Operational research and mosquito control

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Operations Research

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Operations Research is the application of analytical methods designed to help the decision makers choose between various courses of action available to accomplish specified objectives

In simple term it describe as

"The Science of better"

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Operations Research - Methodology

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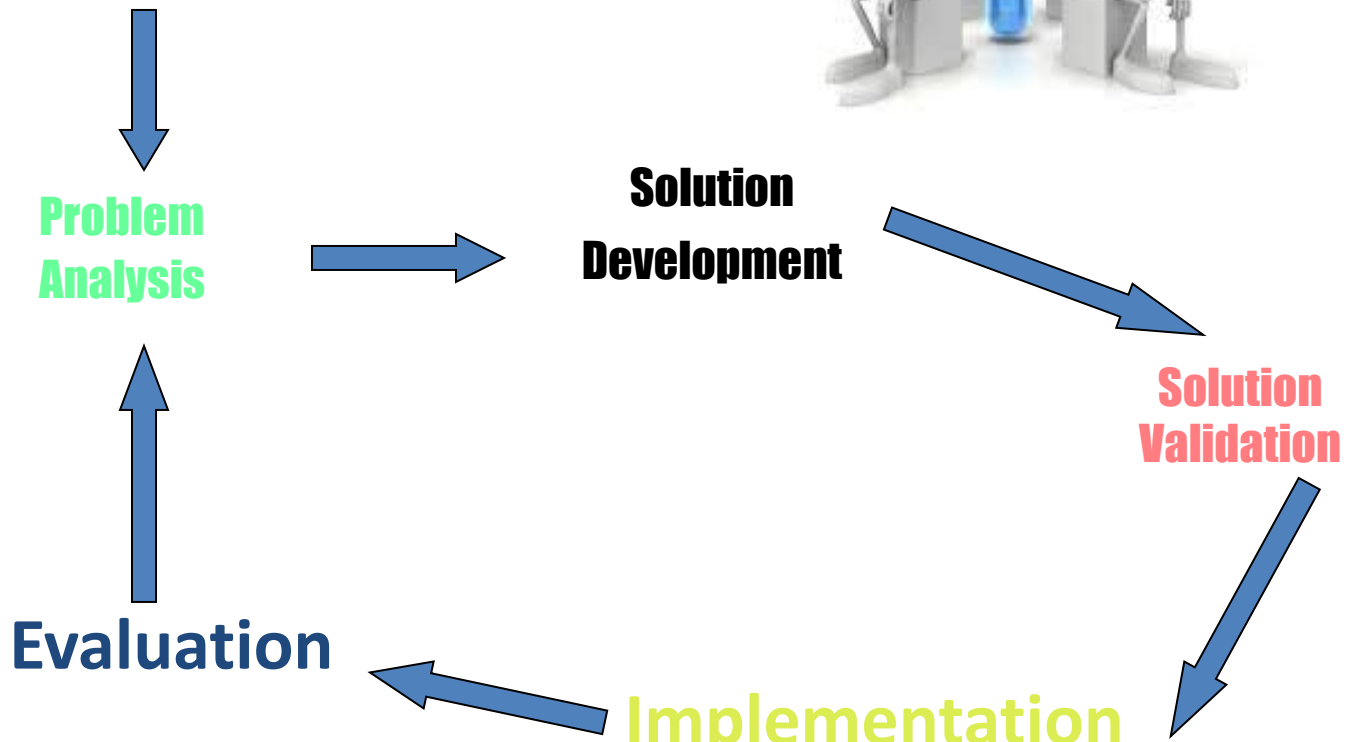

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Malaria burden in India

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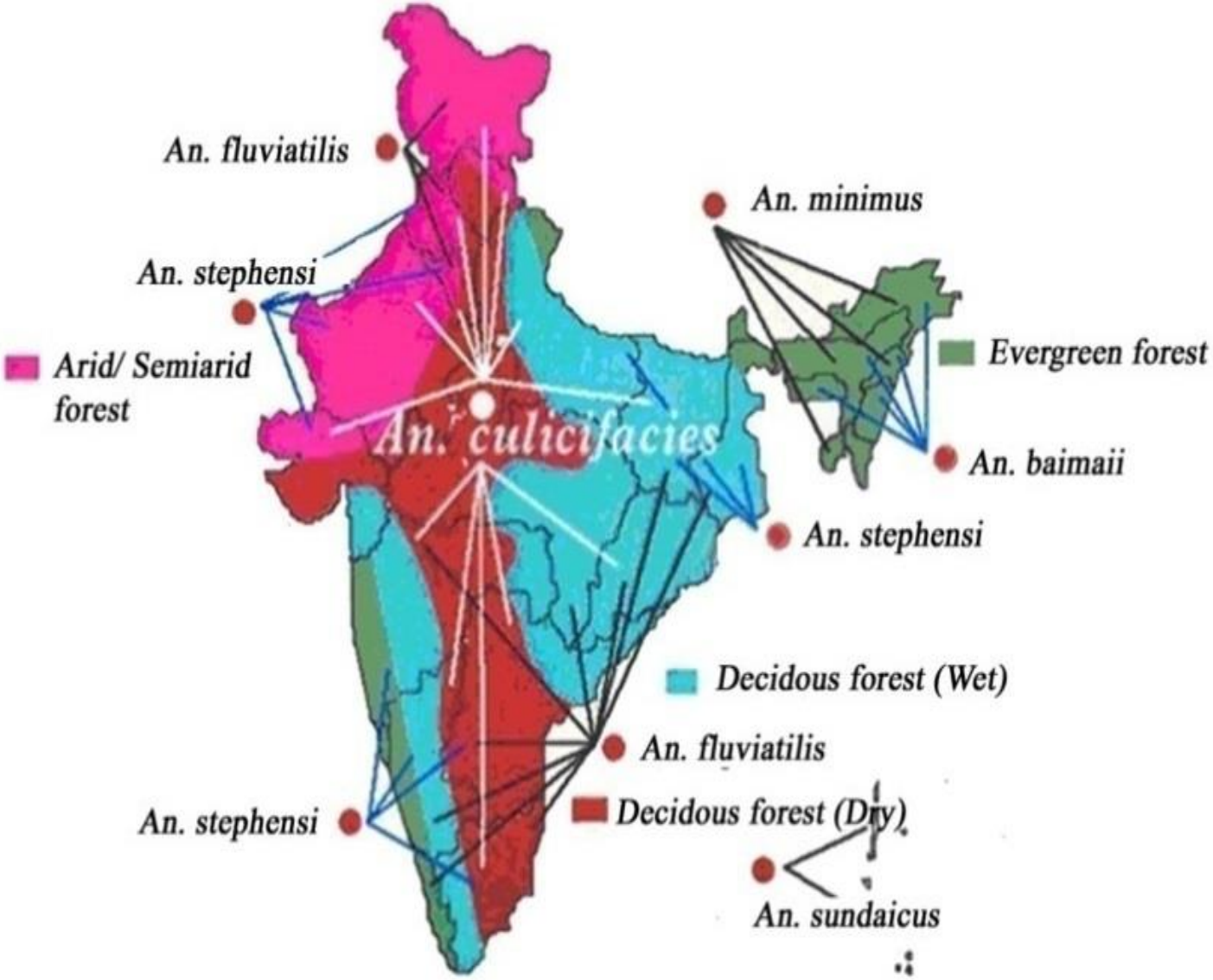
- Malaria continues to remain among the top three infectious diseases (Malaria, tuberculosis and HIV) affecting billions of people globally.
- The World Health Organization estimates that over 300 million new cases of malaria arise every year in world.
- 80.5 % of the 109 billion population of India lives in malaria risk region.
- in India, NVBDCP, (National Vector Borne Diseases Control Programme) indicate 1.5–2 million confirmed cases and about 1,000 deaths annually.

