Research Article

Sericulture and the Development of Resistance to Various Insecticides in *Xenopsylla cheopis* (Rodent flea), Efficient Vector of Human Plague in Active Enzootic Plague foci of Kolar District, Karnataka, and Chittoor District, Andhra Pradesh, India

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Abstract

The intensive use of various insecticides in agriculture has caused concern for increased selection pressure for insecticide resistance development in insect vector population. Selection at different life stages of rodent fleas is usually assumed to arise because of indiscriminate use of agricultural pesticides or indoor residual spray for anti-flea or anti-mosquito measures. Susceptibility status of rodent fleas to various insecticides was studied during 2007 to 2009 in sericulture and non-sericulture villages of Kolar, Karnataka, and Chittoor district, Andhra Pradesh, to study the selection pressure of various insecticides used in agriculture and public health sectors. Mortality rate in synthetic pyrethroids is found to be significantly higher in Palamneru (A.P.) areas in both sericulture and non-sericulture areas compared to Kolar (Karnataka) areas. However, there is no significant difference in mortality rate in other insecticides used in public health programs between sericulture and non-sericulture areas of Kolar and Palamneru area. Silk farmers resist to indoor residual insecticide spraying for mosquito or flea control due to its toxic effect on silk worms. As a result, malaria incidence in the area was high in early nineties. Due to non-acceptance of indoor residual spray in silk-rearing villages of Kolar and Chittoor districts, selection pressure of various insecticides on flea population breeding indoor is negligible but the selection pressure from the insecticide-treated mulberry plant leaves on indoor resting flea population was always there.

Keywords: *Rattus rattus, Bandicota bengalensis, Bandicota indica, Tatera indica, Xenopsylla cheopis, Xenopsylla astia,* Bubonic plague, Insecticide resistant, Susceptible population, Sericulture.

Introduction

Insecticide resistance to insect vector species is visible manifestation of prolonged failure to use insecticides rationally in both agriculture and public health sectors. The intensive use of insecticides in agriculture has caused concern for increased selection pressure for insecticide resistance development in disease vectors. This may have negative implications for vector-borne disease control program.[1]

Resistance to various insecticides in rodent fleas may be selected at either the larval or adult stage. Mature and immature stages of rodent fleas are always found in loose soil of rodent burrows in peri-domestic and feral situations or animal/ human dwellings in domestic situation. Selection at the larval and adult stage of rodent fleas is usually assumed to arise because of indiscriminate use of agricultural pesticides or indoor residual spray for anti-flea or anti-mosquito measures. Since the first case of DDT

resistance in 1947, the incidence of resistance has increased annually at an alarming rate. It has been estimated that there are at least 447 pesticide-resistant arthropods species in the world today.[2]

DDT resistance was first confirmed in *Xenopsylla cheopis*, rat flea in Pune district of Maharashtra state in 1960. In 1960 again both *X. cheopis* and *X. astia,* the predominant flea species in India were found to be resistant to DDT and tolerant to BHC and Dieldrin in Nilgiri hills, Tamil Nadu. Different authors reported susceptibility status of fleas against organochlorine, organophosphate and synthetic pyrethroids in different ecological zones in India.[3]

The persistently high vector fleas and rodent infestations emphasize the need for rigorous surveillance against plague. High degree increase in vector flea density (critical level of *Xenopsylla cheopis* index: ≥1.0) in an active enzootic plague foci is one of the major factors for possible outbreak of bubonic plague and rodent epizootics or rat falls.[3]

Human Plague Transmission in Karnataka and Andhra Pradesh

The decennial death rates due to plague in India per one lakh population during 1898-1918 were 183.3 and 133.8 respectively. The principal plague-affected states in India since 1939 were Bihar, Maharashtra, Andhra Pradesh, Madhya Pradesh and Karnataka. During 1949-1958, mortality rate due to plague was calculated to be 1.8 per one lakh population. Since then mortality due to plague had declined and reached zero level in 1967.[4,5] There was a resurgence of plague in bordering districts of Tamil Nadu, Andhra Pradesh and Karnataka during 1959 to 1966.[4-,6] The active enzootic foci of plague were, however, confined to Hosur area (Krishnagiri district of Tamil Nadu), Attibele (Bangalore rural district), Kolar (Kolar district, Karnataka), Tangnu and Rohru area (Shimla district, Himachal Pradesh) and Palamneru (Chittoor district, Andhra Pradesh). The last human plague case in India during the period was reported from Mulbagal in Kolar District, Karnataka, in 1966.

