhamy *et al*. (1996) reported the field population of *P. papatasi* was susceptible DDT, benzene hexachloride, dieldrin, malathion, permethrin, deltamethrin, and propoxur. This species in Sudan was also found to be susceptible to both DDT and dieldrin (Schmidt and Schmidt 1969b).

Susceptibility status of phlebotomine sand flies have been compared between insecticides sprayed area and unsprayed area in India. In DDT sprayed area *P. argentipes* showed different level of resistance to insecticide (DDT). But this species showed 100% mortality in unsprayed area (Dhiman *et al*., 2003; Kishore *et al*., 2004). Furthermore Chandra *et al*. (1995) tested susceptibility of *P. argentipes* in West Bengal India, where no insecticides has been sprayed for 25 years and observed 100% mortality to DDT, malathion and dieldrin. These studies indicate that development of resistance may be attributed to extensive and indiscriminate use of insecticides for the control of vector borne diseases. As a result these diseases still continues to be a major public health problem in India.

The only report of insecticides resistance in the genus *Lutzomyia* in the New World involved *Lu. youngi* in Venezuela (Scorza *et al*., 1995; cited in Alexaner and Maroli, 2003). This species developed resistance to propoxur and deltamethrin. However, *Lu. longipalpis* in Venezuela was found to be susceptible to deltamethrin, λ-cyhalothrin, propoxur, and malathion (Mazzarri *et al*. 1997). In Brazil, Falcao *et al*. (1988) reported

effectiveness of deltamethrin against *Lu*. *longipalpis* in even at low concentration of active ingredient.

Insecticide resistance mechanisms in insects have a biochemical basis. The two major forms of biochemical resistance are target-site resistance, which occurs due to alteration of amino acids responsible for insecticide binding at its site of action. This causes the insecticide to be ineffective and no longer binds to its target. The target of organophosphorus (e.g., malathion) and carbamate insecticides is acetylcholinesterase in nerve synapses, and the target of organochlorines (DDT) and synthetic pyrethroids are the sodium channels of the nerve sheath. Any mutation in the target site of a gene caused by a given insecticide usually induces cross-resistance to all insecticides acting on the same site. The other form of biochemical resistance is detoxification enzyme-based resistance, which occurs when enhanced levels or modified activities of esterases, oxidases, or glutathione S-transferases (GST) prevent the insecticide from reaching its site of action by metabolize insecticides or by binding to it so tightly that it cannot function (a process known as sequestration) (Brogdon and McAllister, 1998; Hemingway and Ranson, 2000).

In sand flies there is no information available about the type of resistance mechanism involved to insecticides except for DDT. El-Sayed *et al*. (1989) have showed that sand flies metabolize DDT to DDE as a major metabolite using the mixed function of oxidase and glutathione-S-transferase (GST) in similar manner to mosquitoes.

1.6. Integrated control of leishmaniasis

To reduce or interrupt transmission of disease, vector control is well suited to integrated approaches. Integrated vector management (IVM) is the rational use of all appropriate vector control methods in a mutually, cost effective and safe manner in order to achieve vector suppression and control of disease transmission. The means may use chemical and non-chemical control measures mentioned above (WHO, 1992). It entails the optimum use of a range of interventions of proven efficacy as well as collaboration within the health sector and with various other sectors such as agriculture and the environment.

Such an intersectoral and inter programmatic approach improves the efficacy, cost effectiveness, ecological soundness and sustainability of disease control (WHO, 2004a). The most successful method in controlling leishmaniasis as reviewed by Lane (1991) is integrating sand fly control and reservoir control. In areas where leishmaniasis is zoonotic, transmission rates to humans can be reduced by targeting the animal reservoir hosts. In the past, this has involved the destruction of rodents by poisoning or by digging up their burrows and culling of dogs (Killick-Kendrick, 1999). In many foci of zoonotic viscreral leishmaniasis (ZVL), domestic dogs are important reservoir host of *L. infantum*. In these areas impregnated dog collars have been reported as the most acceptable and effective alternative to control ZVL. Killick-Kendrick *et al*. (1997) and Halbig *et al*. (2000) found that, the dog collars weighing 20g impregnated with 40 mg/g deltamethrin, worn by dogs, protected dogs against 96% of the bites of *P. perniciosus* in Italy and against 79% of the bite of *P. papatasi* in Iran, respectively. Based on these results collar would protect a dog by reducing blood feeding rate of female sand fly. As a result, it would reduce contact between vectors and reservoir hosts sufficiently to diminish the risk of infection for humans.

1.7. Vector control of leishmaniasis in Ethiopia

In Ethiopia the information on leishmaniasis vector control is very scarce. However MOH (2006) set IRS and ITNs as a useful strategy to control indoor biting phlebotomine sand flies. In Ethiopia as the most endemic sites are malarious, promotion of increased coverage and consistent use of ITNs is beneficial to combat both diseases. To control *P. martini*, one of the important vector of visceral leishmaniasis in southern Ethiopia, attempts were made by spraying lambda-cyhalothrin and DDT on termite mounds (Lane, 1991). MOH (2006) of Ethiopia also recommended selective outdoor spraying of insecticides on the preference resting and breeding sites of sand flies.

In the country, leishmaniasis have been reported at least from 40 localities to Ministry of Health (MOH) and recently new foci are being reported (MOH, 2006). The disease is expected to spread to places previously unknown to be endemic. New foci like Libo-

Kemkem are coming to the picture of being endemic for VL and Wolayeta Sodo and Gurage highlands for CL (MOH, 2006; SNNP Regional Health Bureau, Person. Commn.).

To combat the burden of the disease in this country, leishmaniasis vector control may be necessary alone or in integrated manner with the control of malaria vector. There are a limited number of insecticides available for public health programs. To use these valuable resources judiciously and to select appropriate insecticides for the control of leishmaniasis, knowledge on the status of insecticides susceptibility of some of the vector of leishmaniasis in Ethiopia is necessary.

Moreover, it has been known that agricultural spraying for the control of pests contributed in the development of resistance in many vector of public health importance (WHO, 1992). Sand fly may also be exposed to various chemicals either during antimalarial or agricultural spraying. Thus it is highly desirable to determine insecticides susceptibility test for effective control of the sand fly vector of leishmaniasis and sand fly fever.

As long as the use of insecticides continue to be the chief control measure in most countries like Ethiopia, one of the most important function of vector control program is insecticides susceptibility testing. Standardized tests have been developed by the World Health Organization to detect insecticide resistance in sand flies (WHO, 1981).

In Ethiopia and the rest of Afrotropical region a susceptibility test against sand flies have not been yet conducted or reported except in Djibouti and in the Sudan. In Sudan the susceptibility of *P*. *papatasi* to DDT and dieldrin was tested 40 years ago (Schmidt and Schmdit, 1969b). But some information is available on susceptibility status of the malaria vectors of the country in some localities. Studies have showed that *Anopheles arabiensis*, the principal vector of malaria in Ethiopia, developed various degrees of resistance to DDT in Gergedi (Upper Awash), Arbaminch, Metahara, Melka-Worer and Koka area. In Methara and Koka area this species also developed resistance to permethrin (Abose *et al*., 1998; Balkew *et al*., 2003; Tadesse, 2006). The second important but secondary malaria

vector in the country, *An*. *pharoensis* has also developed high resistance to DDT in Ziway and Gorgora, Gondar (Wezam and Seulu, 1994; Balkew *et al*., 2006).

The practice of using insecticides until resistance becomes a limiting factor in that it rapidly erodes the number of suitable insecticides for vector control. Hence, the present study is aimed to monitor and determine susceptibility or resistance status of eastern and northern Ethiopia population of *P*. *orientalis* and *P*. *bergeroti* to some representative insecticides of the organochlorine, organophosphate and synthetic pyrethroid. These areas have been under high insecticidal pressure either from antimalarial or agricultural spraying or both for many years.

2. OBJECTIVES

2.1 General objective

To determine the susceptibility/resistance level of *P. orientalis* and *P. bergeroti* field population of the Middle Awash valley and *P. orientalis* of the recent epidemic area Libo-Kemkem to six insecticides representing and recommend possible insecticides towards the control of leishmaniasis and sand fly-fever in the Awash valley and Libo-Kemkem area.

2.2. Specific objectives

1. To determine the susceptibility level of Melka-Worer (Middle Awash Valley) population of *P. orientalis* to insecticides.
2. To determine the susceptibility level of *P.bergeroti* to insecticides.
3. To determine the susceptibility level of Libo-Kemkem population of *P. orientalis* to insecticides.
4. To compare the susceptibility levels of different population of *P. orientalis* and *P. bergeroti* to the various insecticides.

3. MATERIALS AND METHODS

This investigation was carried out in Melka-Worer (Eastern Ethiopia) and Libo-Kemkem (Northern Ethiopia) between December 2007 and June 2008.

3.1. Study area

3.1.1. Melka-worer

Melka-Worer wereda is located in the Middle Awash Valley at an altitude of 740m.a.s.l in the Afar region, Eastern Ethiopia. Due to fertility of the land, the Awash valley is one of the highly developed agro-industrial areas of the country. The whole valley (Upper to Lower Awash) has been home to large scale irrigated agricultural developments of various cash crops for over forty years. In the Melka-Worer plantation, cotton is the major cash crop produced throughout the year by irrigation. As a result, the valley has been under intense insecticide pressure mainly from agricultural pesticides for many years. At least 19 chemical insecticides based on their common names (see Appendix 5) are currently on use for cotton pest management in Melka-Worer area (Ato Ermias Shonga, Melka-Worer Agricultural Institute, Person. Commn.). Houses are also sprayed with DDT to control malaria vectors.

The Awash valley is characterized by several sand fly species of both *Phlebotomus* and *Sergentomyia* species. Of these, *P. orientalis* is highly abundant and anthropophagic throughout the valley. It is also a highly suspected vector of VL in the valley (Gemetchu and Fuller, 1976; Gebre-Michael *et al*., 2004a). *Phlebotomus* (*Phlebotomus*) *bergeroti* is the second most abundant species throughout the valley especially in the Middle and Lower Awash Valley (Gemetchu and Fuller, 1976; Gebre-Michael *et al*., 2004a). It is a suspected vector of sand fly-fever (papatasi-fever) in Ethiopia (Lewis, 1982).

3.1.2. Libo-Kemkem

Libo-Kemkem wereda is situated at an altitude of 1800-2000 m.a.s.l in the Amhara Region of northwestern Ethiopia. Addis Zemen, the wereda capital, about 630 km north of Addis Ababa located on the road between Bahir Dar and Gondar. It is an area of recent

VL outbreak where, the disease has affected the lives of many people (MOH unpublished report; Alvar *et al*., 2007).

Cultivation, grazing, wood cutting and other human activities have reduced the natural vegetation of the wereda to a few scattered clumps of *Acacia* trees. The area is mostly a flat plain of a broad expanse of black soil. Most of the area is water-logged by floods from the surrounding hills during the rainy season but the land dries up during the dry season (November-May), resulting in deep cracks in the soil surface (Gebre-Michael *et al*., 2007). Sand flies were recently collected in the epidemic area, where *P. orientalis* was the only abundantly encountered and thus strongly suspected as the vector of VL in the area (Gebre-Michael *et al*., 2007). In the area, there is very little insecticide pressure from agricultural spraying. However, the area being malarious, DDT is sprayed regularly in addition to recent ITN distribution (Ato Mitiku Reta, Addis Zemen Health Office, Person. Commn.).