Distribution of major malaria vectors in India



Source: Dev & Sharma, 2013

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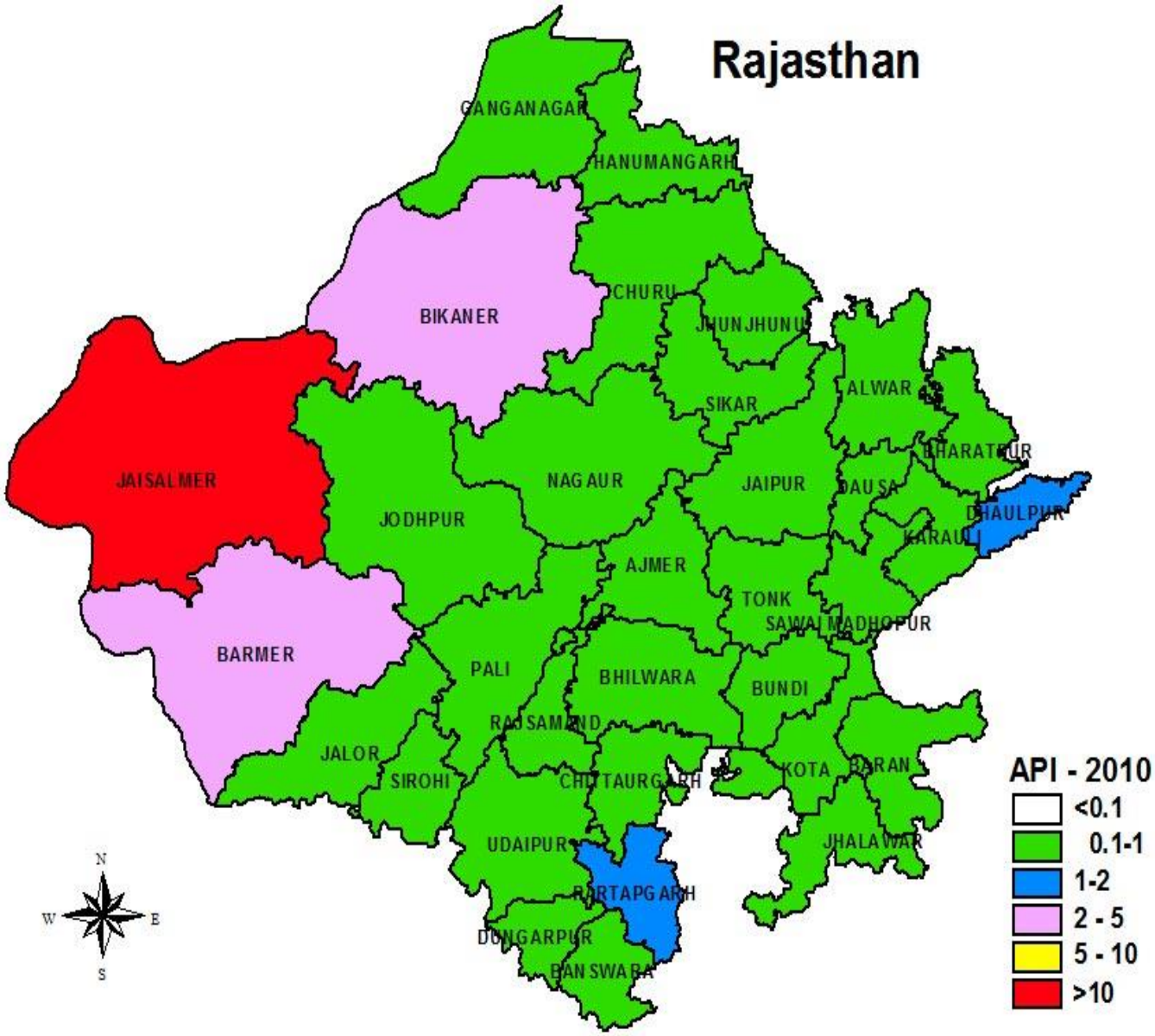
Map showing intensity of malaria in Rajasthan on the bases of Annual Parasite Index (API) in 2010) (Data source: NVBDCP).



