

World Health Organization

Eastern Mediterranean Region

Final Technical Report

Project No: SGS04-76

Title of the Project:

Impact of Olyset long lasting nets to control transmission of anthroponotic cutaneous leishmaniasis in central Iran

Investigators:

M.Motovalli Emami

M.Yazdi

N.Bashardoust

1.Head of Sepahan Green-Thou Plantpathology and Medical Entomology Clinic.

Pars Ave. Masror St.Plaque:147. Isfahan.IRAN

Email:Emamy2002@yahoo.com

Abstract:

A large-scale intervention field trial to control cutaneous leishmaniasis by using Olyset long lasting insecticidal bednets was carried out in two cities, Sedeh and Shiraz, Islamic republic of Iran. Twelve urban sectors of two cities with ACL selected. Six sectors were randomly chosen and were considered intervention areas and the other six were the control areas. A total 8620 individuals in 3000 households were enrolled in the study.

During April 2004, a house-to-house survey was conducted to collect census, socioeconomic and baseline epidemiology data. Olyset nets were distributed to household members of the intervention sectors. Monthly entomological surveys of the vector *Phlebotomus sergenti* were conducted using oily papers and light traps. Epidemiological and entomological surveys were repeated post-intervention.

Results showed significant ($P < 0.05$) reduction in cutaneous leishmaniasis incidence in the intervention areas from 1.6% to 0% in Sedeh and from 3.5% to 0.099% in Shiraz compared to a 6% increase in the control areas. Because cutaneous leishmaniasis in Iran occurs in many areas, the use of Olyset nets could provide a high degree of personal protection against this infection.

Key words: Olyset nets-Anthroponotic cutaneous leishmaniasis-Control-Iran

Introduction:

The leishmaniasis – cutaneous leishmaniasis (CL), mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL)- are parasitic diseases with a wide range of clinical symptoms. Leishmaniasis currently threatens 350 million people in 88 countries around the world, with 1-1.5 million new cases of CL reported annually(1).

Both zoonotic cutaneous – leishmaniasis (ZCL) and anthroponotic cutaneous leishmaniasis (ACL) are found in scattered foci in various parts of the Islamic Republic of Iran. In recent years, due to the increase in population, increase in areas on the southern slopes of the Zagros mountains, many areas in Darab district and Fars province(2) and new foci are recently found in central Iran such as Sedeh in Isfahan province (reported by Isfahan University of Medical Sciences). Previously the diseases was rare in these areas.

In this focus, intensive transmission occurs, so that many indigenous residents acquire the disease before the age of nine.

Phlebotomus (Paraphlebotomus) sergenti is the most predominant sandfly. This species constitutes 82% of the total sand flies captures in indoor.

Use of insecticide – treated mosquito nets (ITNs) for protection against mosquito bites during sleep is a highly effective and cost – effective intervention against vector born diseases such as malaria(3). Results from previous trials have shown insecticide – treated bed nets and curtains reduce mortality in children less than five years old by 18% in sub-saharan Africa(4).

A case – control study in Nepal showed that people using untreated nets were 70% less likely to develop visceral leishmaniasis than people without nets(5). Protection provided by wide mesh nets is enhances by treating then with pyrethroid – reducing sandfly biting rates by 64% - 100%(6-7). The is also evidence from Colombia that sandfly bits are not diverted to people sleeping outside insecticide treated nets: "unprotected" people in the same room as someone sleeping a deltamethrin treated net received 42% less sandfly bites than people in houses without nets(6).

A net treated at factory level with insecticide either incorporated into or coated around fibers, resisting to multiple washes and whose biological activity last as long as the net itself (3 to 4 for polyester nets) is a long lasting insecticidal net (LLIN)(8).

Olyset net is a polyethylene net with 2% permethrin incorporated within fiber. Over time, insecticide migrates to the surface of the yarn, replacing the one that has been removed against malaria vectors for at least 5 years and its use recommended by WHO(8).

In a study Olyset nets showed some deterrency against *An. gambiae* (44% reduction by the new net, 20% by the dirty nets, none by the washed net)(9).

. In the past, various control measures, carried out mainly in the Shiraz and Isfahan area failed to permanently decrease the number of cases. Therefore, we carried out a study in an urban area of two provinces with intense transmission of anthroponotic cutaneous leishmaniasis. We assessed any reduction in abundance of sandflies indoor and of clinical cases in areas with houses protected by ITNs compared with areas with houses using non-ITNs

The study area

This study was carried out during July 2004- July 2005 in 2 cities in Islamic Republic of Iran : 1- Sedeh ,
2 – Shiraz.