3.2. Collection of sand flies for rearing and subsequent testing

3.2.1. Collection and rearing of sand flies from Melka-Worer (Eastern Ethiopia)

Phlebotomus orientalis and *P. bergeroti* are known to abundantly occur in Melka-Worer. For rearing the two sand fly species and subsequent testing the F_1 progenies in the laboratory, wild *Phlebotomus* species were collected using the Center for Diseases Control and Prevention (CDC) miniature light traps (Jhon W. Hock, Gainesville, FL) (Plate 1) set in *Acacia* forest and cotton farm field near villages to run the whole night from 18:00 to 06:00 hours. In the morning, the sand flies collected were individually placed in small tubes to separate only blood fed, semi-gravid and gravid *Phlebotomus* females from the rest of the sand flies (*Sergentomyia*, male *Phlebotomus* and unfed female *Phlebotomus*) under a dissecting microscope. Thus, only fed, semi-gravid and gravid females of the genus *Phlebotomus* were individually placed in small rearing glass vials (Plate 2), the bottom of which were previously lined with plaster-of-Paris for oviposition and covered with fine mesh screened snap tips. The females were given sterile 10% sugar solution soaked in small cotton pads placed on the screened snap tips. The plaster-of-Paris was also wetted with 4-5 drops of distilled water to keep the

substrate moist for oviposition and to maintain high moisture in the vials. The vials containing the female were placed in larger plastic box which in turn were placed in large ice box with moist tissue paper to maintain high humidity and avoid or minimize death of the flies during transport to main laboratory in Addis Ababa (Aklilu Lema Institute of Pathobiology).

The plastic boxes were placed in an incubator at 27^0C. The flies in vials were daily supplied with fresh pad of cotton soaked with 10% sugar solution and 2-3 drops of distilled water on the bottom of the glass vials with plaster-of-Paris (Killick-Kendrick *et al*., 1977). Oviposition was also checked daily under the microscope. After oviposition, each female was mounted on a glass slide using gum chloral mountant (Gebre-Michael and Lane, 1996b) for identification of the parent female sand fly based on the key by Lewis (1982). After identification, eggs of the same species and age were counted and picked with a fine brush into larger rearing pots (150-250ml) which had been perforated at the base and lined with plaster-of-Paris and kept in humidified plastic boxes (Plate 3). Between150-250 eggs were placed in each rearing pots depending on the availability of eggs. These were put in an incubator at 27^0C. After hatching, larvae were provided with a diet basically that of Modi and Tesh (1983) but modified to consist a mixture of equal weight of **i**. pulverized laboratory animal food pellets and **ii**. dried powdered hyrax faces collected from hyrax habitats (Gebre-Michael, Person. Commn.).

After emerging of the adults, F_1 female of the same species (*P. bergeroti* or *P. orientalis*) were put in a large sand fly cage (35cmx35cmx35cm) (Plate 4) until testing with the insecticides. However, wild gravid and fed *P. orientalis* were unexpectedly low in our collection on several trips made to Melka-Worer. The small number of *P. orientalis* collected and placed for oviposition and rearing resulted in very few eggs and progenies which were insufficient for the test. Moreover, the constant power interruption also affected the progress of the rearing process of both species in general. It was thus decided to conduct the test with *P. orientalis* on wild collected flies in the field (Melka-Worer).

For this purpose, *P. orientalis* in Melka-Worer was collected using CDC light traps as described above. The collected sand flies were brought to the field laboratory (Melka-Worer Health Center). Each individual female *Phlebotomus* was put in small test tube and *P. orientalis* female were differentiated visually from *P. bergeroti* on the basis of body color and size. *Phlebotomus bergeroti* is usually yellowish in color and larger in size than *P. orientalis* which is grayish and smaller in size (Gebre-Michael and Balkew, Person. Commn.). *Phlebotomus* species were also separated from *Sergentomyia* species by the arrangement of abdominal hairs which is an erect in the former and recumbent in the later (Abonnenc and Minter, 1965). Thus unfed and non gravid females recognized as *P. orientalis* were used for the test. In addition portion of the test population of *P. orientalis* were mounted on slide to confirm their identity.

3.2.2. Collection in Libo-Kemkem Woreda (Addis Zemen) (Northern Ethiopia)

In the VL epidemic area of Libo-Kemkem Woreda near Addis Zemen town, sand flies were similarly collected using CDC light traps. In this area only *P. orientalis* of *Phlebotomus* species is known to exist (Gebre-Michael *et al*., 2007). Thus, all the *Phlebotomus* females collected in CDC light traps were regarded *P. orientalis*. Those unfed and non gravid flies were used for insecticides bioassay tests in the field laboratory (Addis Zemen Health Center). Portions of the test population were also mounted on slide ffor confirmation of their identity.

Plate 1. CDC light trap set in the field.

Plate 2. Small glass vials used in the study for oviposition.

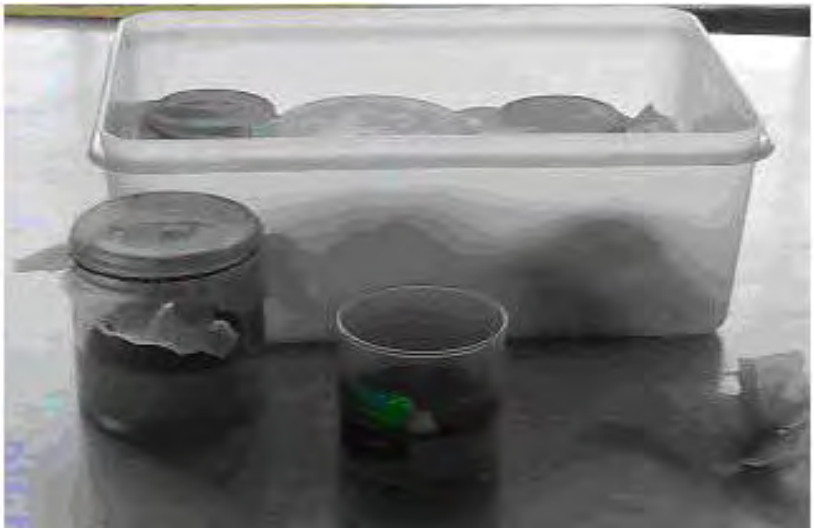


Plate 3. Sand fly rearing pot and plastic box.

Plate 4. Cage (35cmx35cmx35cm) covered with plastic bag.

3.3. Insecticides impregnated papers

Insecticides impregnated papers of WHO discriminating dose were used. DDT and malathion impregnated papers were obtained from Dr. Howard Engirs, the former director of AHRI and from Vector Control Reference Unit of the National Institute for Communicable Disease, Johannesburg, South Africa. Permethrin and deltamethrin impregnated papers were obtained from Vestergaard Company, Denmark. Lambda-cyhalothrin and alpha-cypermethrin impregnated papers were obtained from Syngenta Agro services AG Ethiopia. Table 1 summarizes insecticide impregnated papers and their discriminating concentration used in this study.

Table 1. Insecticides impregnated papers to test susceptibility of test insects in this study.

Insecticide class	Insecticides	WHO discriminating doses
Organochlorine	DDT	4%
Organophosphate	Malathion	5%
Pyrethroids	Permethrin	0.75%
	Alpha-cypermethrin	0.1%
	Deltamethrin	0.05%
	Lambda-cyhalothrin	0.05%

3.4. Bioassay tests for susceptibility and condition of tests

Susceptibility tests were performed using WHO test kits for measuring insecticide resistance/susceptibilities as per the standard guideline of WHO (1981) (Fig. 1). Insecticide susceptibility assays were performed on field population of adult unfed and/or non-gravid female *P. orientalis* that were collected from Melka-Worer and Libo-Kemkem as described above (3.2). In the case of *P. bergeroti* F_1 of 2 to 5 days old and sugar fed female that were reared from field-collected (Melka-Worer) females as described above were used.

For each test 20 female adult sand fly of each species (described above) were introduced into holding tube marked with green dot and held for one hour to verify that the insects were in good condition. Any dead or moribund insects were aspirated out before initiations of test. Those active sand flies were then transferred by gentle blowing into the exposure tubes lined with insecticides impregnated paper and held vertically for one-hour exposure duration. In the case of DDT (organochlorine); permethrin, deltamethrin, lambda-cyhalothrin and alpha-cypermethrin (synthetic pyrethroids) knock down was recorded after 5-, 10-, 15-, 20-, 30-, 40-, 50-, and 60-minute of exposures until all insects were knocked. Knock down time for malathion was not recorded as it has no knock down effect. At the end of the exposure time, sand flies are gently blown back into the holding tube, which is placed vertically for 24 hours with sugar solution. Dead sand flies were counted after 24 hours. The tests were conducted in four replicates for each species and each insecticide. A two replicate of twenty insects for each species in each tube were kept in a tube containing insecticides free paper to serve as control for each insecticide. *Phlebotomus bergeroti* were not tested against insecticides (alpha-cypermethrin) due to the difficulty to find sufficient number of F_1 adult females of test insects within the study period.

Tests in the laboratory with *P. bergeroti* were carried out in a room where holding tubes and exposure tubes were placed in large cartoon (30x35x50cm) covered with moist towel. Temperature inside was about 20^0C and relative humidity 70%. Tests in the field with *P. orientalis* were conducted under shade where the tubes were placed in a cool box covered with moist piece of sack inside which the temperature was 23-25^0C and RH was 76-83%.

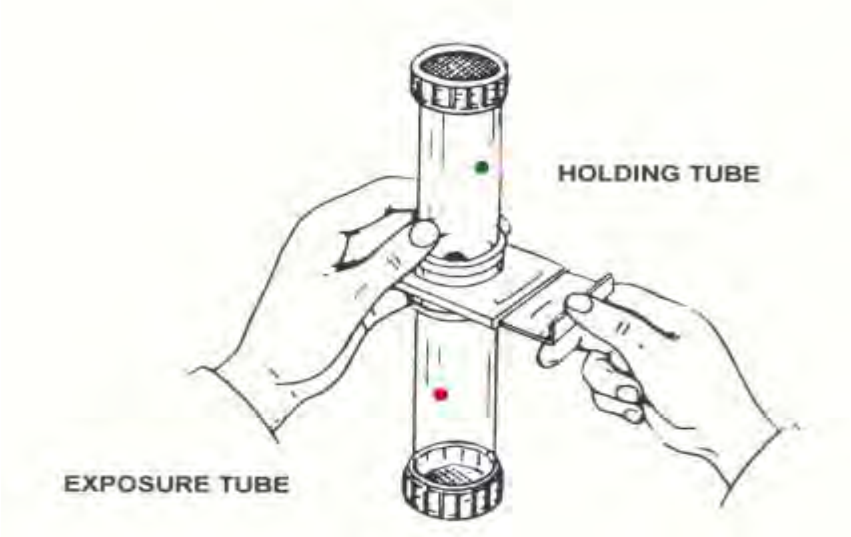


Fig. 1. WHO test kit for measuring insecticides susceptibility tests (Source WHO, 1981)

Plate 5.The cool box used in the field containing the exposure tubes and holding tubes.

3.5. Data analysis

Mean mortality was determined across all sand flies tested for a particular insecticide and the WHO (1992) criterion was used to evaluate the resistance/susceptibility status of sand fly species tested. According to this criterion, resistance is indicated by mortality rates of less than 80% after 24 hour exposure to insecticide while mortality rates greater than 98% are indicative of susceptibility. Mortality rates between 80–97% suggest the possibility of resistance that needs to be confirmed (WHO, 1998).

Since no significant control mortality was observed in the study, there was no need to use Abbot's correcting formula shown below (WHO, 1981).

$$\frac{\%\ \text{test mortality} - \%\text{control mortality}}{100 - \%\text{control mortality}} \times 100$$

Fifty and ninety five percent knockdown times (KT_{5o} and KT_{95}) of the insecticides were estimated using computer soft ware for probit analysis by SPSS 13.0 windows. The KT_{5o} and KT_{95} values of different insecticides were considered significantly different if their 95% confidence intervals did not overlap.