Malaria in Udaipur

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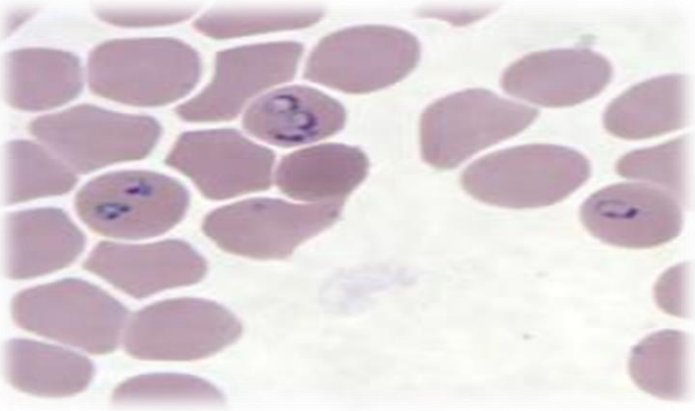
CMHO report of Udaipur

Particular	Year 2014	Year 2015
Total blood slides	288867	281206
Positive	629	624
Total PV	592	596
Total PF	37	28



Mosquito control research in our laboratory

- Bionomics of Anopheline mosquitoes in south Rajasthan
- Bacterial control of Anopheline mosquitoes
- Mycopesticide control of Anopheline mosquitoes
- Phytopesticide control of Anopheline mosquitoes

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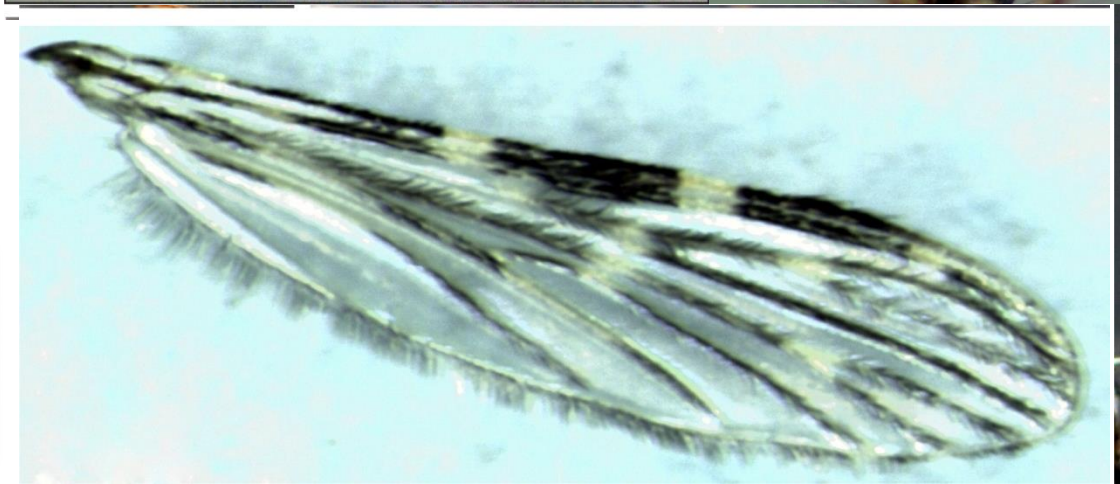
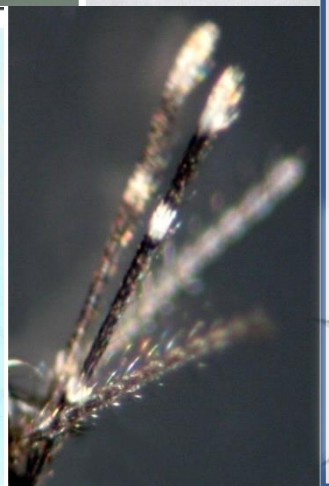
Bionomics study of Anophelines





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Hilly with stream areas of Udaipur district

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Plane with stream area

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Plane with non-stream area

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Adult mosquito collection using aspirator in cattle shades

Adult mosquito collection using aspirator in human dwellings

Mosquitoes collection

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Breading habitat of Anophelines




Cattle tanks


Clay pots



Tree holes

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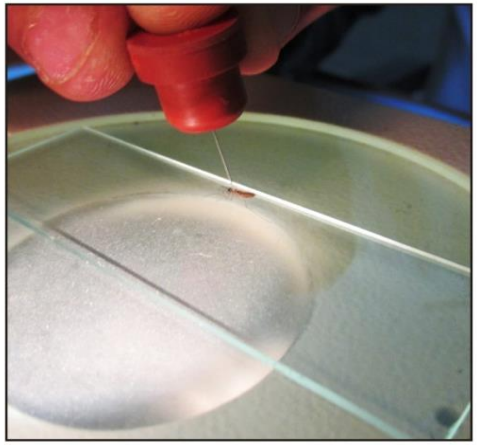

