

6. Vector Biology & Control

6.01 Can high ambient temperature modulate the gut microflora and vectoral competence of mosquito?

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Vectoral competence of mosquitoes encompasses, susceptibility to infection and transmission efficiency. It is influenced by both extrinsic and intrinsic factors. External factors such as, higher ambient temperature, is known to influence arbovirus susceptibility of mosquitoes due to reduction in extrinsic incubation period of virus. Elevated temperatures can also modulate the gut environment of mosquitoes, by altering the expression of genes involved in virus/pathogen - host interaction as well as the gut bacterial consortium. Mosquitoes, like many other insects harbour a large number of bacteria in their mid guts. Reports on mosquitoes so far, have mainly focussed on the impact of these gut microbiota in parasite transmission. There are however, limited reports on mosquito-microbe-virus relationship and influence of temperature if any, on this relationship. Our current study focusses on this relationship using *Aedes aegypti* mosquito and *Chikungunya* virus as a model system. Our findings showed that, *Serratia*, a Gram negative bacterium as a common inhabitant of midgut of *Aedes aegypti* larvae, isolated from environmental niches and laboratory maintained colonies. Interestingly, this larval midgut inhabitant survived during metamorphosis and could be detected in the midgut of imagoes. When this bacterium was fed to adult females of *Aedes* along with virus and bloodmeal, there was a significant enhancement in susceptibility of these females to *Chikungunya* virus. This possibly can be attributed to the interaction of one of bacterial polypeptide (40KDa), identified to be periplasmic membrane protein with midgut brush border membrane fraction of *Aedes aegypti*. Further this bacterium also survived and the expression of its 40KDa protein was enhanced when larvae were exposed to higher temperatures to mimic external environmental conditions. The selective retention of such a microbe, and its influence on viral susceptibility of the vector is an important factor that could be considered for intervention of virus / parasite transmission cycle. Such an association of *Serratia* with *Aedes aegypti* could be explored for introducing desired genes, which could inhibit host-virus interaction in the midgut. Our results thus suggest a novel role of gut inhabitant in enhancing the vectoral competence of mosquito.

6. Vector Biology & Control

6.02 Emerging trends in Pest Control: Role of *Bacillus thuringiensis*

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Bacillus thuringiensis(Bt) is a ubiquitous Gram positive, sporulating bacterium that forms parasporal crystal inclusions normally during stationary phase of its growth. It is well documented that the crystal protein or delta endotoxins contained in the crystals are toxic to insects belonging to the orders Lepidoptera, Diptera and Coleoptera. In our laboratory, extensive work has been carried out to characterize a large number of Bt isolates obtained from different geographical locations in India. The results revealed wide variations in the spectrum of activity against lepidopteran and dipteran insect pests due to the presence of cry genes in different permutations and combinations. The efficacy of a few *Bt* strains far surpass the activity spectrum reported by various workers in the world.

Bt formulations when applied in the field to combat mosquito menace are known to lose their efficacy due to inactivation by UV rays in the sunlight. However in recent times a few groups in the world have attempted to develop transgenic *Deinococcus radiodurans* (Dr) containing cry toxin genes from *Bti*. Dr a gram positive bacterium has a complex cell envelope similar to that of gram negative organisms and is able to survive exposure to 15000 Gy γ -radiation. In terms of DNA damage 6000Gy gamma radiation is reported to induce approximately 200 double strand breaks, over 3000 single strand breaks, and greater than 1000 sites of base damage per Dr genome. Yet the organism is able to repair the damaged DNA precisely much to the astonishment of biotechnologists. Therefore it was thought fit to use this organism for cloning the cry toxin genes for expression of cry toxin proteins for field application in the mosquito abatement program.

Bti isolates were obtained from the culture collections of our department and from the most efficient strain (PBT-602) active against *Aedes sp*, *Anopheles sp* & *Culex sp*, the cry4B gene was PCR amplified using appropriate primers and cloned in a shuttle vector pRAD1. On transformation

6. Vector Biology & Control

Contd.

the transgenic *Dr* containing the cry toxin gene insert was able to code for 130KDa protein. However, when tested in-vitro for biological activity against *C. quinquefasciatus*, *Ae. aegypti* and *An. stephensi*, the transgenic *Dr* failed to show larvicidal activity, raising the possibility of failure due to the 3D folding pattern of 130KDa protein, or resistance of transgenic *Dr* cell walls for digestion by mosquito gut photolytic enzymes or reduction in the virulence of cry proteins produced. The results of our work to delineate the exact possible mechanism underlying non toxicity of transgenic *Dr* against mosquitoes will be discussed.

6. Vector Biology & Control

6.03 Successful malaria vector control using indoor residual spraying in Southern Africa

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Malaria remains a major public health problem in South and southern Africa but the statistics prior to the advent of indoor residual spraying (IRS) paints a grim picture. South Africa lies on the edge of the malaria distribution on the continent but dramatic decreases in malaria morbidity and mortality have been achieved through the use of effective insecticides as part of a structured malaria control strategy. In 2000, the entire southern African sub-region experienced a severe epidemic which was exacerbated by drug and insecticide resistance. In South Africa, the problem was resolved through the re-introduction of DDT as well as through the introduction of artemisinin-based combination therapy (ACT). Further reductions in malaria morbidity and mortality were achieved through collaboration with neighbouring countries with regards to strengthening IRS capacity in southern Mozambique and strengthening control efforts in Swaziland. After determining the insecticide resistance profile of vector mosquitoes in Mozambique, effective IRS was implemented using a carbamate insecticide. Reductions in morbidity were immediately apparent and influenced the malaria transmission dynamics in the neighbouring countries. In Mozambique, ACT was introduced six years after indoor residual activities began. In South Africa, low levels of DDT resistance was found in localised populations in KwaZulu-Natal province. This was eliminated through the use of a pyrethroid insecticide. Carbamates were introduced in this province to counteract the potential of DDT and pyrethroid resistance. It has been decided that carbamates will be one of the insecticides that would be used in a rotational method of spraying in KwaZulu-Natal. This method of spraying will minimise the development of insecticide resistance and prolong the life of available insecticides being used for malaria control.

6. Vector Biology & Control

6.04 Update on the main malaria vectors in Asia: Implications on vector control

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In Asia, the main malaria vectors belong to complexes in which species are morphologically indistinguishable. Recent advances in molecular systematics have allowed a clarification of phylogenetic relationships within species complex and between main malaria complexes of *Anopheles* on the Asian Continent. This is of primordial importance for applying appropriate vector control programs. The work presented here illustrates the importance of precisely identifying the species, assessing relationships among members of complex, and testing phylogenetic hypotheses involving closely related *Anopheles* species including main malaria vectors.

The *An. minimus* Group is widely distributed on the Oriental region and is composed of 13 species among which main malaria vectors are in SE Asia. In spite of cytogenetic and morphological characters showing a close relationship with the *funestus* Group distributed in Africa, these two Groups (*minimus* and *funestus*) are considered distinct based only on their geographical separation. Therefore, based on molecular and morphological characters and a complete phylogenetic work, a new systematic scheme is presented which reflects the evolutionary relationships within and between both Groups.

The *An. sundaicus* Complex is distributed along the coast of Asia and is known as one of the main malaria vectors in southern Asia. Recent molecular works have allowed the recognition of at least three species for which we present a phylogeographic evolutionary scenario and the malaria risk linked to specific human activities.

Other complexes are discussed such as the *dirus*, *fluviatilis*, *culicifacies*, and *maculatus* in relation to systematics and malaria transmission.

6. Vector Biology & Control

6.05 Impact of policies against indoor residual spraying on malaria control in the America

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Malaria endemic countries of the America underwent three phases of malaria and malaria control activities during the last century. The first phase was a period of disease control that was largely confined to larger and wealthier urban areas. Malaria is primarily a disease of rural populations. Thus, the disease was relatively uncontrolled through control measures applied in urban areas. This first phase of malaria control lasted until the mid-1940s. The second phase was a period of strict control in both urban and rural areas and it corresponds to the time from 1945 to 1979. During this second phase malaria was reduced to extremely low levels throughout endemic areas of Central and South America. The second phase of control ended when the World Health Organization changed its strategy to deemphasize indoor spraying of residual insecticides in houses. This occurred in 1979 and the WHO continually reinforced its changed strategy in subsequent years. After 1979, with de-emphasis of house spray programs, malaria started to reemerge as a major health burden in endemic countries. This presentation will dwell on quantitative associations of increasing disease and changes in WHO strategies and World Health Assembly resolutions. Specific quantitative models of associations will be presented for selected countries of South America.