Though human plague had not been reported from India since 1967, yet sporadic cases of suspected human plague had been reported from Tangnu, Himachal Pradesh during 1966, 1983 and 1984 and Attibele, Bangalore Rural District Karnataka (adjacent to Kolar district) during 1984 and at times localized sylvatic plague incidence encountered in the last decade from the trijunction of Karnataka, Andhra Pradesh and Tamil Nadu in peninsular India. From 1989 to 1994, active zoonotic foci of plague were detected from the trijunction of Tamil Nadu (Krishnagiri district), Andhra Pradesh (Chittoor district) and Karnataka (Kolar and Bangalore rural district). Of the total rodents collected (473, 736), *Tatera indica cuvieri* (Hardwicke), the Indian gerbil was by far the most numerous (41.9%) followed by *Rattus rattus rufescens* Gray, *Rattus rattus wroughtoni* Hinton and *Bandicota bengalensis* (Gray). Hemagglutinating antibodies were detected in 243 sera samples from three different rodent species, i.e., *Tatera indica, Bandicota bengalensis* and *Rattus rattus.* Sero-positivity of sylval/ peri-domestic rodents, abundance of vector fleas and hilly terrain in these study zones, which offer protection to rodent population from unnatural deaths from floods and human encroachment to their habitats, supported perennial transmission of sylvatic plague in these regions.[4]

History of Insecticide Used in Public health Sector in the Study Areas

Insecticides used in National Malaria Control Program under NMEP (now NVBDCP) gave a collateral benefit for the control of rodent fleas and dramatic reduction in human plague cases in the erstwhile plague endemic areas in India. In Kolar and Chittoor region, DDT was being used as Indoor residual spray under NVBDCP from 1953 to 1990. From 1991 to 1993, DDT was used along with synthetic pyrethroids. From 1994 to 2006, DDT alone was used for indoor residual spray (IRS) in the region. There was no indoor residual spray from 2007 to 2009 due to non-acceptance of IRS by the sericulture farmers. DDT and BHC were used for anti-flea measures in plague-affected areas of Kolar and surrounding areas from 1959 to 1963. BHC 50% wdp as IRS and BHC 10% dust powder for insufflations of rodent burrows were used in the areas.

Study Areas

Kolar District

Kolar district is located in the southern region of Karnataka state and is the eastern most district of the state. The district is bounded by the Bangalore rural district in the west, Chikballapur district in the north, Chittoor district of Andhra Pradesh in the east and on the south by Krishnagiri and Vellore district of Tamil Nadu. Kolar district belongs to semi-arid drought-prone region. It lies between 77° 21' to 78° 35' east longitude and 20° 46' to 130° 58' north latitude, extending over an area of 8225 km². The major sources of employment are agriculture, dairy, sericulture, and floriculture; hence it is popularly known as the land of "Silk, Milk and Gold." Major soil types are red loamy, red sandy, and mixed

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red. Average annual rainfall is 2169.4 mm. The predominant crops grown are finger millet, groundnut and pulses. The important irrigated crops are paddy, mulberry, sugarcane, potato and other vegetables. It experiences a semi-arid climate, characterized by typical monsoon tropical weather with hot summers and mild winters.

Chittoor District

Chittoor district is located in the extreme south of Andhra Pradesh, between 12°37' and 14°8' north latitudes and 78°3' and 79°55' east longitudes. The district is spread over 15,152 km^2. The temperatures in the western parts of the district like Punganur, Madanapalle, Horsley Hills are relatively lower than the eastern parts of Chittoor district. This is because of the higher altitude of the western parts compared to the eastern parts. The summer temperature touches 46 °C in the eastern parts whereas in the western parts it ranges around 36 ºC to 38 °C. Similarly, the winter temperatures of the western parts are relatively low ranging around 12 °C to 14 °C and in eastern parts it is 16 °C to 18 °C. Chittoor district receives an annual rainfall of 918.1 mm.