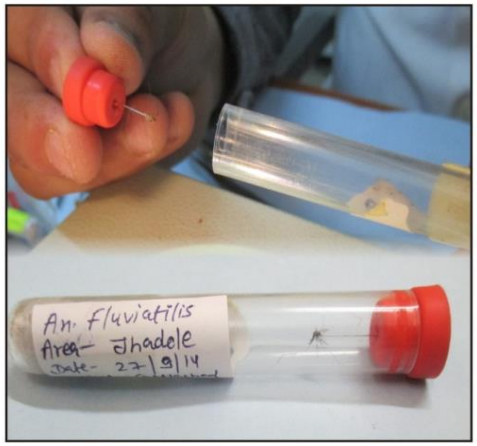

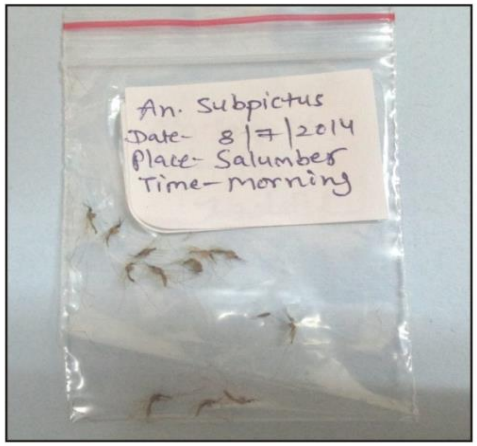

Identification & preservation of Adult mosquitoes

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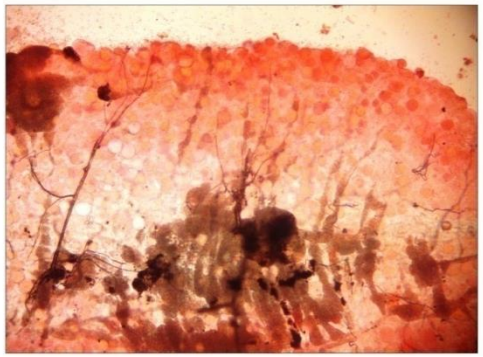
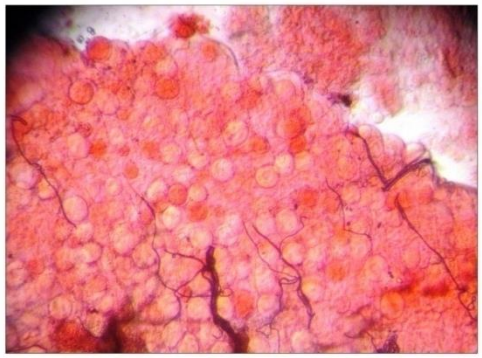
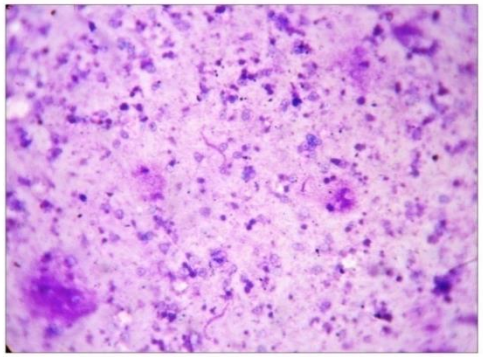
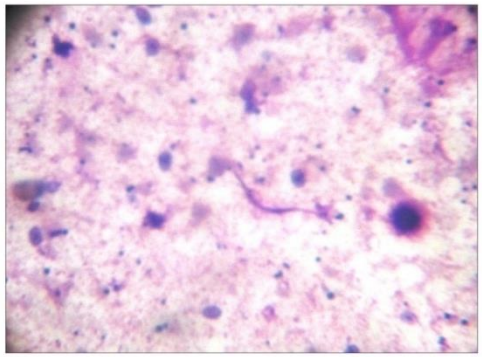




Detection of oocyst from Mid gut dissection of Mosquito

Vector Incrimination study





Detection of sporozoites from Salivary gland dissection of Mosquito

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Incidence of Anophelines in Udaipur (Southern Rajasthan)

S.No.	Mosquitoes collected	Season						Vector Infectivity rate
		Rainy	% OC	Winter	% OC	Summer	% OC	(%)
1.	*An. stephensi*	172	9.16	68	4.42	218	14.47	0.46
2.	*An. culicifacies*	357	19.01	257	16.70	547	36.30	0.61
3.	*An. fluviatilis*	79	4.21	158	10.27	26	1.73	
4.	*An. subpictus*	592	31.52	167	10.85	462	30.66	
5.	*An. annularis*	422	22.47	640	41.59	201	13.34	
6.	*An. splendidus*	141	7.51	104	6.76	18	1.19	
7.	*An. barbirostris*	66	3.51	78	5.07	23	1.53	
8.	*An. jemesai*	33	1.76	41	2.66	7	0.46	
9.	*An. pellidus*	7	0.37	10	0.65	1	0.07	
10.	*An. tessellatus*	1	0.05	4	0.26	0	0.00	
11.	*An. nigerrimus*	8	0.43	11	0.71	4	0.27	
12.	*An.turkhudi*	0	0.00	1	0.06	0	0.00	
	Total	1878	100	1539	100	1507	100	

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Season wise species specific breeding preference of Anophelines from different habitats in of Udaipur, South Rajasthan (July, 2012-June, 2014)

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Season	Mosquitoes collected	Stream & its pits	Rocky pools	Grassy ponds	Seasonal ponds	Tree holes	Cattle tanks	Clay pots	Tanka	**Total breeding sites**
Rainy	*An. stephensi*	+		+			+	+	+	**05**
	An. culicifacies	+		+	+	+				**04**
	An. fluviatilis	+	+	+						**03**
	An. subpictus	+		+	+	+	+	+	+	**07**
	An. annularis	+		+	+	+				**04**
	An. splendidus	+		+		+				**03**
	An. barbirostris	+		+	+					**03**
	An. jemesai			+	+					**02**
	An. pellidus	+		+						**02**
	Total species	**08**	**01**	**09**	**05**	**04**	**02**	**02**	**02**	**33**
Winter	*An. stephensi*			+			+		+	**03**
	An. culicifacies	+	+	+	+	+				**05**
	An. fluviatilis	+	+	+						**03**
	An. subpictus	+		+	+		+	+		**05**
	An. annularis	+		+	+	+				**04**
	An. splendidus	+	+	+						**03**
	An. barbirostris	+		+	+					**03**
	An. jemesai			+	+					**02**
	An. pellidus			+						**01**
	An. nigerrimus			+						**01**
	Total species	**06**	**03**	**10**	**05**	**02**	**02**	**01**	**01**	**30**
Summer	*An. stephensi*	+		+			+	+	+	**05**
	An. culicifacies	+	+	+	+					**04**
	An. subpictus	+		+	+		+		+	**05**
	An. annularis	+		+						**02**
	An. barbirostris			+	+					**02**
	Total species	**04**	**01**	**05**	**03**		**02**	**01**	**02**	**18**

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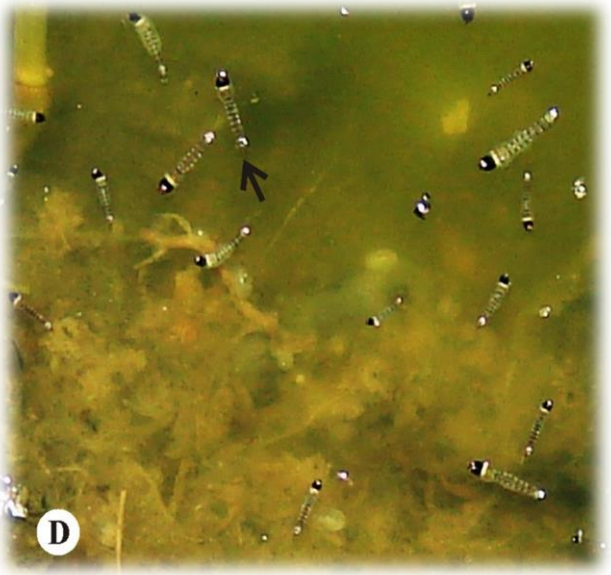
Goal of vector control

- Reduce vector-human contact by targeting the different stages of *Anopheles*.
- Control mosquitoes in safe, efficient and cost-effective manner.
- Prevent damage to humans, animals, land and natural environment.