6. Vector Biology & Control

6.06 Some biological features of *Anopheles (Anopheles) peditaeniatus* (Diptera: *Culicidae*) in endemic malaria foci, Southeastern Iran

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The previous intensive studies coinciding long malaria campaign in Iran, three species of the Hyrcanus group comprises *Anopheles (Anopheles) hyrcanus pseudopictus*, *An. (Ano.) hyrcanus hyrcanus* and *An. (Ano.) hyrcanus nigerrimus* has been morphologically described and documented by various authors. Recently a new species of the Hyrcanus group, *An. (Ano.) peditaeniatus* is identified by Azari-Hamidian, S. (in press). The adult mosquito samples were captured in moderate density using human and animal night bite collection methods at Ghasserghand area, an endemic malaria foci in Sistan & Baluchistan province, southeastern Iran. *An. (Ano.) peditaeniatus* is encountered as a new species for Iranian mosquito fauna. The larvae of this species were found in shaded part of rice-lands. Biting rhythms of *An. (Ano.) peditaeniatus* occurred in midnight (24.00-01.00) and the females collected both on human and cow baits with density 1 and 0.5 per animal and man per night respectively. Majority (50%-80%) of *An. (Ano.) peditaeniatus* was caught in the first half of night during July. The pyrethrum spray sheet collection as well as pit shelter collection methods did not reveal the presence of this species at indoor and outdoor shelters respectively. The accompany species in latter area includes *An.culicifacies*, *An.fluviatilis*, *An.stephensi*, *An.pulcherrimus*, *An.sergenti* and *An.turkhudi*. The ratio of species composition in *An. (Ano.) peditaeniatus* compared with other *Anopheles* species was 4.2% and 30.1% respectively at deltamethrin treated and untreated villages in Ghasserghand County.

6. Vector Biology & Control

6.07 A simple expert system for rapid identification of mosquito species

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Classification and identification of species of mosquitoes in control operation of mosquito-borne diseases is of paramount importance. Pictorial keys of mosquito species, which are currently used for identification of new mosquito species are often time consuming, error-prone and difficult to be followed by a non-taxonomist. Artificial Intelligence programs that achieve expert-level competence in solving problems in task areas by bringing to bear a body of knowledge about specific tasks are called knowledge-based or expert systems. Expert systems provide pre-selected rules for decision-making within specialized domains of knowledge. Here, we describe the use of expert system utilizing ID3 algorithm for the identification of female *Anopheles* and *Culex* mosquito species. The rule-based system is user friendly, menu driven and even a novice can make use of it in identification of unknown mosquito species with little practice. Its application can be extended to new areas of interest in taxonomic problems related to identification of other vector species.

6. Vector Biology & Control

6.08 Quality control of mosquito insecticide treated net as a preventive measure

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Nigeria have adopted the quality control of mosquito Insecticide Treated Net as a measure to prevent the incidence of malaria in the country. Since year 2002, The National Agency for Food and Drugs Administration and Control (NAFDAC) has incorporated into their program, the monitoring and regulation of the manufacturing, importation and sales of Insecticide Treated Net. This monitoring and regulation was adopted after various survey and research of samples sold in the market. It was found that some of the Insecticide Treated Net sold in the market has no insecticide effect on the mosquitoes. Some bed nets are substandard and are not treated with the insecticide. Some of them do not last long, some times it develop holes which allows malaria vector to penetrate the net. The World Health Organization Pesticides Evaluation Scheme (WHOPES) has approved six net insecticide products, which has been endorsed for use by Federal Ministry of Health (FMOH) in Nigeria. All approved insecticides have been registered with the Government Regulatory Authority. The regulatory agencies responsible are the National Agency for Food and Drugs Administration and Control (NAFDAC) and Standard Organization of Nigeria (SON). The control and regulation of insecticide treated Net has been supported by roll back malaria partners like USAIDS, UNICEF, WHO, DFID and the state malaria program in the country. The campaign against substandard Insecticide Treated Net has made the roll back malaria initiative more effective. Non-governmental organizations (NGOs) and Community Based Organizations (CBOs) has focussed their programs more in promoting of the standard Insecticide Treated Net as a way to reduce the incidence of malaria in urban and rural area of the country. The promotion has been adopted through education and communication strategy. Through education, the proper use of Insecticide Treated Net has reduced infant mortality rate and complications in pregnant women.

6. Vector Biology & Control

6.09 Industrial malaria control : Results of a 15-year study on malaria control at five major industrial complexes of India

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Most of the industrial complexes are located in areas with moderate to high risk of malaria. Malaria control in industrial areas is problematic not only because of poor conduct of antimalaria operation but also due to imported ones of migratory population contacts infection in endemic areas and brings into industrial areas. Development of insecticide resistance in vector population, harmful effects on beneficial fauna and concern about possible environmental pollution have created serious impediments in the fight against malaria using residual insecticides. A bio-environmental strategy on malaria control was successfully demonstrated in some major industries in India viz., BHEL Hardwar, IDPL Rishikesh, IOC Mathura, NTPC Shaktinagar District Sonbhadra and NTPC, Rihandnagar District Sonbhadra. The strategy was aimed to utilize the existing infrastructure of the industries by involving the civil maintenance, water supply and sewage, medical and publicity departments as well as other government organizations and local community in vector control programme. The bio-environmental control of malaria strategy was found feasible, appropriate, sustainable in long run and made economic sense. Moreover, a major reduction was also resulted in insecticide pollution as a result of shifting to the bio-environmental control strategy for malaria control.

6. Vector Biology & Control

6.10 Design, identify safe and effective target specific vector countermeasures by 3D pharmacophore modelling

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We developed an *in silico* pharmacophore model for repellent activity from a set of known insect repellents that include N,N-diethyl-m-toluamide (DEET) and its analogs and performed similarity analysis of stereoelectronic properties of the compounds with natural insect juvenile hormone (JH) and a synthetic insect juvenile hormone mimic (JH-mimic) using Catalyst 3D-(QSAR) and AM1 quantum chemical methods. The experimental repellent activity of the compounds was taken from an earlier published study. The pharmacophore consists of two aliphatic and one aromatic hydrophobic sites, and a hydrogen-bond acceptor site and shows an excellent correlation ($R=0.9$) between experimental and predicted repellent activity. The model was validated against a variety of insect repellents. The stereoelectronic similarity analysis of the compounds with JH-mimic and JH indicates a consistency with the pharmacophore model. The observed localized negative electrostatic potential region by the amide/ester moiety and an extended weak negative potential region on both sides of it are clear reflections of the above pharmacophore of hydrogen bond acceptor region and hydrophobic regions, respectively. The remarkable similarity of these features with the JH receptor suggests an electrostatic bioisosterism of the amide group of the DEET compounds and JH-mimic and thus, a model for molecular recognition at the JH-receptor. The pharmacophore template was used to search our in-house database to discover new insect repellent candidates. We plan to present the results of the potential repellency activity of the candidate new repellent compounds.