4. RESULTS

4.1. Susceptibility status of *Phlebotomus bergeroti* from Melka-Worer

4.1.1. Knock down effect of insecticides on *Phlebotomus bergeroti* (F_1)

All the four insecticides (DDT, permethrin, lambda-cyhalothrin and deltamethrin) resulted in 100% knock down to F_1 generation of *Phlebotomus bergeroti* during the one hour exposure period although time variation was noted.

Table 2 summarizes KT_{50} and KT_{95} values of the four insecticides against *P. bergeroti* based on probit analysis. The lowest KT_{50} and KT_{95} was caused by DDT (18.9minutes; 95% CI = 15.8-20.8 and 31.1minutes; 95% CI = 27.3– 37.9) while the highest was that of deltamethrin (30.6minutes; 95% CI = 27.6-33.9 and 46.3 minute; 95% CI =41.7-53.6). The KT_{50} of the latter insecticide was 1.7 fold greater than the former. The second lowest KT_{50} and KT_{95} was that of permethrin followed by lambda-cyhalothrin.

Since the 95% confidence limits of DDT, permethrin and lambda-cyhalothrin were overlapping, knock down effect of these insecticides were similar at both values. Deltamethrin was significantly different from all other insecticides based on KT_{50}. But at KT_{95} there was no significant difference between deltamethrin and lambda-cyhalothrin.

4.1.2. Mortality rates of *Phlebotomus bergeroti* (F_1)

The percentage mortality rates of *P. bergeroti* after exposure to various insecticides are tabulated in Table 3. All the insecticides produced 100% mortality to F_1 generation of *P. bergeroti*. However, a single sand fly survives from exposure to malathion resulting in 98.8% overall mortality. In all the tests there was no control mortality.

Table 2. Results of KT_{50} and KT_{95} of insecticides on *Phlebotomus bergeroti* (F_1) from Melka-Worer (2008).

Insecticides	KT_{50} (minutes)	KT_{95} (minutes)	$\chi 2$ (df=6)
DDT (4%)	18.2 (15.8-20.8)	31.1 (27.3-37.9)	6.636
Permethrin (0.75%)	19.5 (17.1-22.2)	32.1 (28.1-39.2)	2.097
Lambda-cyhalothrin (0.05%)	23.4 (20.6-26.5)	39.2 (34.6-46.9)	1.616
Deltamethrin (0.05%)	30.6 (27.6-33.9)	46.3 (41.7-53.6)	1.040

Figures in parentheses are 95% confidence interval, χ^2 values are for the test of fit of the log-time probit model used to estimate the KT_{50} and KT_{95} values; df =degree of freedom

Table 3. Results of mortality of *Phlebotomus bergeroti* (F_1) from Melka-Worer exposed to various insecticides (2008).

Insecticides	Number of replicates	Number tested	Percent mortality	S.E
DDT (4%)	4	80	100	± 0.00
Permethrin (0.75%)	4	80	100	± 0.00
Lambda-cyhalothrin (0.05%)	4	80	100	± 0.00
Deltamethrin (0.05%)	4	80	100	± 0.00
Malathion (5%)	4	80	98.75	± 0.25
Control	10	200	0	± 0.00

S.E = standard error

4.2. Susceptibility status of wild caught *Phlebotomus orientalis* from Melka-Worer

4.2.1. Knock down effect of insecticides on *Phlebotomus orientalis*

The knock down effect of DDT, deltamethrin, alpha-cypermethrin and permethrin was achieved within 40 minutes whereas lambda-cyhalothrin took 30 minutes.

Table 4 reveals the result of KT_{50} and KT_{95} of insecticides that were used in this study on *P. orientalis*. There was variation in KT_{50} and KT_{95} among the insecticides. The KT_{50} ranged from 8.2 (95% CI= 4.1-10.7) minutes for permethrin to 20.1 (95% CI=17.9-22.8) minutes for DDT. But the KT_{95} ranged from 21.3 (95% CI=19.0-27.4) minutes for lambda-cyhalotrin to 31.6 (95% CI= 27.9-38.3) minutes for DDT. The KT_{50} of DDT was 2.5 fold higher than permethrin. Thus, permethrin showed the highest knock down effect at KT_{50} and lambda-cyhalothrin at KT_{95}.

The 95% confidence intervals of KT_{50} of DDT and pyrethroids did not overlap indicating that the knock down effect of DDT and pyrethroids were significantly different at KT_{50} But DDT had equal KT_{95} time with deltamethrin and permethrin. The KT_{95} the difference was not significant among all pyrethroid insecticides. But at KT_{50} permethrin was significantly different from lambda-cyhalothrin and alpha-cypermethrin.

4.2.2. Mortality rates of *Phlebotomus orientalis*

Table 5 shows results of percentage mortality of *P. orientalis* to various insecticides. All *P. orientalis* exposed to DDT, permethrin, lambda-cyhalothrin and alpha-cypermethrin failed to survive. However, of those exposed to deltamethrin, a single sand fly was unaffected. Mortality from malathion was lower (93.8%) than the rest. Mortality on the control was 0% for all the insecticides.

Table 4. Results of KT_{50} and KT_{95} of insecticides on *Phlebotomus orientalis* from Melka-Worer (2008).

Insecticides	KT_{50} (minutes)	KT_{95} (minutes)	χ^2 (df=6)
Permethrin (0.75%)	8.2 (4.1-10.7)	21.7 (18.0-29.6)	8.024
Deltamethrin (0.05%)	12.48 (10.4-14.5)	22.2 (19.2-27.9)	1.803
Alpha-cypermethrin (0.1%)	12.7 (10.8-14.6)	21.9 (19.1-27.3)	3.027
Lambda-cyhalothrin (0.05%)	12.7 (10.8-14.7)	21.3 (19.0-27.4)	0.573
DDT (4%)	20.1 (17.9-22.8)	31.6 (27.9-38.3)	0.926

Figures in parentheses are 95% confidence interval, χ^2 values are for the test of fit of the log-time probit model used to estimate the KT_{50} and KT_{95} values; df = degree of freedom

Table 5. Results of mortality of *Phlebotomus orientalis* from Melka-Worer exposed to various insecticides (2008).

Insecticides	Number of replicates	Number tested	Percent Mortality	S.E
DDT (4%)	4	80	100	± 0.00
Permethrin (0.75%)	4	80	100	± 0.00
Lambda-cyhalothrin (0.05%)	4	80	100	± 0.00
Alpha-cypermethrin (0.1%)	4	80	100	± 0.00
Deltamethrin (0.05%)	4	80	98.75	± 0.25
Malathion (5%)	4	80	93.75	± 0.25
Control	12	2 40	0	± 0.00

S.E = standard error

4.3. Susceptibility status of wild caught *Phlebotomus orientalis* from Libo-Kemkem

4.3.1. Knock down effect of insecticides on *Phlebotomus orientalis*

The knock down effect of the majority of pyrethroids and DDT against *P. orientalis* from Libo-Kemkem was detected within 40 minutes. Permethrin however, took 10 minute less.

The results of KT_{50} and KT_{95} of the test insecticides on field population of *P. orientalis* in Libo-Kemkem are shown in Table 6. In general variations in KT_{50} and KT_{95} were observed among insecticides. The lowest KT_{50} was that of permethrin (11.7 minutes; 95% CI = 9.5-13.8) followed by alpha-cypermethrin (12.1minutes; 95% CI =10.1-14.0) and deltamethrin (13.3 minutes; 95% CI = 11.3-15.1). Moreover, the lowest KT_{95} was by alpha-cypermethrin (21.1 minutes; 95% CI = 18.4-26.1) followed by permethrin (22.0 minutes; 95% CI = 18.9-28.2) and deltamethrin (22.3 minute; 95% CI = 19.5-27.5). But the highest KT_{50} and KT_{95} was that of DDT (19.1 minutes; 95% CI = 16.9-21.7 and 30.2 minutes; 95% CI = 26.5-36.9).

At the level of KT_{50} insecticides (DDT and lambda-cyhalothrin) were significantly different from the remaining insecticides based on the 95% confidence interval, although both were not significantly different from each other. The differences at KT_{95} were not significant between DDT and other insecticides except with alpha-cypermethrin.

4.3.2. Mortality rates of *Phlebotomus orientalis*

The mortality results of field population of *P. orientalis* from Libo-Kemkem exposed to six insecticides are depicted in Table 7. The pyrethroids and malathion caused 100% mortality to *P. orientalis* form Libo-Kemkem. However, a single sand fly survived from DDT exposure giving mortality rate of 98.8%. No mortality was observed in control.

Table 6. Results of the KT_{50} and KT_{95} of insecticides on *Phlebotomus orientalis* from Libo-Kemkem (2008).

Insecticides	KT_{50} (minutes)	KT_{95} (minutes)	χ^2 (df=6)
Permethrin (0.75%)	11.7 (9.5-13.8)	22.0 (18.9-28.2)	0.397
Alpha-cypermethrin (0.1%)	12.1 (10.1-14.0)	21.1 (18.4-26.1)	9.872
Deltamethrin (0.05%)	13.3 (11.3-15.1)	22.3 (19.5-27.5)	6.840
Lambda-cyhalothrin (0.05%)	18.0 (16.1-20.2)	26.9 (23.9-32.6)	3.504
DDT (4%)	19.1 (16.9-21.7)	30.2 (26.5-36.9)	0.644

Figures in parentheses are 95% confidence interval, χ^2 values are for the test of fit of the log-time probit model used to estimate the KT_{50} and KT_{95} values; df = degree of freedom

Table 7. Results of mortality of *Phlebotomus orientalis* from Libo-Kemkem exposed to various insecticides (2008).

Insecticides	Number of replicates	Number tested	Mortality (%)	S.E
Permethrin (0.75%)	4	80	100	± 0.00
Deltamethrin (0.05%)	4	80	100	± 0.00
Alpha-cypermethrin (0.1%)	4	80	100	± 0.00
Lambdacyhalothrin(0.05%)	4	80	100	± 0.00
Malathion (5%)	4	80	100	± 0.00
DDT (4%)	4	80	98.75	± 0.25
Control	12	240	0	± 0.00

S.E = standard error

4.4. Comparisons of knock down times between species and insecticides in the study areas

For comparisons of the KT_{50} values of the insecticide with regards to *P. orientalis* population from Melka-Worer and Libo-Kemkem and *P. bergeroti* from Melka-Worer, results are depicted in Fig. 2.

The KT_{50} time difference when both species were exposed to DDT was negligible. *Phlebotomus orientalis* from both localities were more affected by deltamethrin and permethrin than *P. bergeroti*. The response of *P. orientalis* in both localities to all insecticides was significantly similar except to lambda-cyhalothrin. The Melka-Worer population of *P. orientalis* was more sensitive to lambda-cyhalothrin than the Libo-Kemkem population. The lowest KT_{50} was recorded for permethrin against *P. orientalis* from Melka-Worer while, the highest KT_{50} was that of deltamethrin against *P. bergeroti*.

Similar comparisons on the KT_{95} values of the insecticides on the two species of sand flies from the respective localities are shown in Fig. 3. With DDT and permethrin at KT_{95}, *P. bergeroti* had similar response with the population of *P. orientalis* from the two localities but had significantly lower response to deltamethrin and lambda-cyhalothrin. The response of *P orientalis* from both localities to all insecticides was similar.