1 – Sedeh city (khomeini Shahr : New name) is situated in an area called Marbin (32°41'N , 51°31' E).
It is located in north western part of Esfahan city (12 km away) in Esfahan province. It is consisted of
three interrelated areas called Varnose - fade – ran, Foroushan and khozan. It is confined to Seyed
Mohammad mountains from the north, to Zayandehrood river from the south, to Borkhar cunty form the
east, and to Najafabad city from the west. It is located 1602^m above the sea level. The climate there is
warm and dry with four distinct seasons. The mean of rainfall has been 117^{mm} during the past 38 years.

The maximum mean of monthly temperature was 48° and the minimum temperature was 5c . The
relative humidity was 44 % .

Sedeh and it's related town has a population of around 300.000 . The recent years immigration has played
an important role to the population increase. There are 1500 stone – cutting factories for buildings in this
industrial city.

2 – Shiraz (29°38'N , 52°34' E) is a city located in southwest Iran with 1050000 inhabitants. It is located
in 1486 meters above sea level admist the Zagros mountains, and it is the capital of Fars province. The
economical base for Shiraz is trading its provincial products, where grapes, citrus fruits, cotton and rice
are produced. In Shiraz itself, industries such as cement, sugar, fertilizer, textile products, wood products,
metal works and rugs dominate. Shiraz has a moderate climate, with a hot – dry summer (July –
September) , a warm wet atumn (October – December) , a mild winter (January – March) and a warm
and rainy spring (April – June) .

In both of the mentioned cities the residential buildings are constructed with clay and brick. Both have old
– structured buildings dating back to over 25 years ago. Houses consisted of three or more rooms
including a living room, hall, small kitchen, bedroom and bathroom. Compounds and houses usually
consist of an entrance leading to a courtyard with a lavatory. Animal quarters are sometimes built at the
furthest end of the same compound opposite the living area but generally they are built under the living
room separated with a cement wall i.e. Sometimes households keep their animals outside the compound

but near enough to be watched closely. The inhabitants performed various activities such as carpetmaking in the courtyards in the daytime during summer. They also watched television and slept in the courtyards using a traditional sleeping sheet on summer nights.

Study population

The people are basically subsistence farmers and animal husbandry. Some people work as laborers in nearby factories and offices. The main activities of the women consist of household duties, carpet waving and arts.

Study design, impregnation and distribution of bednets

In each city six urban sectors were selected (three sectors were interventional groups and three other sectors were control groups). For each of the six pairs we were randomly allocated one sector (using computer created random numbers) to the intervention group blind toward the group allocation. Each sector was distanced at least 2 km from other. We included 12 sectors of two cities, with 3000 houses and 8620 inhabitants. One sector in each pair randomly allocated to receive Olyset Net[®] while the other sector no nets (control group). Between April 2004 and July 2004 two pre-intervention survey were conducted in chosen operational areas. The interviewers examined any scars and ulcers. They paid particular attention to new cases that going to occur during the nine months before the interview. Subsequently interviewers checked for any new cases that were been reported in the leishmaniasis clinic in the past nine months. Reduction in incidence of new cases was the main indicator.

We used a questionnaire to gather data on the demographic (age and sex) and socioeconomic characteristic (normal education, occupations, housing conditions) of the population as well as on people's individual experience with ACL, the sleeping patterns of families and according to these patterns, the number of bednets that would be distributed to householders. In two city all of primary school students examined for ulcer or scar.

Olyset Net[®] is made of knitted polyethylene thread with permethrin 2% (w/w) incorporated during fibre extrusion. The standard Olyset Net[®] weighs ~ 750 gr with surface area 14 m² (length 1.80 m, width 1.80m, height 1.50 m) distributed to 1500 households in the intervention settlements in August 2004. All the bednets ordered were white and made by Changzhou Jiusi Fibre Product Co, China.

To the correct use of the nets, 59 regular training sessions for families in interventional group in schools

and mosques have been formed. Health education messages were disseminated to ensure the population's compliance with the proper use of bednets. Also visiting homes to coordinate, answer the people's questions in the intervention group, not to use other ways in attacking sandflies, use of ITNS, coverage of ITNS and community acceptability was carried out.