6. Vector Biology & Control

6.11 Role of household insecticides in mosquito control

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Malaria is an economic burden of the century incurring an annual expenditure of 3.2 billion dollars. Significant effort to combat this disease is yet to bring forth lasting solutions. Research funding is more inclined towards the parasite rather than insect vector. Incidentally man should learn to live with both mosquito and parasite by disrupting the weakest link of vector-host-parasite complex. Invariably all epidemics find their host in poor people and malaria is no exception to this rule. Urbanization, new irrigation projects and mass migration of workmen have given vectors a dominant role in the spread of malaria. Bio-smartness of the pest coupled with disruption in the ecosystem by human interaction, has posed a greater challenge to public health pest management. The community is unable to demand for a higher level of service from governmental agencies as this model is built on indirect contribution from taxpayers. In addition, poor participation by the people becomes an impediment to mass driven programmes. Undoubtedly blanket control becomes inevitable in disease hot spots. People are more concerned for their comforts to avoid mosquito bites rather than fear of disease transmission. The relevance and spread of any technology needs a high degree of consumer acceptance. Thanks to the household insecticide industry which has grown to \$6 billion globally playing a pivotal role in controlling mosquitoes. Annually 2.5 million households in India are protected from mosquito bites through use of repellents. The importance of this role is yet to be recognized by health officials, physicians' researchers, administrators and media. We can win a war against mosquitoes by an integrated approach involving public pesticide application as well as in-house use. Household insecticide industry has to focus more on developing affordable products which will have wide spread use at the bottom of the pyramid. Mosquito control devices are based on controlled release technology which offer longer hours of protection in the night with a 100% safety factor. Our industry looks forward to an active co-operation from the government to popularize usage of household insecticide. Public health spending needs to be rationalized by confining its usage to disease hot spots.

6. Vector Biology & Control

6.12 In Indian scenario, the vector control is the most powerful weapon against demon like Malaria

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Malaria is a complex disease, and its distribution and intensity vary place to place. Urban and peri-urban malaria are on increase in many areas of India. The vector control is still one of the primary weapons to control malaria in endemic areas. For this, the continuous spraying of the indoor surfaces of houses with residual insecticides is still most effective measure to kill the adult mosquito. Indoor spraying reduces the longevity of the vector. The space application has proved to be effective and economical, which reduces vector population quickly when used as outdoor space sprays. Man-vector contact can be effectively reduced by the use of repellents, protective clothing, bed nets (preferably ITMN), mosquito coils, mats, liquids and screening of houses, but cost is the issue for all of these. The methods of personal protection are of great value when properly employed. The older methods with some of new larvicides such as temephos, which confer long effect with low toxicity and the use of larvivorous fishes and bio-larvicides have now become promising for mosquito control, however, these are comparatively costly operation because of repetition at frequent intervals. Source reduction techniques are among the classical methods of vector control. Integrated control approach is necessary because there is no single and simple method that would ensure control of transmission. The significant reduction of transmission can be obtained only when proper anti mosquito's measures are introduced. There is strong urge for more research on vector bionomics and behaviour including bioenvironmental methods of vector control. The priorities and approaches of vector control must be based on economy, technical feasibility, functional resources, human resources, community participation and exploring the cost effectiveness of the use of remote sensing for mapping the breeding habitats of mosquitoes and prediction of densities of vector species, specially in remote hilly and tribal areas.

6. Vector Biology & Control

6.13 Sibling species prevalence in Angul district

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Angul district of Orissa has reported high malaria deaths during last five years. The district has eight PHCs having a total population of 11,39,341 (census 2001). It has forest, riverine and plain ecotype and it has also developmental dam project areas as well as mining areas. Out of eight PHCs, three PHCs viz. Bantala, Godibandh and Kaniha, each of which represent separate ecotype were selected for entomological studies. *An. culicifacies*, *An. fluviatilis* and *An. annularis* are found to be the main vectors in Angul district. Out of five sibling species of *An. culicifacies* (A, B, C, D, & E), species B and C and from three sibling species of *An. fluviatilis* (S, T & U) only S and T were collected from the district. *An. culicifacies* B and C and *An. fluviatilis* T were found in all the PHCs. The percentage of *An. culicifacies* C were 80 %, 55% and 70% in Bantala, 85 %, 68%, and 75% in Godibandh and 78 %, 65% and 72% in Kaniha PHC during rainy, winter and summer seasons respectively. Molecular identification of *An. fluviatilis* was also done. In the molecular analysis the D3 region of the ribosomal DNA were analyzed using primers developed by MRC (Singh *et al.* 2004) The result of the molecular study revealed the composition of *An. fluviatilis* S were 78%, 88% and 50% in Bantala PHC and 75%, 98% and 66% in Kaniha PHC during rainy, winter and summer seasons respectively. In Godibandh PHC, all the *An. fluviatilis* collected were found to be only *An. fluviatilis* S. Detection of sporozoite was done in 256 anopheline mosquitoes by dissection and in 327 anophelines by PCR method by (Snounou *et al.*, 1993). Two specimens of *An. culicifacies* and one species of *An. annularis* were found positive for *P. falciparum* sporozoite by PCR method.

6. Vector Biology & Control

6.14 The *minimus* and *fluviatilis* Complexes of *Anopheles*, main malaria vectors in Asia: How close are they?

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The *minimus* and *fluviatilis* Complexes widely distributed in Asia, are composed of major malaria vectors which are closely related. The *minimus* Complex includes two sibling species, A and C, over the Southeast Asian mainland, whereas the three sibling species S, T, and U of the *fluviatilis* Complex, are known to occur more westward from India to the Arabian Peninsula. *Anopheles minimus* species C has always been considered as a poor malaria vector in Southeast Asia compared to species A, whereas *An. fluviatilis* S is a main vector in India. No complete comparative studies have been carried out between these two complexes since the *minimus* and *fluviatilis* Complexes have been independently recognized, based respectively on fixed chromosomal inversions in India and isoenzymes patterns in Vietnam. However, morphological and molecular observations tend to prove the synonymy of *An. minimus* C with *An. fluviatilis* S. This finding improves our knowledge on the bionomic and distribution of main vectors in Asia and raises the issue of the vectorial capacity of each sibling species which has practical implications on malaria epidemiology and vector control in Southeast Asia.

6. Vector Biology & Control

6.15 The validity of estimating *Anopheles* populations by hand-catch method

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The efficiency, advantages and limitations of the hand catch sampling method in monitoring indoor resting anopheline densities were studied in five Thenpennai riverine villages of Tamil Nadu State. Hand catch has yielded 16% of *Anopheles culicifacies* and the corresponding percentages were 8.3 for *An. subpictus* and all anophelines taken together respectively. Hand catch of other anophelines like *An. vagus*, *An. varuna*, *An. annularis* etc., amounted to less than 3% of the total catch. The male to female sex ratio obtained in the hand catch of *An. culicifacies* was 1: 2.5 and likewise, the sex ratio recorded for both *An. subpictus* and all anophelines was 1:3.7.

A significant linear relationship could be shown between relative density obtained by hand catch and absolute density for *An. culicifacies* in each of the village studied. There was significant differences among villages, among seasons and among years in respect to per man-hour indices of *An. culicifacies*, *An. subpictus* and all anophelines. The efficiency of three experienced collectors in collecting the indoor resting anophelines was statistically analyzed and found that the collecting efficiency of three experienced collectors are on par for capturing *An. culicifacies*, *An. subpictus* and all anophelines. The advantages and limitations of the hand catch as a sampling method are also discussed.

6. Vector Biology & Control

6.16 Prospecting for botanical pesticides against mosquitoes

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Development of insecticide resistance in mosquito vectors of malaria and other diseases to commonly used synthetic chemical insecticides, have limited the scope of available vector control options. There are only few alternatives available for future vector control needs. This has necessitated the need for the search and development of alternative methods and new molecules for vector control. Phyto-chemicals obtained from huge diversity of plants species are important source of safe and biodegradable chemicals, which could be screened for their activity against mosquitoes. With this background, the present study on prospecting for botanical pesticides, under the multi-institutional mode was undertaken in collaboration with other laboratories. The project is funded by National Bioresource Development Board, DBT. Over six hundred coded plant extracts obtained from different extraction laboratories have been evaluated for their bioactivity against larvae and adult mosquitoes, particularly *Anopheles stephensi*. Of these, 117 extracts have shown larvicidal action within 24 hours at lethal concentration (LC₅₀) below 250 ppm, and 42 extracts showed larvicidal action at lethal concentration (LC₅₀) below 100 ppm. Twenty-nine extracts have shown activity against adult mosquitoes and five extracts have also shown repellent action against *An. stephensi*. These results will be discussed in the light of their vector control potential.