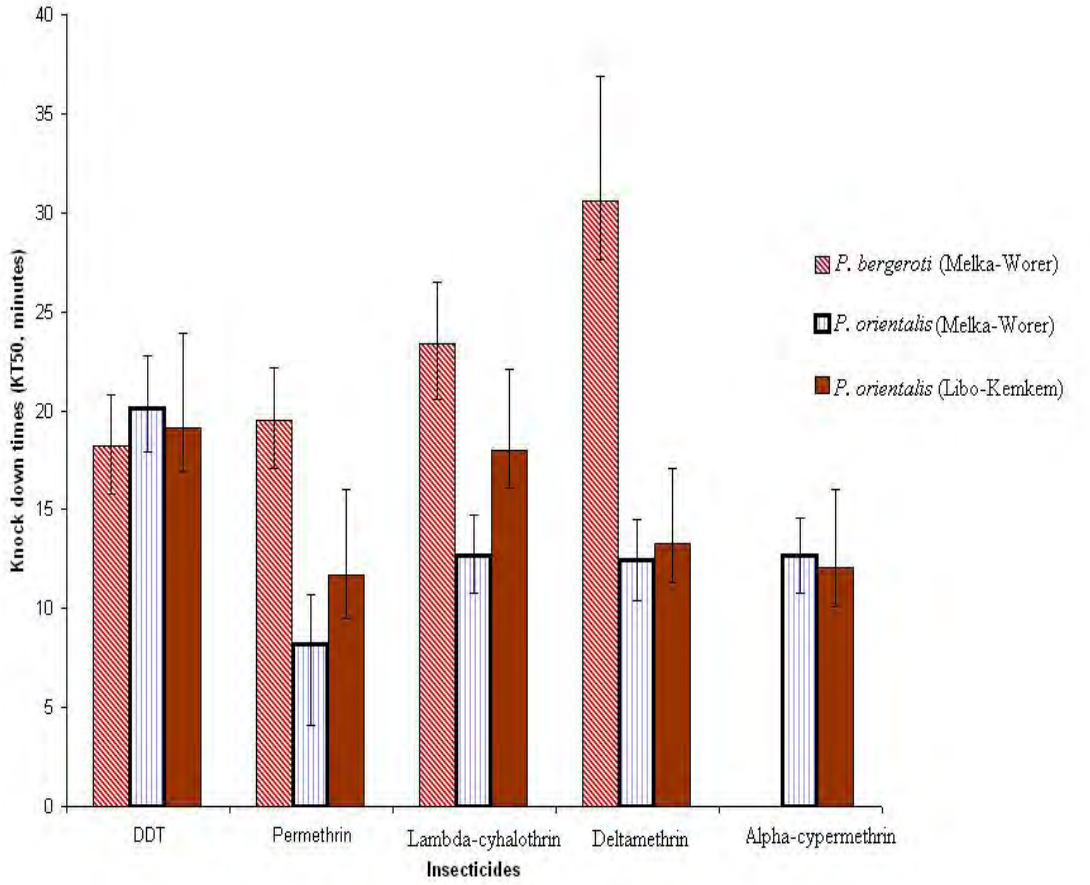


Fig. 2. KT_{50} values of insecticides on *Phlebotomus bergeroti* and *P. orientalis* from Melka-Worer and Libo-Kemkem (2008). Bar indicates 95% confidence interval of KT_{50}

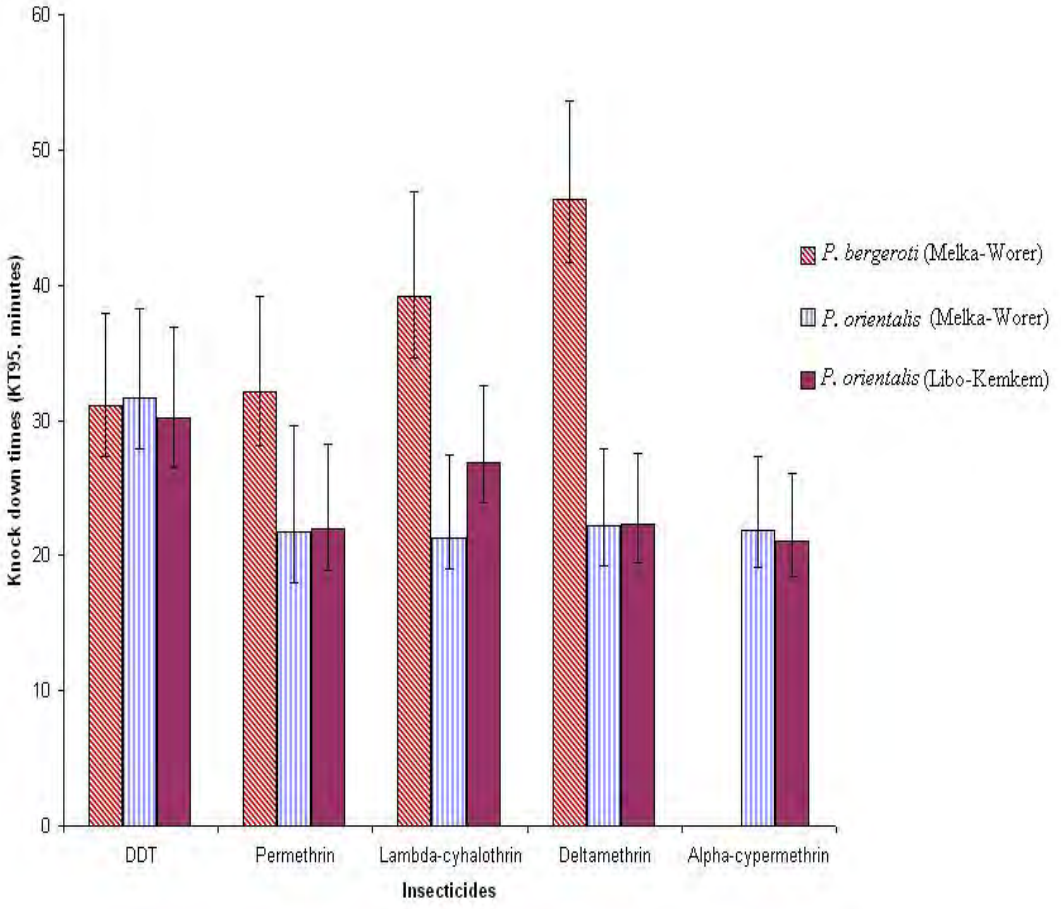


Fig. 3. KT_{95} values of insecticides on *Phlebotomus bergeroti* and *P. orientalis* from Melka-Worer and Libo-Kemkem (2008). Bar indicates 95% confidence interval of KT_{95}

5. DISCUSSION

Pesticides have been the cornerstones for the prevention of vector-borne diseases for the past 50 years; however, use of chemicals extensively and indiscriminately at increased rate has led to the widespread development of resistance as a result of selection for certain genes (WHO, 2006). Therefore detection, monitoring and evaluation of the susceptibility status of vectors are important to delay the build up of vector resistance. Insecticide resistance is commonly assessed by exposing adults to a diagnostic dosage (concentration x time), which normally induces 100% mortality of susceptible individuals (WHO, 1998).

In the present study the status of susceptibility of *P. bergeroti* and *P. orientalis* to 4% DDT, 5% malathion, 0.75% permethrin, 0.05% lambda-cyhalothrin, 0.05% deltamethrin and 0.05% alpha-cypermethrin (0.1%) was investigated. Based on the conventional criteria for characterizing insecticides resistance/susceptibility, where susceptibility is defined by mortality rates greater than 98% after 24 after exposure (WHO, 1998), no evidence for resistance to any of the insecticides tested namely DDT, permethrin, lambda-cyhalothrin, deltamethrin and malathion was found in *P. bergeroti*. The results obtained in this study suggest good susceptibility of *P. bergeroti* in the study area to all the five insecticides tested. A similar study in Egypt on *P. bergeroti* laboratory colony established from females collected in the Republic of Djibouti, showed high susceptibility to DDT, permethrin and malathion (Tetreault *et al*., 2001).

No other information available on the susceptibility status of *P. bergeroti*. Among the sand fly species, *P. papatasi* which belongs to the same subgenus (*Phlebotomus*) with *P. bergeroti* and which is the principal vector of *L. major* is the most studied species on its susceptibility to insecticides in various parts of the world (Alexander and Maroli, 2003). So, *P. papatasi* has been found resistant in different regions (states) of India to about six insecticides (see Appendix 4). The species was reported to have a high degree of resistance to DDT in different areas of India (Rahman *et al*., 1982; Mukhopadhyay *et al*., 1987; Saxena, 1987). Kaul *et al*. (1978), working in the kala-azar epidemic area of Bihar State (India) reported the existence of DDT-resistant populations of *P. papatasi*.

Exposures of one hour to 4%DDT resulted in very low mortalities of two field populations: 20.8% in one population and 2.3% in another population.

Moreover in Pondicherry, southern India, *P. papatasi* were resistant to permethrin and lamda-cyhalothrin and it showed a low level of resistance to malathion and deltamethrin (Amalraj *et al*., 1999). However in Rajastan, India it was found to be susceptible to malathion and permethrin (Bansal and Singh, 1996). The variation in susceptibility between *P. papatasi* from India and *P. bergeroti* in our study could be due to different resting behavior of the two sand flies. *Phlebotomus papatasi* have an endophilic habit (Lewis, 1982), as a result it has more chances of contact with indoor spray of residual insecticides which could impose selection pressure. On the other hand, *P. papatasi* has been found susceptible to the same and other insecticides in different region of India itself as well as many other countries evaluated (Alexander and Maroli, 2003).

Published data on insecticide susceptibility status of *P. orientalis* has not been available to date. To our knowledge the present investigation on *P. orientalis* (one of the principal vectors of VL in Eastern Africa) is the first of its kind. Results from the present study showed that this species from both localities was highly susceptible to all insecticides tested. However it only showed a low level of resistance (6.2%) to malathion in Melka-Worer which suggests an incipient altered susceptible status as per WHO (1998) criteria. Elsewhere, other sand fly species, which exist in the same subgenus (*Larroussius*) with *P. orientalis* were studied for their susceptibility to insecticides. *Phlebotomus langeroni* the most related species to *P. orientalis* in Egypt showed high susceptibility to DDT, permethrin and malathion (Tetreault *et al*., 2001). *Phlebotomus perniciosus*, the main vector of human and canine VL caused by *L. infantum* in Italy was susceptible to DDT, malathion, permethrin and lambda-cyhalothrin (Lavagnino and Ansaldi, 1991; Maroli *et al*., 2002). A wild caught *P. perfiliewi*, which also found in the same subgenus with the former species, was susceptible to DDT and permethrin in Italy. The detection of this low level of resistance (6.2%) to malathion in *P. orientalis* in the present study might be the first record in this subgenus as a whole.

In Melka-Worer Balkew *et al*. (2003) reported that *An*. *arabiensis* was resistant to DDT (42.5%) and 100% susceptible to permethrin. This is in contrast with what has recorded in sand flies with respect to DDT in this area. This variation in susceptibility between mosquitoes and sand flies might be due to the following factors. According to Hemingway and Ranson (2000) the major factor influencing insecticide resistance development in insects are the time of duration in the life cycle, and the number of young produced by insect vectors. Hence the variation in the development of resistance in mosquitoes and lack in sand flies in study area to DDT may be due to the long life cycles and the production of very small numbers of young by the sand flies and short life cycles with abundant progeny by mosquitoes (Service, 2000). Another factor responsible for rapid development of resistance in insects according to WHO (2006) depends on the behavior of the insect. The most endophilic behavior of *An*. *arabiensis* than sand flies in the study area increases the time of exposure of mosquitoes to DDT than sand flies. Therefore in contrast to sand flies, mosquitoes have all the characteristics suited to rapid development of resistance.

It is interesting to note that, despite the high level use of pesticides for a long period of time, due to an extensive agricultural practice in the Awash Valley and particularly in Melka-Worer, the *Phlebotomus* species have not developed any resistance to the chemical insecticides evaluated.

Since mortality rate in *P*. *orientalis* of Libo-Kemkem was above 98.8%, no evidence for resistance to any of the insecticides tested was found according to the standard of WHO criteria. Both Libo-Kemkem and Melka-Worer are expected to have the same history of DDT use for antimalarial spraying. However, Libo-Kemkem appears to be under low insecticides pressure from agricultural pesticides compared to Melka-Worer, which has a long history of agricultural pesticides use due to the extensive mechanized cotton farms (see Appendix 5). Yet population of *P*. *orientalis* from the two localities exhibited almost equal high level of susceptibility to almost all insecticides tested. However, the development of very low level of resistance (6.2%) to malathion in Melka-Worer, although needs further evaluation and confirmation might be due to selection pressure of

the agricultural pesticides. In Melka-Worer, malathion has been used for a long time to control agricultural pests like onion thrips and sesame pests (Ato Ermias Shonga, Melka-Worer Agricultural Institute, Person. Commn.).