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Biological control

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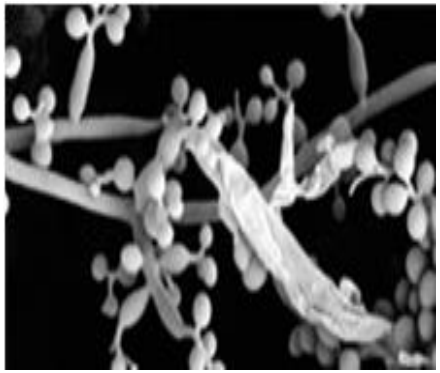
Bio-pesticides are certain types of pesticides derived from natural material such as animals, plants, bacteria, and certain minerals. Many bio-pesticides are less toxic and pose a lower risk than conventional pesticides which can be quite toxic.



Beauveria fungus

Bacillus bacteria

Botanical

Larva killed by NP Virus

To study the occurrence of Anopheline mosquito larvae in relation to their habitat in different zones of Udiapur (South Rajasthan)

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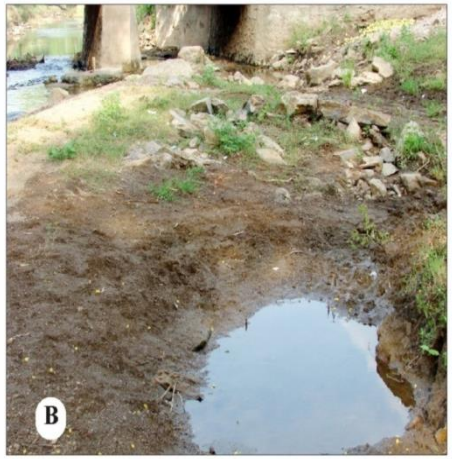
➢Udaipur city of South Rajasthan provided good favorable sites for malaria vector larvae to breed.

Rear view of larval colletion site

Closer view of water body

Anopheline larvae (closer view)



Rear view of larval colletion site

➢The abundance and occurrence of anopheline mosquito fauna was maximum in semi urban areas (followed by urban and rural areas.

Effect of various abiotic factors on the different developmental stages of Anopheles stephensi (L.)

Serial No.	Incubation period	Temperature	RH	Larval Period	Temperature	RH	Pupal Period	Temperature	RH	Adult	Total Life Cycle	Temperature	RH
1	2	27.02	65.21	12	29.42	66.49	3	28.09	65.22	5	22	28.18	65.64
2	3	28.09	69.72	12	27.06	67.82	2	27.65	66.37	4	21	27.60	67.97
3	3	29.42	71.6	10	27.42	71.88	2	28.42	68.29	6	21	28.42	70.59
4	2	27.82	71.02	11	28.69	73.66	2	28.26	68.44	6	21	28.26	71.04
5	2	27.20	72.79	12	27.75	69.89	3	27.49	70.28	5	22	27.48	70.99
6	2	28.73	68.44	10	26.98	70.03	2	27.64	72.89	3	17	27.78	70.45
7	3	27.45	69.82	12	27.5	72.62	3	28.43	71.33	3	21	27.79	71.26
8	2	28.92	71.09	13	28.06	71.69	2	28.59	70.38	5	22	28.52	71.05
9	2	27.49	70.44	13	28.44	71.45	2	28.63	69.82	4	21	28.19	70.57
10	2	28.05	74.02	11	27.89	69.83	3	27.37	69.88	5	21	27.77	71.24
Mean	2.30	28.02	70.42	11.60	27.92	70.54	2.40	28.06	69.29	4.60	20.90	28.00	70.08
R		0.263	-0.010		0.381	-0.096		-0.382	-0.042			0.224	-0.170

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Rearing of mosquitoes

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- Larvae were collected from different water bodies; they were be brought to the laboratory and kept for rearing.

- The larvae were fed on dog biscuits and yeast powder in 3:1 ratio.

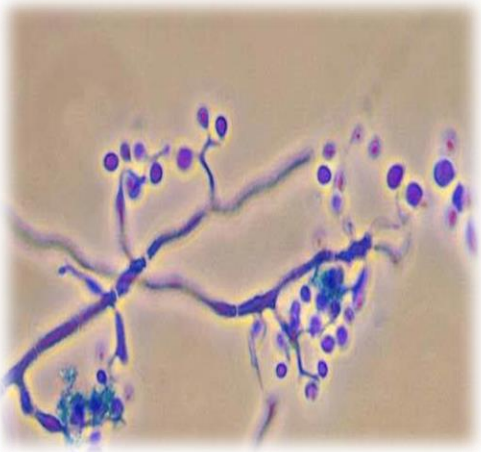
- Adults were provided with 10% sucrose solution

- Mosquitoes were held at 28 ±2^{0}C temperature, 70-80% RH, with a photo period of 14h light: 10h dark

Mycopesticide

Beauveria bassiana (Balsamo)

- An ubiquitous,
- Insect-pathogenic,
- Hemibiotrophic,
- Mitosporic fungi
- With a very diverse and large host range
- Pathogenic to insects
- Very diverse and large host range
- Low virulence against non target insects
- Grows naturally in soil



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Experimental plane

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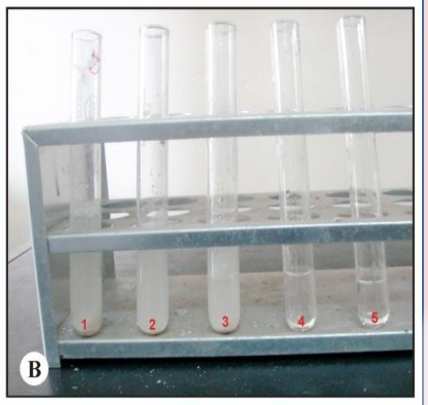
Rearing of mosquites in Rearing Room

Rearing of *Anopheles* Larvae in BC Incubator

Development of *Beauveria bassiana* (Balsamo) in the Laboratary

Larval Treatment

Experimental Observations



Serial Dilutions of *Beauveria bassiana* (Balsamo)

Larval mortality

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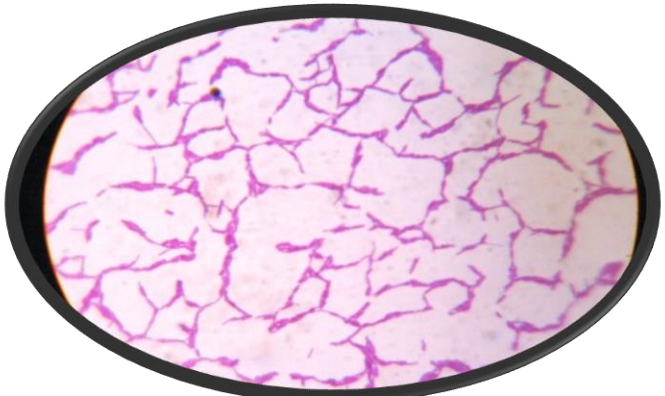
Probit Analysis