6. Vector Biology & Control

6.17 *Anopheles fluviatilis* species S-related malaria in hyperendemic districts of Orissa state, India

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A large part of the Orissa state in eastern peninsular India is hilly and forested with meso- to hypermalaria endemicity. Orissa contributes over a third of all *Plasmodium falciparum* cases and half the number of malaria deaths in India. Entomological investigations carried out in four highly malarious districts namely Keonjhar and Sundergarh in north and Koraput and Malkangiri in the southern part of the state revealed prevalence of *An. fluviatilis* and *An. culicifacies*. Both these taxa have been identified as species complexes and their members vary in their biological characteristics and vector potential. Observations on the sibling species composition revealed that species S comprised >90% of the total *An. fluviatilis* population in the hilly forested areas of these districts. *An. fluviatilis* species S preferred to rest indoors predominantly in human dwellings and was highly anthropophilic with anthropophilic index ranging between 95-100%. A good number of species S were found positive for sporozoites confirming its vectorial efficiency. Among the members of *An. culicifacies* complex, species B and C were prevalent in all the ecotypes and were zoophilic. These observations reveal that *An. fluviatilis* species S is the principal malaria vector in the above mentioned districts. Therefore, keeping in view the biological characteristics of species S, suitable vector control measures should be adopted.

6. Vector Biology & Control

6.18 Life table analysis of *Anopheles* malaria vectors: Generational mortality as tool in mosquito vector abundance and control studies

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Vector control will for sometime remain a primary weapon in the war against vector borne diseases. Malaria is of paramount importance in this with its associated high morbidity and mortality especially in sub-Sahara Africa. This study on generational mortality associated factors in *Anopheles* mosquitoes life table analysis was designed to investigate the fecundity, levels of mortality and mortality associated factors at the aquatic stages of anopheline malaria vectors. Mortality associated factors were investigated at the eggs, 1st and 2nd instars larva, 3rd and 4th instars larva and pupa stages of two anopheline spp: *Anopheles psuedopennis* and *Anopheles gambiae* life cycles in screen cages. Adult male and female mosquitoes were membrane filter fed and algae in culture medium formed the bulk of food substances for the larva stage. Environmental temperature of culture media, pH and some associated physiochemical factors were also determined. Our results showed a significant mortality rates at the various aquatic stages. Infertility, cannibalism and environmental factors were the major factors responsible for mortality at the eggs, larva and pupa stages respectively. The aquatic stages of *Anopheles* mosquito mortality factor K and the mortality factors at the various stages investigated K1, K2, K3 and K4 are discussed. Our recommendations include further studies on the possible genetic modification of predacious. *An. psuedopennis* larva and/or its modification for the production of sterile/infertile eggs as possible alternatives in the reduction and control of anopheline malaria burden.

6. Vector Biology & Control

6.19 Behaviour and population dynamics of *Anopheles gambiae* s.l. in a malaria endemic area in southwestern Nigeria

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Anopheles gambiae Giles and *Anopheles arabiensis* Patton are good vector of malaria parasites and known to coexist in most part of Africa. Here we report the population dynamics and their behaviour in a malaria endemic area where little information exist. Mosquitoes were sampled in four villages in a forest area in southwestern Nigeria. Sampling was done monthly indoor and outdoor using standard mosquito collection techniques from January to December, 2004. Specimens were preserved on desiccated silica for both morphological and PCR identification. Of the 920 *Anopheles gambiae* group collected, 490 (53.3 %) were *An. gambiae* Giles while 430 (46.7%) were *An. arabiensis* Patton. The outdoor collections showed 60.2% of the overall collection to be *An. arabiensis* while daytime indoor resting collections were predominantly (>55%) *An. gambiae* s.s. The overall man-biting rate of *An. gambiae* s.s. was 17.5 bites/man/night as against 6.7 bites/man/night for *An. arabiensis*. *An. gambiae* also accounted for >67% of the wet season collections while *An. arabiensis* was predominant in the dry season (P<0.01). The differences in the density and behaviour of these species highlight the need for an integrated approach to malaria vector control where both species coexist.

6. Vector Biology & Control

6.20 Impact of urbanisation on the bionomics and distribution of malaria vectors around Lagos, south-western Nigeria

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A longitudinal study was carried out in order to evaluate the impact of climatic change and urbanization within the past three decades on the bionomics and distribution of malaria vectors in Lagos. The patterns of annual rainfall as well as average daily temperature have not changed drastically since 1960 when detailed base line entomological surveys were carried out. However, the increase in human population from 1.2 to 10 million has resulted in both expansions of land and tremendous ecological and environmental change. Malaria vectors densities as well as species composition have changed drastically. Preferred larval habitat of malaria vectors has shifted to water reservoirs. The breeding of this vector around human dwelling indicates an increase in man-vector contact creating a high level of risk to this crowded urban population.

6. Vector Biology & Control

6.21 Understanding bionomics of main malaria vectors in irrigated areas of southern Baluchistan, for implementation of impregnated bednet in Iran

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Distribution of insecticide impregnated bed nets is one of the national strategy for malaria vector control in Iran. Prior to any intervention, a comprehensive study on the composition and ecology of malaria vectors is essential. Following the annual report of malaria cases from different part of Chabahar district, Baluchistan province, an investigation on some ecological aspects of main malaria vectors was carried out during 2004-2005. Mosquitoes were collected biweekly from indoor resting places using the pyrethroid spray catch method and from natural outdoors using aspirators. Overall, *An. culicifacies* Giles s.l. predominated (52.6%), followed by *An. stephensi* Liston s.l. (39.4%), *An. pulcherrimus* Theobald (2.0%), *An. dthali* Patton (1.6%) and *An. fluviatilis* James (0.4%). *An. sergenti* Theobald (4.0%) also found in the collection although this species is not a malaria vector. Most mosquitoes (98.8%) were collected from indoor resting-sites whereas collections from potential resting-sites outdoors accounted for only 1.2% of total anopheline densities. *An. culicifacies* populations reaches its peaks of activity in April-May and September-November, respectively, i.e; two main transmission season. In the recent years high temperatures and low rainfall negatively affected seasonal abundance in the area. There were interesting differences in anopheline fauna among villages, with *An. culicifacies* occurring almost exclusively in the village at the head of the irrigation canal, where banana irrigated fields prevailed. Our observations indicated that, in south Baluchistan, irrigation-related sites support the breeding of anopheline mosquitoes. Malaria vectors mainly found indoor.

6. Vector Biology & Control

6.22 Insecticide resistance in *Anopheles culicifacies* : Options for control and management

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Anopheles culicifacies is the major vector of malaria responsible for transmission of 60-70% of malaria cases in the country annually. The major intervention for the control of this species is indoor habitat sprays with residual insecticides and subsequent to the use of different insecticides. The species developed multiple resistance and is an impediment for effective disease control. The only option left for the control of this species is alternate interventions or management of resistance. In early 1980s, this species was discovered to be a complex and studies have shown existence of differential distribution, vectorial capacity and insecticide susceptibility including behavioral aspects. Based on these aspects a modified strategy was suggested for the control of this species. Use of larvivorous fish and insecticide treated nets has shown promise for disease control. These and other aspects of management of resistance including the use of DDT and possible use of rotation of insecticides will be discussed.

6. Vector Biology & Control

6.23 The chemical actions of DDT and other insecticides recommended for indoor residual spray programs

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Malaria is a re-emergent disease in endemic countries of the America. Disease re-emergence is largely a product of official WHO strategies and World Health Assembly resolutions that encourages countries to abandon DDT and indoor spraying of houses. These detrimental strategies and resolutions evolved partly as a result of failure to understand how DDT functions to control disease opposed to its presumed exclusive role in killing malaria mosquitoes. The chemical actions of DDT and other insecticides will be described. Specific spatial repellent, contact irritant and contact toxicity data will be used to describe the actions of DDT and other insecticides presently recommended for indoor spraying. Data from experimental hut studies will be presented to contrast the actions of two classes of chemicals against New World malaria vectors. We will also present detailed comparisons of different chemical actions of recommended insecticides in laboratory assays and experimental huts with the major vector of the dengue viruses, *Aedes aegypti*. *Aedes aegypti* is currently used as the model for testing experimental compounds as possible replacements for DDT in malaria control programs. This testing program is designed to identify chemicals with similar or better (in descending order of priority) spatial repellent, contact irritant, and contact toxicity actions as exhibited by DDT.