After the intervention, to determine the fluctuation of CL incidence and to compare settlements to each other, follow-up questionnaires and examinations were conducted every month between August 2004 and July 2005.

Entomological survey

Sandflies were collected once a month from fixed sites indoors (bedrooms, stables, lavatories, etc.) and outdoors (courtyard), using 30 sticky traps (castrol oil-coated white papers 20 cm * 30 cm) from the beginning to the end of the active season (July - October). For the sampling of exophilic species, light traps were maintained. A total of 20 light traps were used for sampling. Light traps were run between the hours 18⁰⁰ - 06⁰⁰ in each sampling station. Light traps were run every month. For species identification sandflies were mounted in Puri's medium (produced in the Sepahan Green - Thous Plantpathology and Medical Entomology Clinic) and identified after 24 hours using the keys of Theodor and Mesghali (10), then they were mounted and segregated by sex.

Statistical analysis

The chi-square test was used for comparison between proportions. Comparison between incidence rates of the disease before and after intervention was performed using paired t-test. Moreover, due to the longitudinal nature of the study design, the relative risk of using Olyset nets in the intervention compared to the control areas and in the intervention areas after the use of nets compared to baseline was computed.

Results

Approximately 15,000 persons were interviewed before the intervention, and 8,620 of them were included in the study.

Sedeh city

The cumulative ACL incidence estimated from October 2003 to July 2004 (9 months before the intervention) was 1.6% in the intervention sectors and 1.5% in the control sectors (Table 1).

During the pre-intervention period, CL prevalence was remarkably high in both control and intervention sectors and the estimated rates do not differ significantly between them. In the same period, the agent of CL in Sedeh city was confirmed to be *L.tropica* by the analysis of 37 isolates, and the main vector species was identified as *Phlebotomus sergenti*, i.e. the proven vector of *L.tropica* (56% prevalence among 2,801 sandflies trapped in Sedeh).

A total of 16 persons was infected for the entire study sectors between July 2004 and July 2005, early prevalence was determined 0.88% in the control sectors and there were no active lesions among the residents in the intervention sectors (Table 2). In the control sectors the most highly infected age group was 31 – 40 and lowest rate was in the age group of 11-20 years. Statistically significant differences were observed in prevalence of ulcer by sex, prevalence in males being more than twice that in females. More than 63% of the people with active lesions had 1 ulcer, 9.1% had 2 and the rest had 3.

Examination of 5 isolates from human indigenous cases in the control area identified them as *Leishmania tropica*.

A significant difference ($P < 0.05$) was observed between the CL incidence of intervention sectors before and after trial. There was a reduction in the incidence of cutaneous leishmaniasis from 1.6% to 0%, respectively. On the other hand, the incidence of cutaneous leishmaniasis decreased in the control sectors from 1.5% to 0.88%, respectively. These results indicated that there was about a 100% reduction in the incidence of cutaneous leishmaniasis in the intervention area and 58% decrease in the control area. The relative risk of using Olyset nets in the intervention districts of Sedeh compared to the control ones was 0 and the efficacy was 1. Olyset nets have prevented 100% cases of ACL in the intervention areas.

Shiraz city

The cumulative ACL incidence estimated from October 2003 to July 2004 (i.e. 9 months before the

intervention) was 3.5% in the intervention sectors and 4.8% in the control sectors (Table 1). During the pre – intervention period, CL prevalence was remarkably high in both control and intervention sectors and the estimated rates do not differ significantly between them.

A total of 103 persons was infected for the entire study area between July 2004 and July 2005, yearly prevalence was determined 0.09% in the intervention sectors and 5.1% in the control sectors (Table 2) .

In the intervention area only two men (27 and 39 years old) were infected whereas 101 persons were infected in the control area that the most highly infected age group was 21 – 30 and lowest rate was in the age group of 50 years. The Fisher test showed no statistically significant difference in the prevalence of ulcers by sex.

Incidence declined greatly in the intervention sectors where Olyset Nets® were distributed. These results indicate that there was about a 97% reduction in the incidence of cutaneous leishmaniasis in the intervention area while it increased by approximately 6/25 % in the control area. The differences in CL incidence were statistically compared for the year 2004-2005, and found that the declining differences ($P < 0.05$) at the end one year period were significant. Also statistically significant difference was observed between intervention and control sectors during the post – intervention .