Instar	Intercept	$\chi 2$	LC_{50}	LC_{90}
Egg	-0.7058 + 0.6180 (Conc.)	21.87	**1.14**	**3.22**
I Instar	-0.8047 + 0.0937 (Conc.)	5.42	**8.59**	**22.27**
II Instar	-0.9148 + 0.5489 (Conc.)	30.71	**1.67**	**4.00**
III Instar	-0.9542 + 0.6725 (Conc.)	50.53	**1.42**	**3.32**
IV Instar	-0.6415 + 0.7395 (Conc.)	19.40	**0.87**	**2.60**
Pupa	-0.5541 + 0.5529 (Conc.)	37.54	**1.00**	**3.32**

Experimental Plan

- Isolation of soil bacteria
- Bioassay of isolated bacterial strains for their insecticidal activities
- Evaluation of three most toxic bacterial strain against different developmental stages (egg, larvae and pupa) of *Anopheles stephensi (L)*



Bacterial pesticide

- *Anopheles stephensi* larvae was tested with bacterial pesticide.
- Most potent bacterial groups was Actinobacteria, Bacillus and Pseudomonas in detail screening procedure.

	LC_{50} Values				LC_{90} Values		
S.No		Actinobacteria strain A_2	Bacillus strain B_1	Pseudomonas strain P_2	Actinobacteria strain A_2	Bacillus strain B_1	Pseudomonas strain P_2
1	Egg	1.662	1.835	2.004	3.301	3.780	3.351
2	Ist Instar	1.648	1.814	1.996	3.427	3.759	4.158
3	IInd Instar	1.618	1.794	1.965	3.373	3.807	4.141
4	IIIrd Instar	1.645	1.858	2.116	3.513	3.990	4.614
5	IVth Instar	1.721	1.891	2.112	3.687	3.975	4.374
6	Pupa	1.805	2.008	2.202	3.473	3.972	4.331

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Bacterial pesticide field bioassay

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The field bioassay was conducted to assess the efficacy of *Streptomyces indiaensis* in the field conditions to control *Anopheles stephensi* (L) mosquito population.

Pupal Emergence

Dose Concentration	Mean	SD	F	df	Result
Control	44.67	1.53	142.44	3, 8	***
1.0 gm	22.67	3.06			
2.5 gm	14.00	2.00			
5.0 gm	7.67	2.52			

Adult Emergence

Dose Concentration	Mean	SD	F	df	Result
Control	41.00	1.00	397.33	3, 8	***
1.0 gm	13.00	2.00			
2.5 gm	8.00	1.73			
5.0 gm	2.00	1.00			

Results obtained from field trial showed that different concentrations of *Streptomyces indiaensis* were highly toxic to larvae of *Anopheles stephensi* (L.).

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According to the report of Xclaris Lab Actinobacteria strain A_2 was identified as *Streptomyces indiaensis* type strain LMG19961 (Gen Bank Accession Number: AJ781344.1).

Similarly Bacillus strain B_1 was identified as *Bacillus subtilis* type strain IF5 (Gen Bank Accession Number: FJ951435.1) .

The sequence of identified bacteria *Streptomyces indiaensis* type strain LMG 19961 and *Bacillus subtilis* type strain IF5 were registered in NCBI (National Center for Biotechnological Information U.S.A).

NCBI assign an Accession Number which is KJ170314 for *Streptomyces indiaensis* strain LMG19961 and KJO22639 for *Bacillus subtilis* strain IF5.

Press Note

MLSU Researchers discover two new strains of Bacteria to kill Mosquitoes

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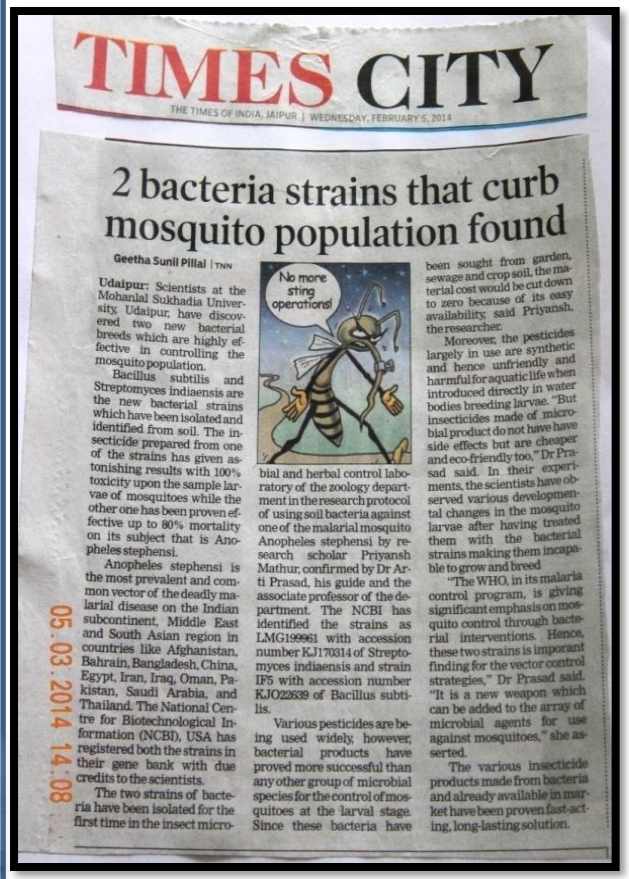

TIMES CITY

THE TIMES OF INDIA, JAIPUR | WEDNESDAY, FEBRUARY 5, 2014

2 bacteria strains that curb mosquito population found

Geetha Sunil Pillai | TNN

Udaipur: Scientists at the Mohanlal Sukhadia University, Udaipur, have discovered two new bacterial breeds which are highly effective in controlling the mosquito population.

Bacillus subtilis and Streptomyces indiaensis are the new bacterial strains which have been isolated and identified from soil. The insecticide prepared from one of the strains has given astonishing results with 100% toxicity upon the sample larvae of mosquitoes while the other one has been proven effective up to 80% mortality on its subject that is Anopheles stephensi.