6. Vector Biology & Control

6.24 Some bionomics of sibling species B and E in the *Anopheles culicifacies* species complex in Sri Lanka

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Anopheles culicifacies Giles *sensu lato* (Diptera: Culicidae) is the predominant vector of malaria in Sri Lanka. Until recently, only sibling species B was reported to be present in Sri Lanka. The conundrum was that species B was reportedly a poor vector of malaria in India. This was resolved that what was described as species B in Sri Lanka is really a mixture of two species, viz B and E, that are also apparently present in Rameshwaram island, India. Species B and E share the same polytene chromosome configuration. However, they differ in that the mitotic Y-chromosome is acrocentric in species B and submetacentric in species E. Differences between *An. culicifacies* species B and E can impact on malaria control measures undertaken in the country. Entomological parameters of species B and E, such as fecundity, longevity, susceptibility to infection by human malaria parasites, and insecticide resistance, that influence vector potential and the dynamics of malaria transmission are presented. The fecundity of the two sibling species though slightly higher for species E, was not significantly different in laboratory studies. The longevity of species E was significantly greater than species B. Species B was significantly more susceptible to malathion than species E. Species E can support the extrinsic cycle of *Plasmodium vivax* and *P. falciparum* at least to the stage of oocyst formation in the midgut. The results confirm that species E can be infected with *P. vivax* as reported for Rameshwaram Island, and show for the first time that it can also be infected with *P. falciparum*. The vectorial capacity of species B is low or nil in Sri Lanka, as it is also the case in India.

6. Vector Biology & Control

6.25 Ecology of *Anopheles fluviatilis* James in a malarious area, Bandar Abbas, Hormozgan province, southern Iran

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In Hormozgan province, an estimated 40% of population living mainly in mountainous areas and are exposed to *Anopheles fluviatilis* biting. This species is one of the most efficient vectors of malaria in Iran, especially in mountainous areas of the province. Ecology of this *Anopheles* was studied in the area to understand the vector behavior and effective control measures. Sampling was conducted using hand catch and dipping methods for adult and larvae, respectively. Susceptibility tests were conducted to a discriminative dose of 10 imagicides and 3 larvicides. Irritability test was carried out to 4 pyrethroids as recommended by WHO. Sibling species identification was used based on molecular method.

An. fluviatilis started to appear indoor in late September, reached two peaks in January and May and disappeared in early June. It is highly exophilic and exophagic species. The maximum larval density was in early April. It is susceptible to all of insecticides and larvicides which are being used in vectors control program in the region. This species exhibited different irritability response to pyrethroids; lambda-cyhalothrin, permethrin, cyfluthrin and deltamethrin. Results of PCR revealed only sibling species T occurs in the region. This study showed that *An. fluviatilis* plays an important role in maintenance of malaria in mountainous regions of Bandar Abbas district so that exophilicity habitat as well as longer age is important factor in transmission of malaria in this country. On the basis of entomological activities in future, a careful systematic checking of the irritability level of this species to pyrethroid insecticides is recommended.

6. Vector Biology & Control

6.26 Integrated Vector Management - An Overview

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Malaria, dengue, filariasis, Japanese encephalitis and leishmaniasis (kala azar) are the main vector borne diseases in India. Vector control is a major component in the control strategies. Around the mid-1970s, resurgence of malaria together with development of insecticide resistance in vectors, low human acceptance of insecticide spraying indoors and environmental concerns led to a global rethinking in vector control strategies. In the early 1980s, WHO proposed an Integrated Vector Control (IVC) strategy using the principles of Integrated Pest Management in agriculture. Since then, the IVC strategy has evolved into a Selective Vector Control approach to an Integrated Vector Management (IVM) approach. IVM is a process for managing vector populations in such a way so as to reduce or interrupt transmission of disease. It is based on the premise that effective control requires the collaboration of various public and private agencies and communities. By reducing the selection pressure for resistance, IVM also serves to extend the useful life of insecticides and drugs. Key elements of IVM strategy include advocacy, social mobilization and legislation, collaboration within the health sector and with other sectors, evidence-based decision making, integrated use of various vector control methods, operational and implementation research, capacity building, and monitoring and evaluation for assessment of impact. The IVM approach aims at addressing the deficiencies in vector control and improving the efficacy, cost-effectiveness, ecological soundness and sustainability and intersectoriality. Conceptual framework and related issues will be further discussed.

6. Vector Biology & Control

6.27 Distribution Pattern of Mosquito in Mainpur Block of Raipur District, Chhattisgarh

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Chhattisgarh is the second state with high incidence of malaria cases in the country. It has incidence of both *Plasmodium vivax* and *P. falciparum*. The incidence of *P. falciparum* is very high in tribal Block of Mainpur which is inhabited by Kamar, Bhugia and Gond etc. tribes, and is situated 120 Km South East of Raipur. The study was focussed on distribution pattern of mosquitoes and was carried out during October, 2002 and March, 2004, with an objective of collecting baseline information on malaria vectors prevalent in five selected villages of Mainpur Block viz., Bhatigarh, Gaughat, Hardibhata, Navmura and Phoolghar.

Indoors resting mosquitoes were collected from five selected villages using aspirator tube in the morning (06:00 - 8:00), identified as per standard key and the vector density represented as per man hour. Meteorological data was also recorded from the locality. A total of 10,959 indoor mosquitoes belonging to 7 species were collected by spending 312 man hours with a mean MHD of 35. Of the 7 species recorded, 5 were of *Anopheles*, one each belonging to *Culex* and *Armigeres*. Among *anophelines*, *Anopheles subpictus* (34.7%) was the dominant species, followed by *An. culicifacies* (16.24), *An. hyrcanus* (13.61%), *An. annularis* (13.57%) and *An. barbirostris* (0.74). Although there was insignificant difference in density pattern in five villages, the average density was high in Hardibhata followed by Navmura, Phoolghar, Bhatigarh and Gaughat. Two peaks of density were recorded, one in February-March, and another in July-October. Blood meal analysis of malaria vectors recorded in region evidently suggested that *An. culicifacies* & *An. annularis* were primarily zoophilic.

6. Vector Biology & Control

6.28 Seasonal prevalence of malaria vectors in Sonitpur District, Assam

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A study on seasonality of malaria vectors was carried out in four areas viz., Lamacamp, Hoograjuli, Behali and Pabhoi of Sonitpur district, Assam for a period of one year from Mar., 2002 to Feb., 2003. Monthly collection of adult mosquitoes was made from human dwelling and cattle shed from dusk to dawn (1800-0600 hrs) with the help of CDC miniature light trap. The study period was grouped into four seasons viz., pre monsoon (February - April), monsoon (May - August), post monsoon (September - November) and winter (December - January). Highest collection of all the vector mosquitoes was made from cattle shed in all the seasons except *A. dirus*, which is highly anthropophagic in nature. *A. philippinensis*, *A. annularis*, *A. minimus* and *A. culicifacies* were found prevalent in all the seasons throughout the study period with varying densities. Highest density of malaria vectors were recorded during monsoon season followed by post monsoon, pre monsoon and lowest in winter season. *A. philippinensis* was found to be the most dominant species in all the four study areas throughout the investigation period followed by *A. annularis*, *A. minimus*, and *A. culicifacies*. *A. dirus* was found available only in Lama camp area with a peak in monsoon season and in winter the prevalence of this species was negligible. *A. varuna* could be recorded from Lamacamp and Behali throughout the study period except in monsoon season at lamacamp area. Highest density of *A. varuna* was recorded in pre monsoon at Lama camp and post monsoon season at Behali areas. Density of *A. fluviatilis* started building up from post monsoon onward and continued up to pre monsoon season. This species could not be detected in monsoon months in all the four study areas. Information about the seasonal prevalence of malaria vectors in this region may pave way for the entomologist to design a suitable control strategy for management of vector mosquitoes.