Impact of Sericulture in Development of Insecticide Resistance in Rodent Fleas

Sericulture, or silk farming, is the rearing of silkworms for the production of silk. India has a rich and complex history in silk production and its silk trade dates back to 15th century. India has the unique distinction of being the only country producing all the five known commercial silks, namely, mulberry, tropical tasar, oak tasar, eri and muga. India is the second-largest producer of silk (16,525 MT, 2006-07) in the world next only to China (93,100 MT, 2006). Karnataka is the leading sericulture state which contributes around 50% of the total silk production in India. Sericulture involves cultivation of mulberry and rearing of silkworm to produce silk cocoons. Andhra Pradesh occupies second position in the country next to Karnataka in production of silk. The mulberry sericulture activity is spread over all 22 districts of Andhra Pradesh, mainly the districts, viz., Chittoor, Ananthapur and West Godavari contributing 75% of the mulberry sericulture activity. The Palamaner area in Chittoor district contributes more than 800 MT of silk annually.

The silkworm-*Bombyx mori* feeds exclusively on mulberry leaves and is highly sensitive to pesticides in general. Like other crops, mulberry is also affected by several pests like insects, pathogens, nematodes, mites, weeds, etc.

Susceptibility Test of Rodent Fleas to Different Insecticides

For the susceptibility test, *Xenopsylla cheopis* (rat flea) collections were made from Kolar (Karnataka) and Palamneru (Chittoor district, A.P.) during the years 2007 to 2009. Keeping in mind the seasonal variation on rodent flea population, all the susceptibility tests in Kolar and Palamner region were undertaken during post-monsoon season, which is the peak breeding season for rodent and fleas in the area, i.e., August to October.[4,7] Male and female samples of rodent fleas (both male and female fleas live on mammalian blood and can transmit plague and other rodent-borne zoonotic diseases) were collected from the trapped rodents. The villages in Kolar and Palamneru area were chosen on the basis of sericulture and non-sericulture areas where there was agricultural and anti-malarial/ anti-plague selection pressure on the flea populations.

Multiple live catch wonder traps with preferred baits were laid in domestic and peri-domestic situations. The retrieved positive traps covered with ventilated cloth bags to avoid escape of live rodent fleas were transported to the local plague laboratory. Subsequently, live rodents were deflead by combing and fleas were collected in a deep white enamel pan. After combing, live fleas were aspirated using flea aspirator tube and were transferred gently into a clean glass jar and kept for 24 hours for observation in the field laboratory maintained at 37 ± 5% RH and 27 ± 2 °C in dark condition. The dead and injured fleas were discarded. The healthy and fully fed fleas, which were suitable for test, were selected.

Susceptibility tests of rodent fleas with various insecticides were carried out following standard procedure.[3] For the susceptibility tests DDT-4.0%, malathion-5.0%, deltamethrin-0.05%, permethrin-0.75% and Lamdacyholothrin-0.05% impregnated filter papers supplied by WHO were used against rat fleas. Strip of the insecticide impregnated papers and untreated papers, measuring 5 cm × 2.5 cm each, folded longitudinally in the form of "Z" were used for the test. Test tubes were marked properly with colored markers. Simultaneously, these tubes were compared with respective control tubes using control papers. A minimum of 15 fleas were introduced into each exposure tube of 15-cm long and 1.5-cm diameter for 1 hour. Fleas were then transferred into clean holding tubes containing a similar untreated filter paper and mortality counts were made after exposure of 24 hours of retrieval period. Abbott's formula was applied to calculate the corrected mortality when control mortality

was observed between 5 and 20%. Susceptibility tests performed during the years from 2007 to 2009 and the results obtained are furnished in Tables 1 and 2. All the tested fleas were kept overnight in 10.0% potassium hydroxide solution, mounted on glass slides with DPX mountant and identified up to species level.[3]

Results and Discussion

Mortality rates of *Xenopsylla cheopis* against organochlorine, organophosphates and synthetic pyrethroids in sericulture and non-sericulture areas in Kolar (Karnataka) and Palamneru (Chittoor district, A.P.) are furnished in Tables 1 and 2. Study revealed that *Xenopsylla cheopis* developed resistance for 1-hour exposure period to DDT-4.0%, malathion 5.0% and deltamethrin 0.05% in Kolar areas, Karnataka, in both sericulture and non-sericulture areas whereas it has developed resistance in DDT-4.0% and Malathion 5.0% only in Palamneru areas, Chittoor district. In Kolar district, rodent fleas are found to be tolerant to all the synthetic pyrethroids in both sericulture and non-sericulture areas. Mortality rates in synthetic pyrethroids are found to be significantly more in Palamneru areas in both sericulture and non-sericulture areas compared to Kolar areas (Table 2). There is no significant difference in mortality rate to various insecticides in both sericulture and non-sericulture areas of Kolar and Palamneru area (Tables 1 and 2).