The relative risk of using Olyset nets in the intervention districts of Shiraz compared to the control ones =0.02 $p < 0.05$ protective and the efficacy was 0.98 Accordingly .Olyset nets have prevented 98% of cases of ACL in the intervention areas.

Entomological results

The total number of sandflies collected in the study areas between July 2004 and June 2005 was 2305 in Sedeh (1497 indoor and 807 outdoor) and 8711(5031 indoor and 3680 outdoor) in Shiraz. The following 4 species were found in Sedeh : *P.sergenti* (53.01%), *P.papatasi* (27.8%), *P.caucasicus* (0.82%) *S.sintoni* (18.37%), and in Shiraz : *P.sergenti* (51.9%), *P.papatasi* (18.03%), *P.major* (4.17%), *P.wenyoni* (0.3%), *P.alexandri* (1.6%), *P.simici* (0.1%), *S.sintoni* (17.91%), *S.dentata* (2.73%), *S.theodori* (1.63%), *S.pawlowsky* (0.93%), *S.clydei* (0.7%).

P.sergenti the major vector of *L.tropica* was the most abundant species comprising 50% of the total number of sandflies caught. Figure 1. shows the seasonal population fluctuations of female *P.sergenti* captured in Sedeh and Shiraz areas during the survey season. There were significant differences in monthly population sizes of *P.sergenti* between control and intervention sectors in both cities ($P < 0.05$) The total number of female *P.sergenti* captured indoor in the control sectors was greater than that of the intervention sectors population. There were two peaks, one during July and another in September in the intervention and control areas. In both areas, intervention and control, the highest

population size was recorded in August (Figure 1). In both cities, dead sandflies, were found in the morning around the Olyset Nets[®] during survey team visits to households in the intervention sectors.

Discussion

Long Lasting Insecticidal Nets are an efficacious method for the control of anthroponotic cutaneous leishmaniasis in endemic areas. Field trials with treated bednets to prevent anthroponotic cutaneous leishmaniasis have been undertaken in Sudan, Syria and Turkey (6,11,12). Our study is the first intended to explore the effectiveness of Olyset Net[®] (LLIT) in the control of ACL in Iran. It shows that use of permethrin – impregnated bednets (Olyset Net[®]) can provide significant personal protection from the bites of sandflies and subsequently reduce the risk of CL infection. Insecticidal bednets have also been employed successfully against *Anopheles* mosquitoes and their recent use in wide – scale control programme has resulted in significant reduction of malaria morbidity and mortality (4,9). For sandflies, deltamethrin – impregnated nets provided significant reduction in the biting rates of *Lu.youngi* in Colombia (13) as well as *P.sergenti* and *P.papatasi* in Syria (6) and Turkey (12). Similar bednets and curtains impregnated with permethrin 100 mg a.i./m² provided significant reduction in the biting activity of *P.papatasi* in khartoum (11). The efficacy of pyrethroid – impregnated bednets observed in this study confirms the findings of another trial carried out in Bam, Iran (14). However their results showed higher CL incidence in females in the intervention area. This was not the case in our study, which showed that there was no difference incidence among males and females in the intervention areas compared with the control areas. These results were confirmed by the study carried out in Syria (6) and Turkey (12). It seems that people using Olyset bednets[®] were still exposed to some sandfly biting activity similar people with no nets, apparently due to sandflies going through the mesh of the bednets. Therefore, an infected sandfly entering the net may still transmit the infection (and die afterwards), but the fact that not a single case was recorded in Sedeh refutes such assumption. Because bednets with finer mesh size may be unpleasant to use in the hot weather, Olyset Nets[®] (50 holes / inch²) would be the most practical way to maximise their protective efficacy.

Although CL infection reduced in Sedeh control sectors after intervention but no patient was in Sedeh intervention sectors and increase in Shiraz control sectors indicate to high efficacy of Olyset Nets[®] in

intervention sectors. It is interesting to note that Sedeh was new focus of ACL and the promising results of this study may allow large scale field trial in an epidemiologically similar area and new foci of ACL. Altogether the use of Olyset nets provides a significant protection against ACL to the populations at risk and its efficacy accounts for 98% (Table 3) The longitudinal design of the study allowed all individuals to be followed up during the study period (July 2004 – July 2005) every month after the distribution of the bednets (intervention period). This helped reduce the margin of error and gradually made the estimate of previously infected individuals more accurate. Although the intervention and control sectors in both city had many characteristics in common, slightly two men were infected in the intervention sectors at the beginning of the trial (August).