In two different districts in Bihar state of India *P. argentipes* showed variation in susceptibility between two places. According to Kishore *et al*. (2004) this variations in two areas were due to different insecticidal pressure as part of the kala-azar control program. These differences emphasize the focal nature of insecticide resistance and the need to carry out situational analyses and monitoring for individual settings.

In addition to mortality, knock down time is a good indicator for an early detection of reduced susceptibility (WHO, 1998). Prior to this study, no published reports were available on the KT_{50} and KT_{95} values of pyrethroids and DDT against various sand fly species that have been evaluated in different countries. The majority of the studies have been performed by using dose-mortality bioassay, and thus it was difficult to compare our results based on knock down bioassay with others. The measurement of knock down time provides initial information on the possible involvement of the *kdr* gene. Thus, our results serve as a reference for further studies for the early detection of reduced susceptibility. The KT_{50} and KT_{95} of all insecticides (except KT_{50} and KT_{95} of deltamethrin on *P. bergeroti*) observed in the present study are similar or less to those observed for *An. gambiae* sensu lato populations that are categorized as susceptible by different investigators (Chandre *et al*., 2000; Etang *et al*., 2003; Kamau and Vulule, 2006). But we observed a significant increase of these values (about 3 fold) in deltamethrin on *P. bergeroti* compared to susceptible reference strain of *An. gambiae* (Etang *et al*., 2003). The increase of KT for deltamethrin suggested that at least a target site mutation (*kdr*) was involved. Although this requires further evaluation, we still recommend the use of deltamethrin for impregnation of nets provided the mortality rate remains high.

The KT_{50} values in the present study indicated that permethrin was found to have high level of knock down effect on Melka-Worer and Libo-Kemkem population of *P. orientalis* of 8.2 minute and 11.7 minute, respectively, than other pyrethroids. However,

permethrin exhibited slightly higher level of knock down, though the 95% confidence limits indicate similar level of activity with deltamethrin in both locality and with alpha-cypermethrin in Libo-Kemkem. In both areas DDT on *P. orientalis* showed lowest knock down effect at both KT_{50} and KT_{95} values. In contrast to *P. orientalis*, DDT showed higher knock down effect followed by permethrin on *P. bergeroti*.

Considering confidence intervals of KT_{50} and KT_{95} of insecticides: permethrin, deltamethrin, alpha-cypermethrin and DDT; the response of *P. orientalis* in both localities to each of these insecticides was similar. But knock down bioassays to lambda-cyhalothrin revealed more than 1.4 folds higher values of KT_{50} in *P. orientalis* from Libo-Kemkem, than populations from Melka-Worer. Even though there was 100% mortality in *P. orientalis* exposed to diagnostic concentration of lambda-cyhalothrin for one hour, the delayed knock-down effect of lambda-cyhalothrin in *P. orientalis* from Libo-Kemkem (KT_{50}: 18minute), may indicate the development of tolerance to lambda-cyhalothrin in this population of *P. orientalis*. According to Chandre *et al*. (2000) a significant increase in knock down times before any change in mortality rates is known to be an indicator for early detection of resistance in populations. Remarkable change in mortality becomes evident only after successive generations.

The KT_{50} value of permethrin (on permethrin susceptible strain) *An. arbabienis* (KT_{50} = 12.5 minutes) that observed in Melka-Worer by Balkew *et al*. (2003) was higher than that obtained in *P. orientalis* and lower than *P. bergeroti* in the same place. However, the KT_{50} value of permethrin (19.4 minutes) and deltamethrin (22.8 minutes) obtained in Gorgora (North Gondar) against *An. pharoensis* by Balkew *et al*. (2006) was higher than that obtained in Libo-kemkem against *P. orientalis*. But the KT_{50} value of deltamethrin obtained in *P. bergeroti* was 1.3 fold higher than that obtained Gorgora. Thus the KT_{50} of deltamethrin against *P. bergeroti* is the highest in the country than mosquitoes studied.

In the present study although *P. orientalis* in both areas were susceptible based on dose mortality bioassay to all pyrethroids and DDT, the KT_{50} of DDT however is about 2.5 fold and 1.5 fold higher than that of permethrin in Melka-Worer and Libo-Kemkem,

respectively. Thus, permethrin provided higher knock down effect than DDT. In respect to *P. bergeroti* the KT_{50} value of deltamethrin is about 1.6 fold higher than DDT and permethrin, though this species was equally susceptible to all insecticides.

6. CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The results obtained in this study suggest the two sand fly species (*P. orientalis* and *P. bergeroti*) from the two localities are highly susceptible to all insecticides tested based on WHO discriminating doses. The low level of resistance of *P. orientalis* to malathion (6.2%) in Melka-Worer does not pose any serious concern on any vector control measure against this species.

The KT_{50} and KT_{95} values for the two species except *P. bergeroti* to deltamethrin were either lower than or equal with those of *An. gambiae* s. l. categorized as susceptible in other studies. In addition, the data (KT_{50} and KT_{95}) obtained will provide baseline information needed in for monitoring the development of resistance (*kdr*) to the insecticides.

6.2. Recommendation

All the insecticides tested could be effective for any control measure against the two species of sand flies in the respective localities for either indoor residual sprays (DDT and malathion) or ITNs (the pyrethroids) if the vectors exhibit significant or some degree of endophilic or peridomestic biting behavior. However, the behavior of the sand fly must be studied before.

Similar studies must be conducted in other major VL endemic areas of Metema and Humera (where *P. orientalis* is the vector) and in Southern Ethiopia (where *P. martini* and *P. celiea* are the vectors). Metema and Humera (similar to the Awash valley) have extensive commercial farms and are expected to be under high insecticidal pressure from agricultural spraying.Similar evaluations must also be carried out against the two major vectors of CL (*L. aethiopica*) in the country (*P. longipes* and *P. pedifer*).

REFERENCES

Abonnenc, E. and Minter, D. M. (1965). Keys for the identification of the sand flies of the Ethiopian Region. *Cahiers Office de la Rechereche Scientifigue et Technique Outre Mer* (*Entomologie Medicale*), **5**: 1-63.

Abose T., Yeebiyo Y., Olana D., Alamirew D., Beyene Y.A., Regassa L. and Mengesha A. (1998). *Re-orientation and definition of the role of malaria vector control in Ethiopia*.World Health Organization, WHO/Mal/1998.1085.WHO, Geneva, 31pp.

Aboul-Ela, R. G., Morsy, T. ., El-Gozamy, B. M. and Ragheb, D. A. (1993). The susceptibility of the Egyptian *Phlebotomus papatasi* to five insecticides. *Journal of Egyptian Society of Parasitology*, **23**: 69-94.

Alexander, B., Cadena, H., Usma, M. C. and Rojas, C. A. (1995a). Laboratory and field evaluations of a repellent soap containing diethyl toluamide and permethrin against phlebotomine sand flies (Diptera: Psychodidae) in valle del Cauca, Colombia. *American Journal of Tropical Medicine and Hygiene*, **52**: 169-173.

Alexander, B., Jaramillo, C., Usma, M. C., Quesada, B. L., Cadena, H., Roa, W. and Travi, B. L. (1995b). An attempt to control phlebotomine sand flies (Diptera: Psychodidae) by residual spraying with deltamethrin in a Colombian village. *Memorias do Instituto Oswaldo Cruz*, **90**: 421-424.

Alexander, B., Usma, M. C., Cadena, H., Quesada, B. L., Solarte, Y., Roa, W.and Travi, B. L. (1995c). Evaluation of deltamethrin impregnated bednets and curtains against phlebotomine sand flies in Valle del Cauca, Colombia. *Medical and Veterinary Entomology*, **9**: 279-283.

Alexander, B. and Maroli, M. (2003). Control of phlebotomine sand flies. *Medical and Veterinary Entomology*, **17**: 1-8.

Ali, A. (1997). Leishmaniasis survey in the Awash valley: Leishmanian skin test profile in the upper Awash and surrounding areas. *Ethiopian Medical Journal*, **35**: 225-233.

Ali, A., Berhe, N., Mengistu, G., Gebre-Michael, T. (2002). Leishmaniasis survey in the Awash valley: the magnitude and of the positive leishmanian reaction and its pattern in the Middle Awash. *Ethiopian Journal of Health Development*, **16**: 157-163.

Ali, A., Gebre-Michael, T., Mengistu, G., Balcha, F. (2004). A survey on leishmaniasis and leishmanian skin test profile in lower Awash valley, northern Ethiopia. *Ethiopian Journal of Health Development*, **18**: 159-163.

Alten, B., Caglar, S., Kaynas, S. and Simsek, F. M. (2003). Evaluation of protective efficacy of K-OTAB impregnated bednets for cutaneous leishmaniasis control in Southeast Anatolia-Turkey. *Journal of Vector Ecology*, **28**: 53-64.

Alvar, J., Bashaye, S., Argaw, D., Cruz, I., Aparicio, P., Kassa, A. Orfanos, G., Parreno, F. Babaniyi, O., Gudeta, N., Cañavate, C. and Bern, C. (2007). Kala-Azar outbreak in Libo Kemkem, Ethiopia: Epidemiologic and parasitologic assessment. *American Journal of Tropical Medicine and Hygiene*, **77**: 275–282.

Amalraj, D. D., Sivagnaname, N. and Srinivasan, R. (1999). Susceptibility of *Phlebotomus argentipes* and *Phlebotomus*. *papatasi* (Diptera: Psychodidae) to insecticides. *Indian Journal of Communicable Diseases*, **1**: 177-180.

Ashford, R. W., Bray, M. A., Hutchinson, M. P. and Bray, R. S. (1973a). The epidemiology of cutaneous leishmaniasis in Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **67**: 568-601.

Ashford, R.W., Hutchinson, M.P. and Bray, R. S. (1973b). Kala-azar in Ethiopia: pidemiological studies in a highland valley. *Ethiopian Medical Journal*, **11**: 259-264.

Ashford, R. W., Seaman, J., Schorscher, J. and Pratlong, F. (1992). Epidemic visceral leishmaniasis in southern Sudan: Identity and systematic positions of the parasites from patients and vectors. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **86**: 379-380.

Asilian, A., Sadeghinia, A. Shariati, F., Jome, M. I. and. Ghoddusi, A. (2003). Efficacy of permethrin-impregnated uniforms in the prevention of cutaneous leishmaniasis in Iranian soldiers. *Journal of Clinical Pharmacy and Therapeutics*, **28**: 175-178.

Balkew, M., Elhassen, I., Ibrahim, M., Gebre-Michael, T. and Engers, H. (2006). Very high DDT-resistant population of *Anopheles pharoensis* Theobald (Diptera: Culicidae) from Gorgora, northern Ethiopia. *Parasite*, **13**: 327-329.

Balkew, M., Gebre-Michael, T. and Hailu, A. (2003). Insecticide susceptibility level of *Anopheles arabiensis* in two agrodevelopment localities in eastern Ethiopia. *Parassitologia*, **45**:1-3.

Bansal, S. K. and Singh K. V. (1996).Susceptibility status of *Phlebotomus papatasi* and *Sergentomyia punjabaensis* (Diptera Psychodidae) to some insecticides in district Bikaner (Rajasthan). *Journal of Communicable Diseases*, **28**: 28-32.

Bern, C., Joshi, A. B., Jha, S. N., Das, M. L., Hightower, A., Thakur, G. D. and Bista, M. B. (2000). Factors associated with visceral leishmaniasis in Nepal: bed-net use is strongly protective. *American Journal of Tropical Medicine and Hygiene*, **63**: 184–188.