During resurgence of plague in peninsular India, DDT and BHC were used for anti-flea measures. From 1959 to 1963, BHC 50% wdp was used as IRS and BHC 10% dust powder for insufflations of rodent burrows (insufflation-the method of treatment of rat burrows and rat runs with 10% DDT or Malathion 5% dust powder (wettable powder) [1 part of 25% w.p. and 4 parts of chalk powder or inert material]. Insecticide dust is blown with rotary plunger type duster or cyanogas pump in the mouth of the rodent burrows and a patch of dusting powder about 0.5 to 1.0 cm thick and 20-25 cm wide is left around the mouth of the burrow, Patches of dust [30 gm per burrow] is applied in the rodent burrows). Sprinkling of dust powder is also done indoor in the corners of the houses and runways. In 1963, NICD team studied the susceptibility status of fleas collected from the commensal rodents in Kolar district to various insecticides and they were clearly shown to be highly resistant to DDT and tolerant to BHC. This degree of resistance to DDT was the result of anti-malaria program under NMEP.[8]

Though the use of pesticides is inevitable in mulberry ecosystem, it has its own limitation owing to toxicity to silkworms. Since newer pesticide molecules are being flooded into the market and the availability of recommended old pesticides is becoming scarce, sericulture farmers unknowingly purchase and spray some non-recommended pesticides to solve the pest problem. Insecticide treated or non-treated harvested leaves are always preserved in a separate room or in a corner of silk worm rearing room. Leaves scattered on floor always come in contact with immature or mature stages of fleas breeding in loose soil indoor or with rodents carrying fleas on their body.

Table 1.Results of Susceptibility Status of *Xenopsylla cheopis* to Various Insecticides in Sericulture and Non-sericulture Villages in Kolar Areas, Karnataka, from 2007 to 2009

Insecticides Used (Diagnostic concentration)	Tests Performed Period	Mortality in Tested Population *Xenopsylla cheopis*						Control Mortality (Percent)		Corrected Mortality		Mean Mortality	
		Total Fleas Exposed		Total Fleas Dead		Percent Mortality (No. of Replicate)							
		S	NS	S	NS	S	NS	S	NS	S	NS	S	NS
DDT-4.0%	2007	57	69	29	37	50.9 (3)	53.6 (4)	9.5	8.7	45.7	49.2	52.0	46.6
	2008	112	87	69	46	61.6 (7)	52.9 (5)	13.3	10.3	55.7	47.5		
	2009	94	103	55	53	58.5 (6)	51.4 (6)	8.5	14.6	54.6	43.1		
Malathion 5.0%	2007	84	107	38	62	45.2 (5)	57.9 (7)	14.2	20.6	36.1	46.9	42.4	53.6
	2008	65	98	39	66	60.0 (4)	67.3 (6)	13.3	17.1	53.9	60.6		
	2009	79	125	37	77	46.8 (5)	61.6 (8)	15.4	17.7	37.1	53.3		
Deltamethrin 0.05%	2007	47	59	29	32	61.7 (3)	54.2 (4)	15.1	-	54.9	-	55.6	51.1
	2009	71	49	44	27	62.0 (4)	55.1 (3)	13.3	13.8	56.2	47.9		
Permethrin 0.75%	2007	32	35	28	30	87.5 (2)	85.7 (2)	13.3	10.0	85.6	84.1	81.5	83.05
	2009	35	38	28	32	80.0 (2)	84.2 (2)	11.8	12.0	77.3	82.0		
Lamdacyholothrin 0.05%	2007	32	35	31	33	96.8 (2)	94.3 (2)	6.3	12.0	96.6	93.5	96.8	95.2
	2009	38	36	37	35	97.4 (2)	97.2 (2)	10.3	11.5	97.1	96.8		