Anopheles stephensi is the most prevalent and common vector of the deadly malarial disease on the Indian subcontinent, Middle East and South Asian region in countries like Afghanistan, Bahrain, Bangladesh, China, Egypt, Iran, Iraq, Oman, Pakistan, Saudi Arabia, and Thailand. The National Centre for Biotechnological Information (NCBI), USA has registered both the strains in their gene bank with due credits to the scientists.

The two strains of bacteria have been isolated for the first time in the insect microbial and herbal control laboratory of the zoology department in the research protocol of using soil bacteria against one of the malarial mosquito Anopheles stephensi by research scholar Priyansh Mathur, confirmed by Dr Arti Prasad, his guide and the associate professor of the department. The NCBI has identified the strains as LMG199961 with accession number KJ170314 of Streptomyces indiaensis and strain IF5 with accession number KJO22639 of Bacillus subtilis.

Various pesticides are being used widely, however, bacterial products have proved more successful than any other group of microbial species for the control of mosquitoes at the larval stage. Since these bacteria have been sought from garden, sewage and crop soil, the material cost would be cut down to zero because of its easy availability, said Priyansh, the researcher.

Moreover, the pesticides largely in use are synthetic and hence unfriendly and harmful for aquatic life when introduced directly in water bodies breeding larvae. "But insecticides made of microbial product do not have have side effects but are cheaper and eco-friendly too," Dr Prasad said. In their experiments, the scientists have observed various developmental changes in the mosquito larvae after having treated them with the bacterial strains making them incapable to grow and breed

"The WHO, in its malaria control program, is giving significant emphasis on mosquito control through bacterial interventions. Hence, these two strains is imporant finding for the vector control strategies," Dr Prasad said. "It is a new weapon which can be added to the array of microbial agents for use against mosquitoes," she asserted.

The various insecticide products made from bacteria and already available in market have been proven fast-acting, long-lasting solution.

05.03.2014 14:08

उदयपुर के वैज्ञानिक ने खोजे मलेरिया को खत्म करने के बैक्टिरिया

सुविवि के शोधार्थी प्रियांश ने की दुनिया की बड़ी खोज

गुपेंद्रसिंह चूंडावत/उदयपुर

अब मलेरिया बढ़ने से पहले ही इस पर काबू संभव होगा। उदयपुर के युवा शोधार्थी वैज्ञानिक ने दो ऐसे जीवाणुओं की खोज की है, जिससे मलेरिया के लार्वा पर काबू करना संभव होगा। गौरतलब है कि दुनिया में अब तक मलेरिया के लिए कोई टीका उपलब्ध नहीं है। जबकि कुनैन की गोली से मलेरिया पर तेजी से काबू संभव नहीं है। युवा वैज्ञानिक ने अपनी खोज को नेशनल सेंटर फॉर बायोलॉजिकल इंफोर्मेशन यूएसए के जीन बैंक में रजिस्ट्रेशन भी करवा लिया है।

ये है खोज: मलेरिया फैलाने के लिए मादा ऐनाफिलीज मच्छर के लार्वा को जिम्मेदार माना जाता है। अपने प्रयोग में कॉलेज ऑफ साइंस के प्राणी विज्ञान के शोधार्थी वैज्ञानिक प्रियांश ने दो जीवाणुओं की खोज की। जिसमें बेसिलस स्फेरिकस और बेसिलस थूरेंजिसिस शामिल है। ये जीवाणु मलेरिया के लार्वा पर हमला कर इन्हें खत्म कर देते हैं। प्रयोग में पहले जीवाणु की मारक क्षमता 100 प्रतिशत व दूसरे जीवाणु की 80 प्रतिशत दर्ज की गई। ■ शेष पेज-13

विश्व स्वास्थ्य संगठन (डब्ल्यूएचओ) भी बैक्टिरिया की मदद से मलेरिया मच्छरों पर काबू पाने की कोशिश करता रहा है। इस तरह से युवा वैज्ञानिक प्रियांश द्वारा इन जीवाणुओं की खोज एक बड़ी खोज है।

डॉ. आरती प्रसाद, एसोसिएट प्रोफेसर, प्राणी विज्ञान विभाग, कॉलेज ऑफ साइंस, सुविवि

Researchers and Zoologists at Mohanlal Sukhadia University, Udaipur have discovered two new bacterial breed effective in controlling the mosquito population.

Experimental Plan

❖Extract preparation from plant (*Moringa Oleifera* L seed, flower and leaves)

❖ Bioassay of extracted plant materials for their insecticidal activity

❖ Evaluation of Repellant properties of extracts of most effective plants against adult mosquito.

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Phyto-pesticide

Evaluation of Repellant properties of extracts of most effective plants against adult mosquito

In *Moringa oleifera* (Lam.), further seeds were most effective among all the three plant parts considered i.e. leaves and flowers

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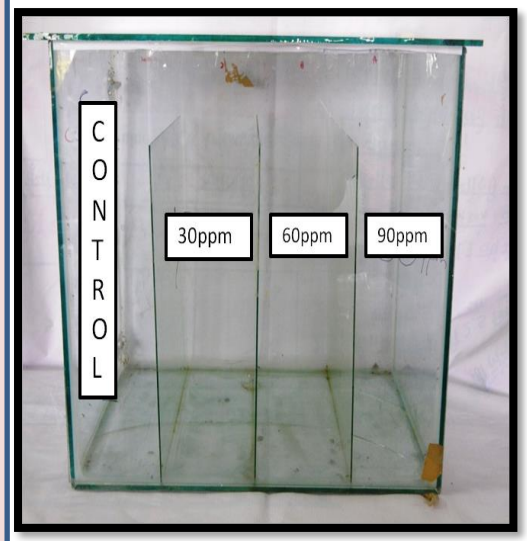
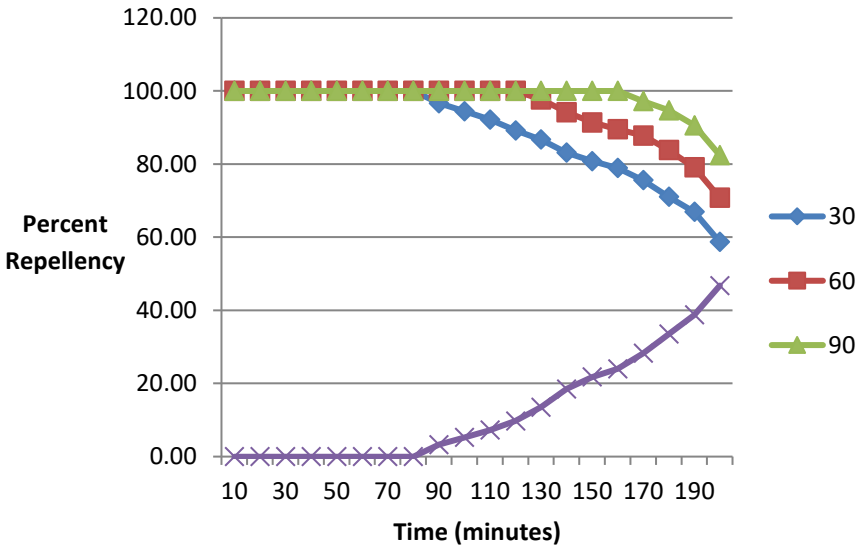
Screen tunneled method