In both areas usually people wake up at 4:00 – 5:00 for prey and than sleep until 6:00 – 7:00 and most of them sleep early at 21:00 – 24:00 in the courtyard.

The present observations demonstrate that Olyset nets[®] are effective in reducing the outdoor man – biting rate of *P.sergenti* . Use of Olyset Nets[®] would provide an invaluable control measure against these vectors of anthroponotic cutaneous leishmaniasis and may have better results with regard to the interruption of transmission of ACL. It provides a practical control method against phlebotomine sandflies and also other biting flies.

References:

1. 1- Desjeux P. *The increase in risk factors for Leishmaniasis worldwide. Trans. R. Soc. Trop. Med. Hyg.* 2001; 95: 239-93.
- 2.
3. 2- Nadim A. Javadian E. Mohebbali M. *The experience of Leishmanization in the Islamic Republic of Iran. <http://www.ermo.who.int/Publications/emhj/0302/11.htm>.*
- 4.
5. 3-Korenromp E.L. Miller J. etal. *Monitoring mosquito net coverage for malaria control in Africa: Possession vs. use by children under 5 years. Trop. Med. Inter. Health.* 2003. Vol: 8(8) 693-703.
- 6.
7. 4- Ter Kuile F.O. Terlouw D. J. Phillips - Howard P.A. and etal. *Impact of permethrin - treated bed nets on malaria and all - cause morbidity in young children in an area of intense perennial malaria transmission in wasteren kenya Am. J. Trop. Med. Hgy.* 2003 (63) 100-107.
- 8.
9. 5- Bern C. Joshi A.B. Jha S.N. etal. *Factors associated with visceral Leishmaniasis in Nepal: bed - net use is strongly protective. Am.J. Trop. Med. Hyg.* 2000; 63: 184-188.
- 10.
11. 6- Tayeh A. Jalouk L. Al-Khiami Acutaneous. *Leishmaniasis control trial using pyrethroid - impregnated bednets in villagas near Aleppo, Syria. Geneva: World Health Organization. 1997 (WHO/ LEISH/97.41).*
- 12.
13. 7- Ashford D.A. David J.R. Freire M. etal. *Studies on control of visceral Leishmaniasis: impact of dog control on canine and human visceral leishmaniasis in Jacobina, Bahia, Brazil. Am. J. Trop. Med. Hgy.* 1998; 59:53-57.
- 14.
15. 8- Pierre F. *Long lasting insecticidal nets: current status & programmatic issues. Guillet vector control. WHO Geneva. <http://www.emro.who.int/rbm/LLITNS.pdf>.*
- 16.
17. 9- Guessan R.N. Darrie F. etal. *Olyset Net efficacy against pyrethroid - resistant Anopheles gambiae and Culex quinquefasciatus after 3 years field use in Cote d'Ivoire. Med. Vet. Entomol.* 2001. 15: 97-104.
- 10-Theodor O.Mesghali A. *On the phlebotominae of Iran.J.Med.Entomol.*1964.1:285-300.
- 11-Elnaiem D.A. Aboud M.A. El Mubarek S.G. Hassan H.K. Ward R.D. *Impact of pyrethroid-impregnated curtains on Phlebotomus papatasi sandflies indoors at Khartoum,Sudan.Med.Vet.Entomol.*1999.13:191-197.
- 12-Alten B. Caglar S.S. Kaynas S. Simsek F.M. *Evaluation of protective efficacy of K-OTAB impregnated bednets for cutaneous leishmaniasis control in Southeast Anatolia-Turkey.J.Vec.Ecol.*2003.4,53-64.
- 13-Alexander B. Usma M.C. CandenaH. Quesada B.L.Solate Y. Roa W. Travi B.L. *Evaluation of deltamethrin impregnated bednets and curtains against phlebotominae sandflies in Valle Del cauca.Colombia.Med.Vet.Entomol.*1995.9:279-283.