S-Sericulture village, NS-Non-sericulture village

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Table 2.Results of Susceptibility Status of *Xenopsylla cheopis* to Various Insecticides in Sericulture and Non-sericulture Villages in Palamneru Areas, Chittoor District, Andhra Pradesh from 2007 to 2009

Insecticides Used (Diagnostic Concentration)	Tests Performed Period	Mortality in Tested Population *Xenopsylla cheopis*						Control Mortality (Percent)		Corrected Mortality		Mean Mortality	
		Total Fleas Exposed		Total Fleas Dead		Percent mortality (No. of replicate)							
		S	NS	S	NS	S	NS	S	NS	S	NS	S	NS
DDT-4.0%	2007	76	82	49	44	64.5 (5)	53.6 (5)	10.0	15.0	60.6	45.4	55.5	49.0
	2008	97	107	57	66	58.8 (6)	61.7 (7)	8.3	12.0	55.1	56.5		
	2009	104	93	61	51	58.6 (7)	54.8 (6)	16.0	17.8	50.7	45.0		
Malathion 5.0%	2007	95	112	58	76	61.0 (6)	67.9 (7)	8.0	9.5	57.6	64.5	52.5	59.4
	2008	85	99	43	56	50.6 (5)	56.6 (6)	10.3	8.0	44.9	52.8		
	2009	85	114	53	74	62.3 (5)	64.9 (7)	16.0	10.0	55.1	61.0		
Deltamethrin 0.05%	2007	67	89	65	81	97.0 (4)	91.0 (6)	14.3	10.7	96.5	89.5	96.9	90.0
	2009	75	46	73	42	97.3 (5)	91.3 (3)	4.0	8.3	97.2	90.5		
Permethrin 0.75%	2007	42	34	41	32	97.6 (2)	94.1 (2)	6.4	18.7	97.4	92.7	97.5	92.6
	2009	45	47	44	44	97.8 (3)	93.6 (3)	7.1	14.3	97.6	92.5		
Lamdacyholothrin 0.05%	2007	35	38	35	37	100.0 (2)	97.4 (2)	9.4	14.2	100.0	97.0	98.5	95.8
	2009	33	38	32	36	97.0 (2)	94.7 (2)	-	4.0	-	94.5		

S-Sericulture village, NS-Non-sericulture village

Moreover, there is continuous intermingling of rodents from sericulture and non-sericulture areas and transferring of fleas which are found to be as ectoparasites on rodent bodies. Silk farmers resist indoor residual insecticide spraying for mosquito or flea control due to its toxic effect on the silk worms. As a result, malaria incidence in the area was high in the early nineties. Due to non-acceptance of indoor residual spray in silk-rearing villages of Kolar and Chittoor districts, selection pressure of various insecticides on flea population breeding indoor is negligible but the selection pressure from the insecticide-treated mulberry plant leaves on indoor resting flea population was always there.

Insecticide Resistance Action Committee (IRAC) defines resistance as the selection of a heritable characteristic in an insect population that results in the repeated failure of an insecticide product to provide the intended level of control when used as recommended. According to this definition, differences in susceptibility apparent in laboratory bioassays may not necessarily constitute resistance if the difference does not result in a change in the field performance of the insecticide. Development of resistance is a complex and dynamic process and depends upon many factors.

Most commonly, when the frequency of resistant insects in a vector population increases, efficacy of the treatment decreases up to the point where the insecticide has to be replaced by another one. Increasing the dosages in an attempt to maintain efficacy is not a recommended option because of environmental and safety concerns, increased cost of the insecticide and the resistance genes can be driven to even higher frequencies. Effective resistance management depends on early detection of the problem and rapid assimilation of information on the resistant insect population so that rational pesticide choices can be made.

Almost all public health insecticides are also used in agriculture. When vectors breed within or close to agricultural crops, they can be exposed to the same or similar insecticidal compounds and develop resistance. This phenomenon is of particular relevance for plague vectors. Moreover, many insecticides are also massively used to control domestic pests, and therefore, impact will be more on the vector species which are breeding and resting indoors like fleas. Furthermore, in some circumstances, resistance can persist in populations for very long periods after regular use of an insecticide has ceased. In these cases, resistance to new insecticides is inherited from the past as a result of the previous use of other insecticides.

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Conflict of Interest: None

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