6. Vector Biology & Control

6.29 Bionomic of malaria vectors in an endemic area, Kahnouj District, Kermaan, South of Iran

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Kahnouj is an exceptional malarious region where five malaria vectors are present. The most important vectors with respect to transmission of malaria actively are *Anopheles fluviatilis*, *An. stephensi*, *An. culicifacies*, *An. superpictus* and *An. dthali*. The district is distinguishably divided to two areas. Hilly area where *An. fluviatilis* is highly dominated species (92%) and flat area where *An. stephensi* shows majority population (95%). This ratio may be slightly changed every year based on rainfall and other physical aspect. However, in hilly area, *An. fluviatilis* an exophil species showed activity during whole year.

In flat area, endophily behavior had observed among *Anopheles* vectors particularly within *An. stephensi* population with two peaks of activities. Only *An. stephensi* shows resistance to insecticide and no resistances have been seen among rest of vectors.

Generally, HBI for females of *An. fluviatilis* was found 2.82%, *An. stephensi* 0.5%, *An. superpictus* 0.9%, and no human blood was observed positive in *An. culicifacies* and *An. dthali*. Residual adulticides was covered approximately 30% of places in whole district.

Malaria incidence with API =3.86 to 3.66 mostly has occurred in hilly area where *An. fluviatilis* was highly active. Thus, this species is more accountable for transmission of malaria in Kahnouj.

In conclusion, control of vectors such as *An. fluviatilis* which are extremely wild and using microclimate needs to emphasise on larviciding as well as reduction of vector contact with human like impregnated bednet particularly when the community participation is low and just allow to spray 30% of indoor places. Thus, control of malaria and its vectors in Kahnouj is so complicated and need long term planning.

6. Vector Biology & Control

6.30 Insecticide resistance in mosquito vectors of diseases and its management

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To monitor the level of resistance in vectors of malaria, dengue and filariasis, WHO adult susceptibility tests were performed in south and central Gujarat districts using wild caught as well as laboratory reared F1 generation females. In Surat City, where *An. culicifacies* has developed triple resistance to DDT, HCH and malathion, the tests showed reduced mortality of *An. stephensi* against deltamethrin, cyfluthrin and lambda-cyhalothrin (87.7-94.6%), *Ae. aegypti* against deltamethrin and lambda-cyhalothrin (95-97%) and *Cx. quinquefasciatus* against cyfluthrin and lambda-cyhalothrin (80-86.7%). *Cx. quinquefasciatus* also showed resistance to deltamethrin (mortality 57.5%). The mean knockdown time of *An. stephensi* (KD_{50}) against the three pyrethroids tested ranged from 10.8-13.4 min., of *Ae. aegypti* from 8.4-14.8 min. and of *Cx. quinquefasciatus* from 18.6-40.9 min. When compared with the results of similar tests on mosquitoes collected where pyrethroids have not been used in the programme, the KD_{50} values varied significantly for *An. stephensi* (KD_{50} 3.9-7.8 min.; $p < 0.01$) and *Cx. quinquefasciatus* (KD_{50} 7.4-13.6 min.; $p < 0.02$). In central Gujarat, *An. culicifacies* showed resistance/tolerance to deltamethrin (mortality 65-83.3%), lambda-cyhalothrin (96.7%) and alphacypermethrin (98.3%). Prospects for resistance management, priorities for detection and surveillance and adoption of a stratified approach for the selection of appropriate and effective insecticide(s) will be discussed.

6. Vector Biology & Control

6.31 Bio-efficacy of Olyset® Net against malaria vectors in Central India

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Insecticide treated nets (ITN) are widely used as a personal protection measure in vector control for their effectiveness in controlling vector borne diseases particularly malaria. Field trials with various insecticides such as deltamethrin, cyfluthrin, permethrin, lambda-cyhalothrin, etc. have demonstrated not only cost-effective control of malaria but also reduced insecticide contamination in aquatic and terrestrial environment. However, low treatment rates, washing, erratic dose of the insecticide resulted in diluted efficacy of the nets. During recent years long-lasting insecticide treated nets (LLIN) have been developed to overcome these problems.

The efficacy of Olyset® Nets (LLIN) were tested in several countries. Results of these studies revealed that these nets have demonstrated cost-effective control of malaria vectors. In view of this it was considered desirable to investigate the bio-efficacy of Olyset® Nets against vector species *An. culicifacies* in Madhya Pradesh.

Laboratory bioassays were performed from May to October, 2004 on field collected *Anopheles culicifacies* in the laboratory of Malaria Research Centre FS Jabalpur following WHO standard bioassay procedure to determine the impact of repeated washing with detergent on the persistence of insecticide. Impregnated mosquito treated nets were washed with surf Excel detergent of which one was dried in shadow (net A), another in sunlight (net B). These washed nets were tested for bioassay tests after 24 hours of each wash. Untreated nets (net C) and one treated net (net D) were not washed.

The results of bioassay tests upto 5th wash revealed 100% corrected percent mortality (CPM) of *An. culicifacies* in both the nets. Then after it gradually decreased and reached to 10% in net A (in 32 wash) and 18.3% in net B (in 27 wash). The treated nets without any washing showed 100% mortality upto 14 weeks.

6. Vector Biology & Control

6.32 Candidate antigens for vector directed malaria transmission blocking/Anti-mosquito immunity against *Anopheles culicifacies* sibling species complex

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Anti-arthropod/Transmission blocking vaccines as a part of multistrategical approach may impart a new lease of life to Malaria Eradication Programme. Anti-mosquito antibodies against 'concealed' antigens have already been postulated to be able to induce drastic changes in the reproductive physiology of the mosquitoes as well as hamper parasite development. Rabbits were immunized with protein extracts of midgut and salivary glands of different members of *Anopheles culicifacies* species complex. The maximum antibody titer raised was in the range of 10^4 - 10^6 as determined by ELISA. Significant reduction in parasite infection (26%) was observed in *Anopheles culicifacies* that ingested anti-midgut antibodies along with the *P. vivax*. The mean number of oocysts per infected mosquito were reduced significantly (87%) and the number of sporozoites reduced by 32%. Anti-midgut antibodies were found to be more effective than antibodies raised against salivary glands.

There was significant reduction in fecundity and hatchability whereas no significant difference was noticed in case of mortality and engorgement. Fecundity reduction was maximum (35%) in third week after immunization that coincided with the highest antibody titer. Eight immunogenic polypeptides were identified in glucose fed *An. culicifacies* complex, whereas twelve immunogenic polypeptides were observed in midguts of blood fed *An. culicifacies* complex. Four immunogenic polypeptides (105, 80, 70 & 43 kDa) were found to be present exclusively in the midgut of blood fed *An. culicifacies* species complex. Similarly, eight immunogenic polypeptides were resolved in the salivary glands of *An. culicifacies* sibling species complex. In addition, cross reactivity with other mosquito species vis-à-vis with other tissues has also been examined. Identifying these candidate antigens/effector molecules that may confer refractoriness to malaria parasite in *Anopheline* vectors.

6. Vector Biology & Control

6.33 Larvicidal activity of plant extracts against *Aedes (S) albopictus* and *Culex quinquefasciatus*

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Methanol and ethanol extract of different parts of five indigenous plants were evaluated as larvicides against *Aedes (S) albopictus* and *Culex quinquefasciatus* mosquitoes under laboratory conditions. Methanol and ethanol extracts of *Aristolochia saccata* (roots) were found to have most promising larvicidal activity against *Aedes (S) albopictus* with LC₅₀ 14.52, 17.30ppm and LC₉₀ 42.68, 58.51ppm respectively followed by ethanol and methanol extract of *Annona squamosa* (leaf) with 20.70, 20.26ppm and 76.73, 86.59ppm respectively. LC₅₀ values of methanol extract of *Gymnopetalum cochinchinensis* (fruit/pericarp and seed), *G. cochinchinensis* (seed), *Caesalpinia* (bark) species and ethanol extract of *Piper* species were obtained at <100ppm while methanol extract of *Piper* species was obtained at >100ppm against *Ae. (S) albopictus* larvae. LC₉₀ values of methanol extract of *G. cochinchinensis* (fruit/pericarp), bark of *Caesalpinia* species and ethanol extract of *Piper* species were obtained at <200ppm but methanol extract of *G. cochinchinensis* (seed) and *Piper* species (stem) gave at >300ppm against *Ae (S) albopictus* larvae. Ethanol extract of leaf of *A. squamosa* was found to have the most promising larvicidal activity against *Cx. quinquefasciatus* larvae with LC₅₀ and LC₉₀ values as 6.96 and 31.80ppm respectively. LC₅₀ values of ethanol and methanol extracts of *Aristolochia saccata* (root), methanol extract of *Caesalpinia* species (bark) and *A. squamosa* (leaf) gave at <50ppm while methanol extract of *Gymnopetalum cochinchinensis* (fruit/pericarp) and methanol and ethanol extract of *Piper* species (stem) showed at <100ppm and methanol extract of *Gymnopetalum cochinchinensis* (fruit/pericarp) gave at <200ppm. Methanol and ethanol extracts of *Aristolochia saccata* (root), methanol extract of *A. squamosa* (leaf) showed LC₉₀ values at <100ppm while methanol extract of *G. cochinchinensis* (fruit/pericarp), methanol and ethanol extract of *Piper* species showed at <200ppm and methanol extract of *Gymnopetalum cochinchinensis* (seed) showed at >300ppm. Further studies on identification of active compounds, toxicity and field trials are needed to recommend these plant extracts for development of eco-friendly chemicals for control of insect vectors.