Boakye, D. A., Wilson, M. D. and Kweku, M. (2005). A review of leishmaniasis in West Africa. *Ghana Medical Journal*, **39**: 94-97.

Bongiorno, G., Panchetti, F., Zaim, M. and Maroli, M. (2005). Laboratory study to investigate the efficacy of cyfluthrin EW treated nets against phlebotomine sand flies. *Annalia dell Istituto Superiore di Sanità*, **41**: 247-252.

Brogdon, W. G. and McAllister, J. C. (1998). Insecticide resistance and vector control. *Emerging Infectious Diseases*, **4**: 605-613.

Chandre, F., Darriet, F., Duchon, S., Finot, L., Manguin, S, Carnevale, P. and Guillet, P. (2000). Modifications of pyrethroid effects associated with *kdr* mutation in *Anopheles gambiae*. *Medical and Veterinary Entomology*, **14**: 81-88.

Chandre, F., Darriet, F., Manga, L., Akogbeto, M., Faye, O., Mouchet, J. and Guillet, P. (1999). Status of pyrethroid resistance in *Anopheles gambiae* sensu lato. *Bulletin of World Health Organization*, **77**: 230-234.

Chandra, G., Bhattachakya, J. and Hati, A. K. (1995). Susceptibility status of *Phlebotomus argentipes* to DDT, dieldrin and malathion in Hoogly, West Bengal. *Journal of Communicable Diseases*, **27**: 247-249.

Courtenay, O., Gillingwater, K., Gomes, P. A., Garcez, L. M. and Davies, C. R (2007). Deltamethrin impregnated bednets reduce human landing rates of sand fly vector *Lutzomyia longipalpis* in Amazon household. *Medical and Veterinary Entomology*, **21**: 168-176.

Davies, C. R., Llanos-Cuentas, E. A, Campos, P., Monge, J., Leon, E. and Canales, J. (2000). Spraying houses in the Peruvian Andes with lambda-cyhalothrin protects residents against cutaneous leishmaniasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **94**: 631-636.

Davies, C. R**.,** Kaye, P., Croft, S. L. and Sundar, S. (2003). Leishmaniasis: new approaches to disease control. *British Medical Journal*, **326**: 377-382.

Dergacheva, T. I. and Strelkova, M. V. (1986). Sensitivity of *Phlebotomus papatasi*, scopli, 1786 (Diptera, Phlebotominae} to DDT in the Mubarek region of the Uzbek SSR. *Medicinskaja Parasitologija*, **4**: 84-85.

Dhiman, R. C., Raghavendra, K., Vijay, K. S. and Kishore, K. (2003). Susceptibility status of *Phlebotomus argentipes* to insecticides in districts Vaishali and Patna (Bihar). *Journal of Communicable Diseases*, **3**: 49-51.

Dhiman, R. C., and Sharma, V. P. (1994). Evaluation of neem oil as sand fly, *Phlebotomus papatasi* (scopoli) repellent in an oriental sore endemic area in Rajasthan. *Southeast Asian Journal of Tropical Medicine and Public Health*, **25**: 608-610.

Elnaiem, D. A., Aboud, M. A., Elmubarek, S. G., Hassan, H. K. and Ward, R. (1999a). Impact of pyrethroid-impregnated curtains on *Phlebotomus papatasi* sand flies indoors at Khartoum, Sudan. *Medical and Veterinary Entomology*, **13**: 191-197.

Elnaiem, D. A., Elnahas, A. M. and Aboud, M. A. (1999b). Protective efficacy of lambda-cyhalothrin impregnated bednets against *Phlebotomus orientalis*, the vector of visceral leishmaniasis in Sudan. *Medical and Veterinary Entomology*, **13**: 310-314.

El-Sayed, S., Hemingway, J, and Lane, R. P. (1989). Susceptibility baselines for DDT metabolism and related enzyme systems in the sand fly *Phlebotomus papatasi* (Diptera: Psychodidae). *Bulletin of Entomological Research*, **79**: 679-684.

Esterre, P., Chippaux, J. P., Lefait, J. F. and Dedet, J. P. (1986). Evaluation of cutaneous leishmaniasis control program in a forest village in French Guyana. *Bulletin of the World Health Organization*, **64**: 559-565.

Etang, J., Manga, L., Chandre, F., Guillet, P. Fondjo, E., Mimpfoundi, R. Toto, J. and Fontenille, D. (2003). Insecticide susceptibility status of *Anopheles gambiae* s.l.

(Diptera : Culicidae) in the republic of Cameroon. *Journal of Medical Entomology*, **40**: 491-497.

Fhamy, A. R., Khater, E. M., El Sawaf, B. and Shehata, M. (1996). *Insecticide susceptibility status of field populations of sand fly Phlebotomus papatasi in the Sinai Peninsula*, *Egypt*. WHO/LEISH/96.38, World Health Organization, Geneva.

Falcao, A. L., Falcao, A. R., Pinto, C. T., Gontijo, C. M. and Falqueto, A. (1991). Effect of deltamethrin spraying on the sand fly populations in a focus of American cutaneous leishmaniasis. *Memorias do Instituto Oswaldo Cruz*, **86**: 399-404.

Falcao, A. R., Pinto, C. T. and Gontijo, C. M. (1988). Susceptibility of *Lutzomyia longipalpis* to deltamethrin. *Memorias do Instituto Oswaldo Cruz*, **83**: 395-396.

Feliciangeli, M. D., Mazzarri, M. B., Lendrum, D. C., Maroli, M. and Maingon, R. (2003). Cutaneous leishmaniasis vector control perspectives using lambda-cyhalothrin residual house spraying in El Ingenio, Miranda State, Venezuela**.** *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **97**: 641-646.

Fuller, G. K., Lemma, A., Hailu, T. and Gemeda, N. (1979). Kala-azar in Ethiopia: survey of south-west Ethiopia. The leishmanin skin test and epidemiological studies. *Annals Tropical Medicine and Parasitology*, **73**: 417-430.

Gebre-Michael, T. and Balkew, M. (2003). *Phlebotomus* (*paraphlebotomus*) *gemetchi* (Diptera: Psychodidae), a new sand fly species from Ethiopia. *Journal of Medical Entomology*, **40**: 141-145.

Gebre-Michael, T., Balkew, M., Alamirew, T., Gudeta, N. and Reta, M. (2007). Preliminary entomological observations in a highland area of Amhara region, northern Ethiopia, with epidemic visceral leishmaniasis. *Annals Tropical Medicine and Parasitology*, **101**: 367-370.

Gebre-Michael, T., Balkew, M., Ali, A., Ludovisi, A., Gramiccia, M. (2004a). The isolation of *Leishmania tropican* and *L. aethiopica* from *Phlebotomus* (*Paraphlebotomus*) species (Diptera: Psychodidae) in the Awash valley, northeastern Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **98**: 64-70.

Gebre-Michael, T. and Lane, R. P. (1996a). The role of *Phlebotomus martini* and *P. celiae* (Diptera: Phlebotominae) as vector of visceral leishmaniasis in the Aba Roba focus, Southern Ethiopia. *Medical and Veterinary Entomology*, **10**: 53-62.

Gebre-Michael, T. and Lane, R. P. (1996b). A new sand fly species, *Phlehotomus* (*Larroussius*) *ashfordi* (Diptera, Psychodidae) from Ethiopia, previously confused with *P*. (*L*.). *aculeatus*. *Annals Tropical Medicine and Parasitology*, **90**: 523-531.

Gebre-Michael, T. Malone, J. B., Balkew, M. Ali, A., Berhe, N., Hailu, A. and Herzi, A. A. (2004b). Mapping the potential distribution of *Phlebotomus martini* and *P. orientalis* (Diptera: Psychodidae), vectors of kala-azar in East Africa by use of geographic information systems. *Acta Tropica*, **90**: 73–86.

Gebre-Michael, T., Pratlong, F. and Lane, R. P. (1993). *Phlebotomus* (*Phlehotomus*) *duboscqi* (Diptera: phlebotominae), naturally infected with *Leishmania major* in southern Ethiopia. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **87**: 10-11.

Gemetchu, T. and Fuller, G. K. (1976). Kala-azar in Ethiopia: the phlebotomid sand flies of the Awash river valley. *Ethiopian Medical Journal*, **14**: 81-85.

Gemetchu, T., Laskay, T., Frommel, D. (1990). Phlebotomine sand flies (Diptera: Psychodidae, Phlebotomiae) of Ochollo, southwestern Ethiopia: species composition and natural infection of *Phlebotomus pedifer* with *Leishmania aethiopica*. *Sinet*: *Ethiopian Journal of Science*, **13**: 43-50.

Gemetchu, T., Zerihune, A., Assefa, A. and Lemma, A. (1975). Observations of the sand fly (Phlebotomidae) fauna of Setit Humera (Northwestern Ethiopia). *Ethiopian Medical Journal*, **13**: 41-51.

Hailu, A. Balkew, M. Bertie, N. Meredith1, S. E.O., and Gemetchu, T. (1995). Is *Phlebotomus* (Larroussius) *orientalis* a vector of visceral leishmaniasis in South-west Ethiopia? *Acta Tropica*, **60**: 15-20.

Hailu, A., Gebre-Michael, T., Berhe, N. And Balkew, M. (2006). Leishmaniasis. **In**: Yemane Berhane, Damene Haile Mariam and Kloos, H., (eds.). *The Epidemology and Ecology of Health and Disease in Ethiopia*, pp.615-634, Shama Books, Addis Ababa.

Halbig, P., Hodjati, M. H., Mazloumi-Gavgani, A. S., Mohite, H. and Davies, C. R. (2000). Further evidence that deltamethrin impregnated collars prevent domestic dogs from sand fly bites. *Medical and Veterinary Entomology*, **14**: 223-226.

Hemingway, J. and Ranson, H. (2000). Insecticide resistance in insect vectors of human disease. *Annual Review of Entomology*, **45**:371–391.

Horst, R., Collin, M., Ritmeijer, K., Bogale, A. and Davidson, N. (2008). Concordant HIV infection and VL in Ethiopia: the influence of antiretroviral treatment and other factors on outcome. *Clinical Infectious Diseases*, **46**: 1702–1709.

Jin, C., He, S., Hong, Y. and Li, G. (2004).The effect of sand fly control on the transmission of visceral leishmaniasis. *China Journal of Parasitology and Parasitic Diseases*, **22**: 338-339.

Kalyanasundaram, M., Srinivasan, R., Subramanian, S. and Panicker, K. N. (1994). Relative potency of DEPA as a repellent against the sand fly *Phlebotomus papatasi*. *Medical and Veterinary Entomology*, **8**: 68-70.

Kamau, L. and Vulule, J. M. (2006). Status of insecticide susceptibility in *Anopheles arabiensis* from Mwea rice irrigation scheme, Central Kenya. *Malaria Journal,* **5**: 46

Kaul, S. M., Sharma, R. S., Dey, P., Rai, N. and Verghese, T. (1994). Impact of DDT indoor residual spraying on *Phlebotomus argentipes* in a kala-azar endemic village in eastern Uttar Pradesh. *Bulletin of World Health Organization*, **72**: 79-81.

Kaul, S. M., Wattal, B. L., Bhatnagar, V. N., and Mathur, K. K. (1978). Preliminary observations on the susceptibility status of *Phlebolomus argentipes* and *Phlebolomus papatasi* to DDT in two districts of North Bihar State (India). *Journal of Communicable Diseases*, **14**: 208-211.

Kebede, Y. (2006). *Evaluation of neem* (*Azadirachita indica* A. Juss) *and chinaberry* (*Melia azedarach L.*) *oils against Anopheles arebiensis*, *Aedes aegypti and Phlebotomus orientalis in Ethiopia*. MSc thesis. Addis Ababa University.