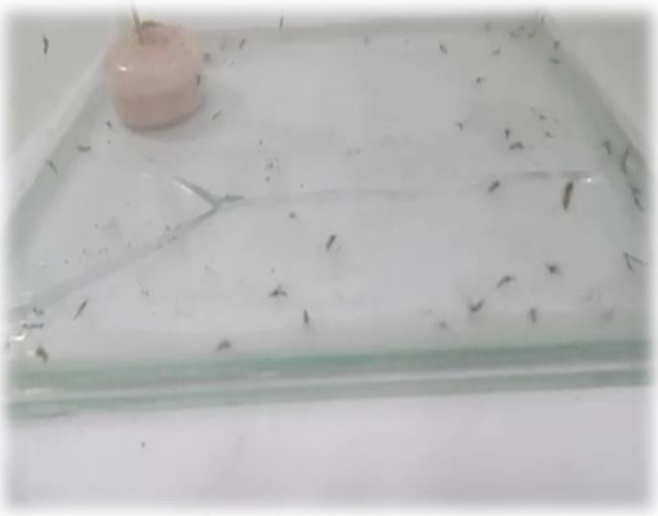
Protection time (minutes)			
	Leaves	Flowers	Seeds
30ppm	10	30	80
60ppm	20	50	120
90ppm	50	90	160





Graphical representation of the *Moringa oleifera* (Lam.) seeds against *Anopheles stephensi* (L.)

Experiment with the *Moringa oleifera* (Lam.) incense sticks



Knock down time (minutes)			
	Leaves	Flowers	Seeds
Sample 1	28	18	14
Sample 2	16	14	8
Sample 3	14	10	4

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Parasite control

An another aspect of malaria control

We have explored the antmalarial properties of some easily available plants with the help of *Plasmodium yoelii* infected Swiss Albino mice.

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Experimental

- Plant Extract preparation
- Dose preparation
- Passaging of mice with plasmodium
- Dosing of extracts
- Monitoring of mice
- Blood slid preparation
- Present suppuration study

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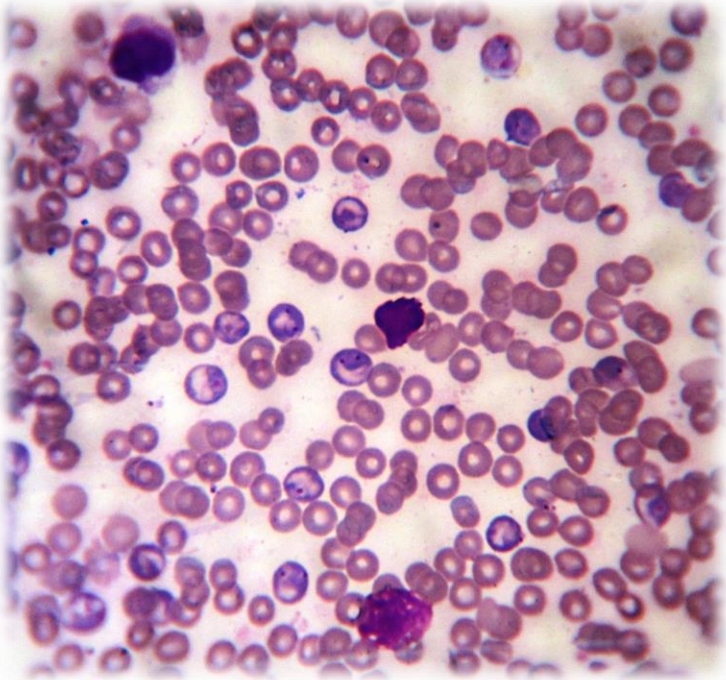

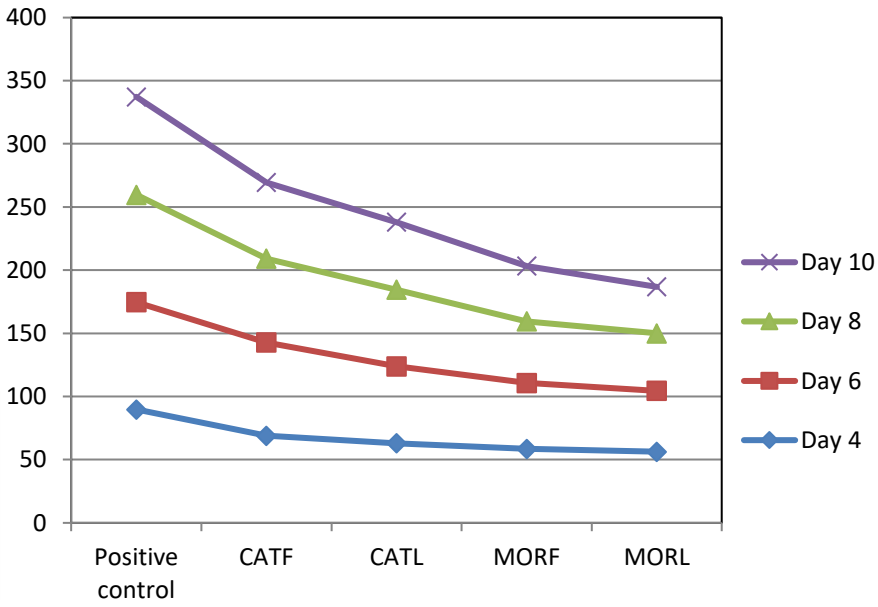

➢ *Catharanthus rouses* flower was found most affective with highest present suppuration followed by *Catharanthus rouses* leaf.

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Thanks

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