6. Vector Biology & Control

6.34 Planning of vector control programme in Bangalore City

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Malaria and dengue are most public health problem in urban cities. Bangalore City is no exception of such problem. A longitudinal study has been carried out in this City from 2000 to find out the mosquitogenic situation. Geographical Reconnaissance (GR) of mosquito breeding habitats indicated that cesspools and drains were major contributor for *Culex quinquefasciatus*, wells and over head tanks for *Anopheles stephensi* and containers and cement tanks for *Aedes aegypti*. A change in the species composition has been recorded where 82% were *Culex*, 7% *Anopheles* and 11% *Aedes*. This indicated that there is a threat of malaria and dengue in the city. A planning has been made based on breeding habitats in each house of the respective wards. Accordingly, control of mosquitoes in each habitat can be carried out effectively. A detailed action plan has been formulated which will be discussed.

6. Vector Biology & Control

6.35 Operational use of larvivorous fishes for Vector Borne Disease Control

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Larvivorous fishes such as *Gambusia affinis*, *Poecilia reticulata* and a few indigenous fish species (*Aphanius*, *Oreochromis*, *Fundulus* and Killi fishes) have been successfully field tested and utilized for mosquito control. Certain other fish species and the young ones of food fish also prey upon mosquito larvae. Fishes viz. *Tilapia zillii* (Somalia), *Oreochromis mossambicus* (Pakistan), *Carassius auratus* (Iran), *Aphanius dispar* (Oman, Somalia), *Nothobranchius palmquisti* and *N. guentheri* (Somalia), *G. affinis* (Jordan, Lebanon, Syria, Iran, Iraq, Afghanistan, Sudan) and *Poecilia reticulata* (Pakistan) are in use for vector control. *Gambusia* was introduced in the US (Hawaii), the Philippines, Japan, Spain, Italy, then to Africa. In India, as far back as in 1904, larvivorous fishes were used in Bombay city in the control of malaria vector *Anopheles stephensi*. *P. reticulata* (Guppy), a native of South America, and *G. affinis* (*Gambusia*) of Texas were imported in India in 1908 and 1928, respectively, for the control of malaria. These fishes were used in Bangalore and Calcutta cities and during the construction of Sharda canal in Uttar Pradesh. Since mid-1980s Malaria Research Centre have been surveying larvivorous fish and has demonstrated their use at different places as a part of the integrated disease vector control strategy.

Large-scale operational use of larvivorous fish for vector control remains under exploited. Adequate knowledge on operational aspects is often lacking in the public health staff. As larvivorous fishes constitute one of the components of urban malaria scheme, there is an urgent need to set up a national level Research and Training Facility on the operational aspects. The use of larvivorous fish is a reasonably effective, simple and inexpensive vector control method and there is need to develop and fully utilize such resource.

6. Vector Biology & Control

6.36 Role of *Anopheles fluviatilis* and *An. culicifacies* in transmitting malaria in the endemic region of District Sundargarh, Orissa

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India being a tropical country is rich in biodiversity and thus favours tropical diseases. Malaria is one of the most devastating diseases which take nearly hundreds of lives per year in India alone. In India, Orissa contributes maximum number of malaria cases (22%). Sundargarh district of Orissa has maximum number of cases (15%) and deaths (12%). The district can be divided into forest ecotype with perennial streams and reverine plain, which are mosquitogenic and increases the complexity of endemicity of disease to manifold. Longitudinal transmission dynamic studies reveal that *An. culicifacies* and *An. fluviatilis* were found as major vector. *An. culicifacies* is found in both ecotypes, while *An. fluviatilis* is restricted to forest only. The prevalent *Plasmodia* species of forest area are: *Plasmodium falciparum* (85%), *P. vivax* (14%) and *P. malariae* (1%); while 75%, 25% and 0% in plain respectively. Mosquitoes were collected during the period 2002-04, from both the ecotype using night bait collection (NBC) and indoor resting collection (IRC) and analyzed for sporozoite positivity using ELISA technique. The number of *An. fluviatilis* collected in NBC are more than *An. culicifacies* and reverse was true for *An. culicifacies*, which may be due to difference in their anthropophagic index. *An. fluviatilis* showed higher sporozoite rate (SR) than *An. culicifacies* throughout the year and in high and moderate transmission seasons. This may be due to difference in their host preference and life span. As *An. fluviatilis* also has higher mosquito landing rate than *An. culicifacies*, the EIR increased manifold and worsens the situation. The EIR of *An. fluviatilis* and *An. culicifacies* in high transmission season is 0.458 and 0.031; moderate 0.204 & 0.009 and in low 0 & 0.008 in the forest ecotype; and 0.005, 0.015 & 0.003 in plain ecotype. Thus, it may be concluded from this study that *An. fluviatilis* is the main vector transmitting malaria in the forest ecotype.

6. Vector Biology & Control

6.37 Effect of *Azadirachta indica* for the control of malarial vector *Anopheles stephensi* (Diptera: *Anophelinae*)

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Investigations have been made to evaluate the larvicidal and pupicidal effect of neem (*Azadirachta indica*) on the malarial vector *Anopheles stephensi*. Methanolic extract of neem seed kernel (NSKE) and leaf extract of neem (NLE) showed higher larvicidal activity on the fourth instar larvae and also having pupicidal mortality higher in neem (NSKE, NLE). The LC50 and LC90 values of NSKE and NLE were observed in the two experiments respectively. This may be due to the toxic impact of larvicidal and pupicidal activity greater than other plant extract. After the toxicity test of NSKE, the larval & pupal duration were extended due to emergency of adult always for 4 to 6 days. This is mainly due to growth regulatory effect of neem active compound, the *Azadirachtin* on the moulting hormone and further arresting moulting process of mosquitoes for NSKE treated insects also laid significantly.

6. Vector Biology & Control

6.38 Engineering methods for malaria control

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It is widely recognized that poor engineering methods increase the mosquito-generating potential of the area. The developmental activities in an area normally result in permanent changes in the biotype of the locality as well as changes in the local malaria endemicity. The National Malaria Eradication Programme tried to achieve its objective by using chemical control as the dominating strategy. Due to rapid development of resistance by mosquitoes to the widely used insecticides, the increasing cost of developing new chemicals, the logistical constraints involved in the implementation of residual-spraying programs and the environmental concerns linked to the use of persistent organic pollutants there was a renewed interest in environmental-management-based approaches for the control of malaria vectors. One among them is the use of engineering methods. The objective of my presentation is to focus on various potential areas of the application of these methods such as site selection for human settlements, development of prudent water supply system, proper sanitary disposal of waste water, water resources development projects and other constructions in urban areas which will play a major role in malaria control operations.