Kelly, D. W., Mustafa, Z. and Dye. C. (1997). Differential application of lambda-cyhalothrin to control the sand fly *Lutzomyia longipalpis*. *Medical and Veterinary Entomology*, **11**: 13-24.

Killick-Kendrick, R. (1999). The biology and control of phlebotomine sand flies. *Clinics in Dermatology*, **17**: 279-289.

Killick-Kendrick, R., Killick-Kendrick, M., Focheux, C., Dereure, J., Puech, M. P. and Cadiergues, M. C. (1997). Protection of dogs from bites of phlebotomine sand flies by deltamethrin collars for control canine leishmaniasis. *Medical and Veterinary Entomology*, **11**: 105-111.

Killick-Kendrick R., Leaney A. J. and Ready P. D. (1977). The establishment, maintenance and productivity of a laboratory colony of *Lutzomyia longipalpis* (Diptera: Psychodidae). *Journal of Medical Entomology*, **13**: 429-440.

Kishore, K., Kumar, V., Kesari, S., Bhattacharya, S.K and Das, P. (2004). Susceptibility of *Phlebotomus argentipes* against DDT in endemic districts of North Bihar India. *Indian Journal of Communicable Diseases*, **36**: 41-44.

Kishore, K., Kumar, V., Kesari, S., Dinesh, D., Kumar, A. J., Das, P. and Bhattacharya, K. (2006). Vector control in leishmaniasis. *Indian Journal of Medical Research*, **123**: 467-472.

Kumar, V., Kesari, S. K., Sinha, N. K., Palit, A., Ranjan, A., Kishore, K., Saran, R. and Kar, S. K. (1995). Field trial of an ecological approach for the control of *Phlebotomus argentipes* using mud and lime plaster. *Indian Journal of Medical Research*, **101**: 154-156.

Lane, R. P. (1991). The contribution of sand fly control to leishmaniasis control. *Annals de la Societe Belge de Mediciene Tropicale*, **71**: 65-74.

Lavagnino, A., and Ansaldi, G. (1991). Susceptibility tests on *Phlebotomus perniciosus* and *Phlebotomus perfiliewi* wild populations in Sicily. *Parassitologia*, **33**:349-51.

Le Pont, F., Padilla, J. M., Desjeux, P., Richard, A., Mouchet, J. (1989). Impact of the spraying of deltamethrin in a focus of leishmaniasis in Bolivia. *Annals of the Society of Belgium Tropical Medicine Exotica*, **69**: 223-232.

Lewis D. J. (1982). A taxonomic review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bulletin of British Museum*, **45**: 121-209.

Majori, G., Maroli, M., Sabatinellp, G. and Fausto, A. M. (1989). Efficacy of permethrin-impregnated curtains against endophilic phlebotomine sand flies in Burkina Faso. *Medical and Veterinary Entomology*, **3**: 441-444.

Maroli, M., Cianchi, Bianchi, R. and Khoury, C. (2002). Testing insecticide susceptibility of *Phlebotomus perniciosus* and *Phlebotomus papatasi* (Diptera: Psychodidae) in Italy. *Annalia dell Istituto Superiore di Sanità*, **38**: 419-423.

Maroli, M. and Majori, G. (1991). Permethrin impregnated curtains against phlebotomine sand flies (Diptera: Psychodidae): laboratory and field studies. *Parasitologia*, **33**: 399-404.

Mazzarri, M. B., Feliciangeli, M. D., Maroli, M., Hernandez, A. and Bravo, A. (1997). Susceptiblity of *Lutzomyia longipalpis* (Diptera: Psychodidae) to selected insecticides in an endemic focus of visceral leishmaniasis in Venezuela. *Journal of American Mosquito Control Association*, **13**: 335-341.

Modi, G. B. and Tesh, R. B. (1983). A simple technique for mass rearing *Lutzomyia longipalpis* and *Phlebotomus papatasi* (Diptera: Psychodidae) in the laboratory. *Journal of Medical Entomology*, **20**: 568-569.

MOH (2006). *National guidelines for diagnosis and treatment of leishmaniasis*. Addis Ababa, Ethiopia: Ministry of Health.

Mukhopadhyay, A., Chakravarty, A., Kureal V. and Shivraj, K. (1987). Resurgence of *Phlebotomus argentipes* and *P. papatasi* in parts of Bihar (India) after DDT spraying. *Indian Journal of Medical Research*, **85**: 158-160.

Mutinga, M. J., Basimike, M., Mutero, C. M. and Ngindu, A. M. (1992). The use of permethrin impregnated wall cloth (mbu) for control of vectors of malaria and leishmaniases in Kenya ii. Effect on phlebotomine sand fly populations. *Insect Science Applications*, **13**: 163-172.

Najera, J. A. and Zaim, M. (2003). *Malaria vector control decision making criteria and procedures for judicious use of insecticides*. WHO/CDS/WHOPES/2002.5 Rev.1. WHO, Geneva, 106pp.

Pener, H. and Wilamovski, A, (1996). Susceptibility of larvae of the sand fly *Phlebotomus papatasi* (Diptera:Psychodidae) to *Bacillus sphaericus*. *Bulletin of Entomological Research*, **86**: 173-175.

Pener, H. and Wilamovski, A. (1987). Base-line susceptibility of *Phlebotomus papatasi* to insecticides. *Medical and Veterinary Entomology*, **1**: 147-149.

Perich, M. J., Hoch, A. L., Rizzo, N. and Rowton, E. D. (1995). Insecticide barrier spraying for the control of sand fly vectors of cutaneous leishmaniasis in rural Guatemala. *American Journal of Tropical Medicine and Hygiene*, **52**: 485-488.

Rahman, S. J., Wattal, B. L., Mathur, K. K., Joshi, G. C. and Kumar, K. (1982). Susceptibility of laboratory reared strain of *Phlebotomus papatasi* to organochlorin insecticides. *Journal of Communicable Diseases*, **14**: 122-124.

Reithinger, R., Brooker, S. and Kolaczinski, J. H. (2007). Visceral leishmaniasis in eastern Africa current status. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **101**: 1169-1170.

Reithinger, R., Davies, C. R, Cadena, H. and Alexander, B. J. (1997). Evaluation of the fungus *Beauveria bassiana* as a potential biological control agent against phlebotomine sand flies in Colombian coffee plantations. *Journal of Invertebrate Pathology*, **70**: 131-135.

Robert, L. L. and Perich, M. J. (1995). Phlebotomine sand fly (Diptera: Psychodidae) control using a residual pyrethroid insecticide. *Journal of American Mosquito Control Association*, **11**: 195-199.

Robert, L. L, Perich, M. J., Schlein, Y., Jacobson, R. L., Wirtz, R. A., Lawyer, P. G. and Githure, J. I. (1997). Phlebotomine sand fly control using bait-fed adults to carry the larvicide *Bacillus sphaericus* to the larval habitat. *Journal of American Mosquito Control Association*, **13**: 140-144.

Rojas, E. and Scorza, J. V. (1991). The use of lemon essential oil as a sand fly repellent. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **85**: 803.

Saxena V. (1987). Sand flies from a kala-azar outbreak area of district Darbhanga (Bihar), India. *Journal of Communicable Diseases*, **19**:405-407.

Schmidt, M. L., and Schmidt, J. R. (1969a). Relative effectiveness of chemical repellents against *Phlebotomus papatasi*. *Journal of Medical Entomology*, **6**: 79-80.

Schmidt, M. L. and Schmidt, J. R. (1969b). Insecticide susceptibilities of *Phlebotomus papatasi* (Scopoli) from Egypt and Sudan. *Journal of Medical Entomology*, **6**: 87-90.

Service, M. W. (2000). *Medical entomology for students*. Cambridge university press, Cambridge.

Sharma, V. P., and Dhiman, R. C. (1993). Neem oil as sand fly (Diptera: Psychodidae) repellent. *Journal of American Mosquito Control Association*, **9**: 364-366.

Sivagnaname, N. and Amalraj, D. D. (1997). Breeding habitats of vector sand flies and their control in India. *Journal of Communicable Diseases*, **29**: 153-159.

Tadesse, K. (2006). *Studies on systematic action of ivermectin on some mosquitoes and insecticide susceptibility of Anopheles gambiae s.l in Koka area*. Msc thesis. Addis Ababa university.

TDR (2005). *Leishmaniasis*. Seventeenth Programme Report Progress 2003-2004.

Tetreault, G. E., Zayed, A. E., Hanafi, H. A., Beavers G. M. and Zeichner, B. C. (2001). Susceptibility of sand flies to selected insecticides in North Africa and the Middle East. *Journal of the American Mosquito Control Association*, **17**: 23-27.

Warburg, A. (1991). Entomopathogens of phlebotomine sandflies: Laboratory experiment and natural infections. *Journal of Invertebrate Pathology*, **88**:189-202.

Wezam, A. and Seulu, F (1994). *Anopheles pharoensis* susceptibility status to DDT and malathion in Ziway area, south-central Ethiopia. *Ethiopian Pharmacological Journal*, **12**: 45-50.

Wilamovski, A**.** and Pener, H. (2003). Efficacy of microencapsulated insecticides against the sand fly, *Phlebotomus papatasi***.** *Journal of Vector Ecology*, **28**: 229-233.

WHO (1981). *Instructions for determining the susceptibility resistance of adult blackflies*, *sand flies and biting midges to insecticides*. WHO/VBC/81.810 World Health Organization, Geneva, 6pp.

WHO (1986). *Resistance of vectors and reservoirs of disease to pesticides*. Technical Report Series 737. World Health Organization, Geneva ,88pp.

WHO (1990). *Control of leishmaniais*. Technical Report Series 793. World Health Organization, Geneva ,158pp.

WHO (1992). *Vector resistance to pesticides*. Technical Report Series 818. World Health Organization, Geneva ,62pp.

WHO (1998). *Test procedures for insecticide resistance monitoring in malaria vectors*, *bio-efficacy and persistence of insecticides*. WHO/CDS/MAL/98.12. WHO, Geneva, 43 pp.

WHO (2004a). *Global strategic framework for integrated vector management*. WHO/CDS/CPE/PVC/2004.10. World Health Organization, Geneva, 12pp

WHO (2004b). *WHO position on DDT use in disease vector control under the Stockholm convention on persistent organic pollutants*. WHO/HTM/RBM/2004.53., Geneva, 2pp.

WHO (2005). *Safety of pyrethroids for public health use*. WHO/CDS/ WHOPES/GCDPP/ 2005.10 WHO/PCS/RA/2005.1, Geneva, 69pp.

WHO (2006). *Pesticides and their application*. *For the control of vectors and pests of public health importance*.WHO/CDS/NTD/WHOPES/GCDPP/2006.1.WHO, Geneva, 114pp.

WHO (2007a). *Control of leishmaniasis*. Sixtieth World Health Assembly A60/10 Provisional agenda item 12.3. WHO, Geneva, 5pp.

WHO (2007b). *Leishmaniasis*. World health Organization, Geneva, (http://www.who.int /leishmaniasis/ en/).

Xue, R., Ali, A. and Barnard, D., R. (2003). Laboratory evaluation of toxicity of 16 insect repellents in aerosol sprays to adult mosquitoes. *Journal of the American Mosquito Control Association*, **19**: 271-274.

Yaghoobi-Ershadi, M. R., Moosa-Kazemi, S. H., Zahraei-Ramazani, A. R., Jalai-Zand, A. R., Akhavan, A. A., Arandian, M. H., Abdoli, H., Houshmand, B., Nadim, A. and Hosseini, M. (2006). Evaluation of deltamethrin-impregnated bed nets and curtains for control of zoonotic cutaneous leishmaniasis in a hyperendemic area of Iran. *Bulletin de la Societe Pathologia Exotique*, **99**: 43-48.