Table 1:

The cumulative ACL incidence estimated from October 2003 to July 2004

(before intervention)

City	Type of sector	No.of.people	New CL	Incidence%
Sedeh	intervention	1812	29	1.6
	control	1820	28	1.5
Shiraz	intervention	2006	71	3.5
	control	1998	96	4.8

**Table 2: The cumulative ACL incidence estimated from July 2004 to July 2005
(after intervention)**

City	Type of sector	No.of.people	New CL	Incidence%
Sedeh	intervention	1809	0	0
	control	1820	16	0.88
Shiraz	intervention	2001	2	0.099
	control	1995	101	5.1

table 3: The summary table comparing the total population exposed to the intervention compared to the control population

Area	Population in Sedeh and Shiraz		Total	RR(95% confidence interval)	Efficacy
	ACL cases	NO ACL cases			
Intervention	2	3810	3812	0.02(0.00-0.07) *	98%
Control	117	3815	3932		

p<0.05*

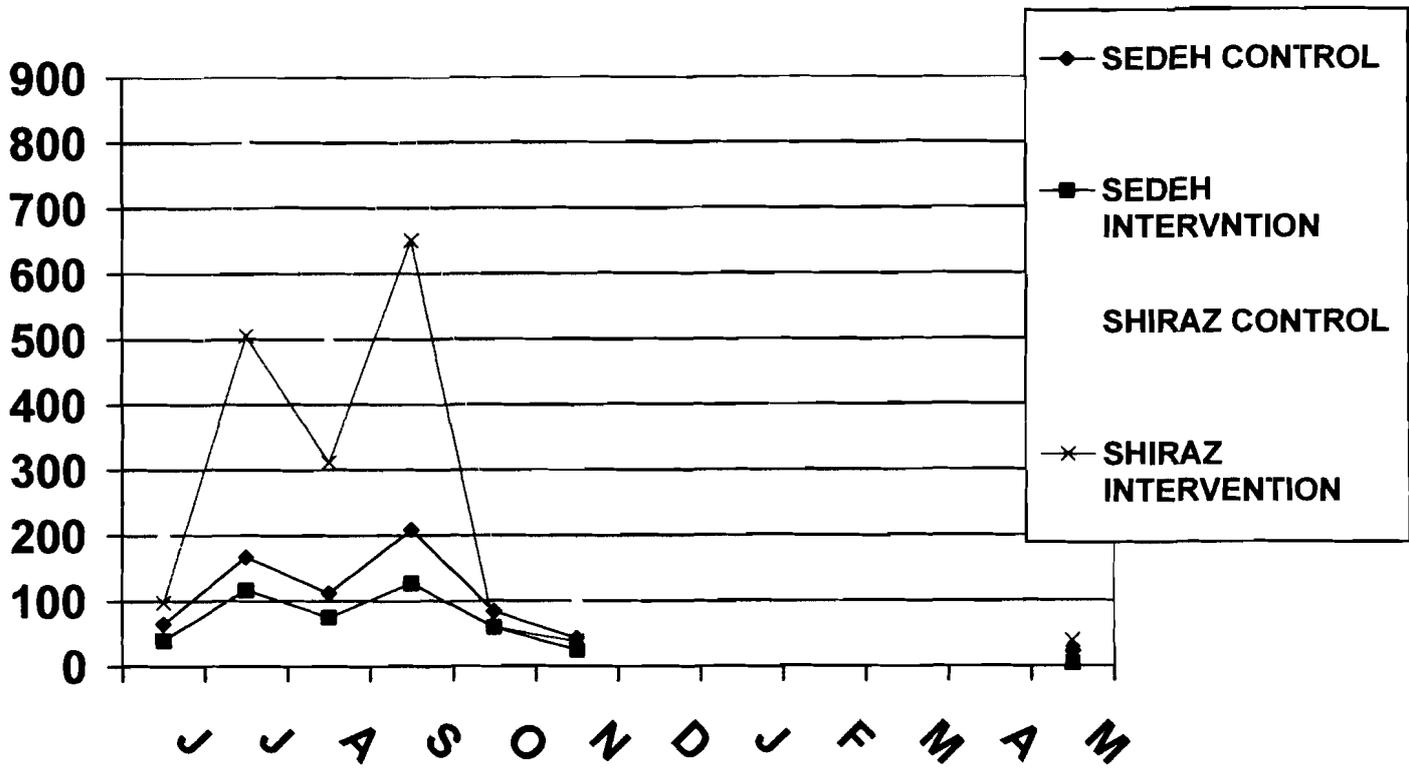


Figure 1 .Seasonal population fluctuations of *P. sergenti* distributing in the Shiraz and Sederh