Yuval, B. and Warburg, A. (1989). Susceptibility of adult phlebotomine sandfiies (Diptera: Psychodidae) to *Bacillus thuringiensis* var. *israeliensis*. *Annals Tropical Medicine and Parasitology*, **83**: 195-196.

Zaim, M., Aitio, A. and Nakashma, N. (2000). Safety of pyrethroid-treated mosquito nets. *Medical and Veternary Entomology*, **14**: 1-5.

Zaim, M. and Jambulingam, P. (2007). *Global insecticide use for vector-borne disease control*. WHO/CDS/NTD/WHOPES/GCDPP/2007.2.WHO,Geneva, 81pp.

APPENDICES

Appendix 1. Knock down rate of *P. bergeroti* at different exposure time of test insecticides from Melka-Worer (2008).

Time (minute)	Mean knock down rate[a]			
	DDT	Permethrin	Lambda-cyhalothrin	Deltamethrin
5	0	0	0	0
10	2	2	2	0.75
15	7.75	7.75	4.5	1.25
20	15.5	10	7.25	2
30	17.25	17.75	14.75	9.25
40	19.75	20	19.5	16.75
50	20	20	19.75	19.75
60	20	20	20	20

[a]mean of four replications

Appendix 2. Knock down rate of *P. orientalis* at different exposure time of test insecticides from Melka-Worer (2008).

Time (minute)	Mean knock down rate[a]				
	DDT	Permethrin	Lambda-cyhalothrin	Deltamethrin	Alpha-cypermethrin
5	0	4.75	1.5	1.75	1
10	1.5	13.25	7	6.5	6.75
15	4.75	17.25	12	14	13.75
20	11	19	18.5	18.25	18.25
30	17.75	19.25	20	19.75	19.75
40	20	20	20	20	20
50	20	20	20	20	20
60	20	20	20	20	20

[a]mean of four replications

Appendix 3. Knock down rate of *P. orientalis* at different exposure time of test insecticides from Libo-Kemkem.

Time (minute)	Mean knock down rate[a]				
	DDT	Permethrin	Lambda-cyhalothrin	Deltamethrin	Alpha-cypermethrin
5	0	2.5	0	0	0
10	2	8.75	0.75	6	9
15	6.25	13.25	6	15.75	15.75
20	10.75	18.25	15	16.5	17.75
30	18.75	20	19	19.75	19.75
40	20	20	20	20	20
50	20	20	20	20	20
60	20	20	20	20	20

[a]mean of four replications

Appendix 4. List of studies on the susceptibility status of phlebotomine sand flies to various insecticides modified from Alexander and Maroli (2003).

Continent	Species tested	Insecticides	Susceptibility status	countries	References
Africa	*P. papatasi*	DDT BHC, propoxur, malathion, permethrin Dieldrin, resmethrin, bendiocarb, cyflulthirn	Susceptible	Egypt, Sudan	(Six references cited in Alexander and Maroli, 2003)
	P. bergeroti *P. langeroni* *P. sergenti*	DDT, malathion, permethrin, resmethrin,bendiocarb cyflulthirn	Susceptible	Egypt, Djibouti	(One references cited in Alexander and Maroli, 2003)
Asia	*P. papatasi*	DDT permethrin	Susceptible	Israel	(One references cited in Alexander and Maroli, 2003)
		Methoxychlor, DDT	Low level of resistance	Israel, Iran	(Two references cited in Alexander and Maroli, 2003)
	P. papatasi	DDT, malathion, dieldrin, permethrin, lambda-cyhalothrin, propoxur	Resistant	India (different states)	(Seventeen references cited in Alexander and Maroli, 2003)
		BHC, bendiocarb, dieldrin, permethrin, malathion, fenitrothion, deltamethrin	Susceptible		
		Deltamethrin, dieldrin, DDT, malathion	Low level of resistance		
	P. argentipes	DDT, malathion, dieldrin, permethrin, deltamethrin	Resistant		
		Bendiocarb, DDT, malathion, dieldrin, deltamethrin	Susceptible		
		Lambda-cyhalothrin, BHC, DDT	Low level of resistance		
Europe	*P. papatasi*	DDT, lambda-cyhalothrin, permethrin, chlorophos	Susceptible	Italy,Uzbekistan Turkmenistan	(Three references cited in Alexander and Maroli, 2003)
	P. perniciosus	DDT, lambda-cyhalothrin, permethrin	Susceptible	Italy	(One references cited in Alexander and Maroli, 2003)

Appendix 4. (Continued)

America	*Lu. longipalpis*	Deltamethrin, DDT, propoxur, chlorpyriphos, malathion, lambda-cyhalothrin	Susceptible	Brazil, Venezuela	(Three references cited in Alexander and Maroli, 2003)
		Fenitrothion, pirimiphos-methyl, permethrin	Low level of resistance		
	Lu. youngi	DDT, lindane, lambda-cyhalothrin, cypermethrin	Susceptible	Venezuela	(Two references cited in Alexander and Maroli, 2003)
		Dieldrin, , malathion, fenthion	Low level of resistance		
		Propoxur,deltamethrin	Resistant		

Appendix 5. List of chemical insecticides used for cotton pest management in Melka-Worer.

Common name	Trade name	Insect pest to be controlled
Primiphos methyl	Actellic	Aphid on cotton
Furathiocarb	Deltanet	Aphid
Carbosulfan	Marshal	Aphid
Dimecron	Phosphamidon	Aphid
Metasytox	Oxydemethon methyl	Aphid
Primiphos methyl	Sybolt	Whitefly on cotton
Profenofose	Curcuron	Whitefly
Mitac	Amitraz	Whitefly, Red spider mite (RSM) and thrips
Endosulfan	Thiodan	Whitefly, African boll worm (ABW) and thrips
	Ethiosulfan	ABW
	Thionex	ABW
Lmbda-cyhalothrin	Karate	ABW
Alpha-cypermethrin	Fastac	ABW
Primiphos-methyl	Bostox	ABW
Deltamethrin	Decis	ABW and leaf hopper
Cypermethrin	Ripcord	ABW , leaf worm and thrips
Bromoprpyl	Neuron	RSM
Monocrotophos	Nuvacron	RSM
Dicofol	Mitigan	RSM
Permethrin	Talstar	RSM and Whitefly
Cypermethrin	Cymbush	Cotton pest
Carbarayl	seivien	Beetel

Source: Melka-Worer Agricultural Institute (2008).

Appendix 6. IUPAC name and chemical structure of insecticides.

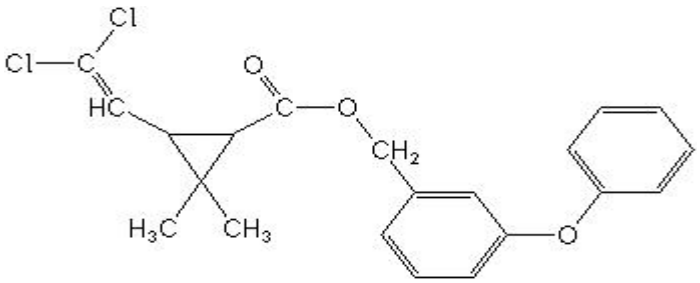
1. Permethrin

IUPAC name

3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

or

3-phenoxybenzyl (1*RS*)-*cis*-*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate



Chemical structure of permethrin

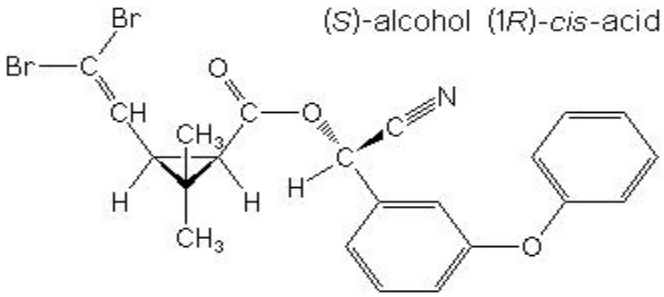
2. Deltamethrin

IUPAC name

(*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

or

(*S*)-α-cyano-3-phenoxybenzyl (1*R*)-*cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

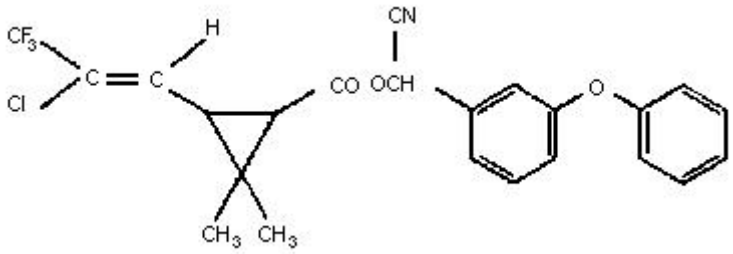


Chemical structure of deltamethrin

3. Lambda-cyhalothrin

IUPAC name

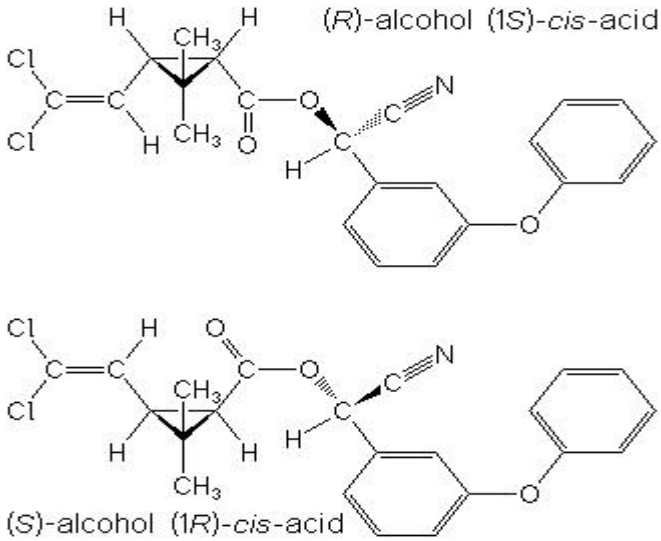
(*R*)-α-cyano-3-phenoxybenzyl (1*S*)-*cis*-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate and (*S*)-α-cyano-3-phenoxybenzyl (1*R*)-*cis*-3-[(*Z*)-2-chloro-3,3,3-trifluoropropenyl]-2,2-dimethylcyclopropanecarboxylate



Chemical structure of lambda-cyhalothrin

4. Alpha-cypermethrin

(*R*)-α-cyano-3-phenoxybenzyl (1*S*,3*S*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate and (*S*)-α-cyano-3-phenoxybenzyl (1*R*,3*R*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate

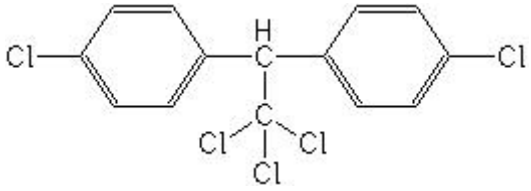


Chemical structure of alpha-cypermethrin

5. DDT

IUPAC name

dichlorodiphenyl trichloroethane

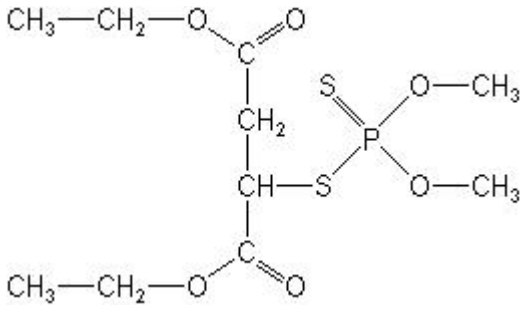


Chemical structure of DDT

6. Malathion

IUPAC name

S-1,2-bis(ethoxycarbonyl)ethyl *O*,*O*-dimethyl phosphorodithioate



Chemical structure of malathion

Source: Compendium of Pesticide Common Name

http://www.alanwood.net/pesticides/structures