6. Vector Biology & Control

6.39 Sporogonic development of plasmodial infection in *An.fluviatilis* species T and U

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Anopheles fluviatilis is considered as a vector of malaria in India, Pakistan and Nepal, although it is found widely in eastern Asia and in parts of western Asia. In India, it is a vector of primary importance in hilly and foothill regions and ranks second in contributing to total malaria cases. Susceptibility to plasmodial infection is one of the major factors for determining any anopheline to be malaria vector. Among many determinants for any species to be a vector, the most important is its inherent susceptibility to plasmodial sporogony. We determine a comparative feeding experiment of *An. fluviatilis* species T and U against rodent plasmodial infection *Plasmodium vinckei petteri* and human malarial parasite *Plasmodium falciparum* under laboratory condition. Susceptibility of the mosquitoes was compared using 3 parameters i.e percent gut positivity with oocysts, geometric mean (GM) among the infected mosquitoes and percent gland positivity with sporozoites. In feeding experiments with *P. vinckei*, better feeding was observed in species U (60%) as compared to T (53%). Significant difference was observed between species T and U in the oocyst rate ($p < 0.05$). The geometric mean number of oocyst differ statistically between species T and U ($p < 0.05$). The sporozoite per cent varied significantly ($p < 0.001$) between species T and U against *P.vinckei petteri*.

In artificial membrane feeding experiment with *P.falciparum*, the feeding percentage was 54% for species T and 51% for species U. No significant differences were observed for oocyst rate between species T and U. The sporozoite rate showed no significant differences between species T and U against *P. falciparum*.

6. Vector Biology & Control

6.40 Efficacy of different insecticides on malaria vector *Anopheles culicifacies* in Central India

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One of the major causes for the setbacks in the global programmes of malaria eradication is the development of resistance among vector anophelines to the commonly used various insecticides. *An. culicifacies* is the principal vector of malaria in rural areas of Madhya Pradesh and Chhattisgarh state. The vector was highly susceptible to DDT when it was used in the beginning. In subsequent years physiological resistance to DDT was observed, then after HCH and Malathion were used. In recent years some synthetic pyrethroids are also in use in Central India. To observe the efficacy of all these insecticides, we carried out insecticide susceptibility tests and bioassay tests on *An. culicifacies* in the year 2002-03 in different districts viz. Sagar, Damoh, Betul, Chhindwara, Khandwa, Jabalpur, Mandla, Dindori and Panna in Madhya Pradesh and Raigarh district in Chhattisgarh. The results of susceptibility tests against DDT 4% and Malathion 5% showed 7.3 to 21.1% and 46 to 94% corrected percent mortality respectively. 98.9 to 100% mortality was observed against Deltamethrin 0.05%, Profoxure 0.1% and Bendiocarb 0.1%.

The bioefficacy of Etofenfrox 20% & Alphacypermethrin 5% on *An. culicifacies* was carried out in Jhabua (Madhya Pradesh) and Korea (Chhattisgarh) using cone bioassay tests on sprayed surfaces. Results revealed 96.3% mortality of *An. culicifacies* (after one week of spray) against Etofenfrox which gradually decreased to 63.8% and 5.3% in 3 weeks and 12 weeks respectively. The mortality in Alphacypermethrin sprayed area was 100% after 2 weeks of spray. However, the mortality in DDT sprayed area by cone bioassay was 25% and 13% after 1 week and 3 weeks of spray respectively.

6. Vector Biology & Control

6.41 Effect of formulation on efficacy of *Metarhizium anisopliae* strain against three mosquito genera

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Three isolates of *Metarhizium anisopliae* were grown on Emerson's YpSs media and the efficacy of non-formulated spores was evaluated against three mosquito species. *M. anisopliae* MRCD3 was found to be more effective than the two strains against the 2nd-, 3rd- and 4th-instar larvae of all the three species. The 2nd-instar larvae were more susceptible than the 3rd- and 4th-instar larvae against *M. anisopliae* MRCD3. When the LC₅₀s were compared, *Culex quinquefasciatus* (1.34×10^3 spores/ml) was found to be more susceptible than *Anopheles stephensi* (2.69×10^3 spores/ml) and *Aedes aegypti* (4.66×10^5 spores/ml). To enhance the efficacy and for a wide spectrum activity *M. anisopliae* MRCD3 was formulated with talc, water chestnut and castor seed coat. The dust formulation of talc and granular formulation of water chestnut and castor seed coat was applied on the surface. A 10% (w/w) formulation of spore with talc, water chestnut and castor seed coat was applied at a concentration of 2g/m². Talc formulation was more effective against *An. stephensi* than *Cx. quinquefasciatus* and *Ae. aegypti* and 100% mortality was observed within 48 hour of application. A significant mortality difference (P=0.01, F=7.55, df=2, 9) among the three species was observed against talc formulation and this formulation is specific against *Anopheles* larvae. The granular formulation, water chestnut was more effective against *Ae. aegypti* and *Cx. quinquefasciatus*. It also accelerated the efficacy against *Ae. aegypti* and 100% mortality was observed within 48 hour of exposure to 2g/m². The castor seed coat formulation was less effective than talc and water chestnut formulation. A nearly equal mortality was observed among the three species against castor seed coat formulation and no significant mortality difference was observed among the species (P>0.05, F=0.67, df=2, 9). Results suggest that *M. anisopliae* MRCD3 has potential for use in mosquito control programmes.

6. Vector Biology & Control

6.42 Resting behaviour *An. stephensi* and to assess its amenability to control through IRS in an Arid Zone, District Jodhpur, Rajasthan, India

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Studies on resting behaviour of *An. stephensi* were carried out to assess its amenability to control through indoor residual spray in an Arid zone district Jodhpur, Rajasthan during March-April, 2002. In the present study, resting behaviour of the species during all its movement rhythms covering 24 hrs. period related to i) swarming/mating, ii) pre and post biting rest iii) after feed resting between hopping movements and iv) night and day time resting and finally v) diel activity movements in response to temperature changes, were carried out in both unsprayed and sprayed villages. Analysis revealed that about 95% and 97% of *An. stephensi* preferred to rest on unsprayable surfaces viz. household objects in unsprayed and sprayed villages respectively. The most preferred resting sites in both group of villages under household objects were hanging cloths, utensils and cupboard. There was no significant difference in resting behaviour of the species in both groups of villages ($p>0.05$). Pre and post biting rest period ranged from 5 to 15 minutes and 15 to 25 minutes respectively. After the biting out door, species starts entering the rooms at around 11.30 pm and conceded with fall in ambient temperature indoor below 30°C. Maximum entry ie.56% and 62% of the species entered the rooms during III quarter ie. 1AM to 4AM in unsprayed and sprayed villages respectively. Statistically there was no significant difference in the entry of mosquitoes ($P>0.05$) in both group of villages. Therefore, control of *An. stephensi* in study area requires an integrated control strategy based upon intersectoral, environmental, larviciding with chemical/ biolarvicide and use of larvivorous fish where ever feasible. Such a control strategy offer cost effective and sustainable option than indoor residual spray.

6. Vector Biology & Control

6.43 Evaluation of larvicidal efficacy of *Blumea lacera* against *Anopheles culicifacies*

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Anopheles culicifacies is the major vector of malaria in India and are highly prevalent in Chhattisgarh region. Due to the development of resistance, synthetic pesticides and larvicidal compounds are losing their importance to control the vectors. Attempts were made to find some eco friendly substitute of these hazardous and unbiodegradable synthetic pesticides. Botanists have shown some good results in this regard besides being biodegradable and ecofriendly. *Blumea lacera* is a commonly occurring herb, distributed through out the plains of India and posses; anti-bronchial, antshelmentic and analgesic properties. It is prevalent as weed in and around Raipur. Larvicidal efficacy of the ethanol, acetone and petroleum ether fraction of the leaf, stem and root extracts of *Blumea lacera* was tested against the early 2nd and 4th instars of *Anopheles culicifacies* under laboratory conditions as per WHO (1996) method with slight modification. Replicates of larvae were exposed to serial concentrations of the extract for 72 hrs and the mortality was recorded every 24 hours. Mortality was found to be dose as well as time dependent. The observations indicate that the petroleum ether fraction of leaf extracts was more effective followed by root and stem, both in 2nd and 4th instars of *Anopheles culicifacies*. In ethanol and acetone fraction, root extracts were more effective followed by leaf and stem, both in 2nd and 4th instars of *Anopheles culicifacies*. The efficacy of the petroleum ether extract was more followed by ethanol and acetone extracts. The LC50 of petroleum ether leaf extract of *Blumea lacera* was 62.87 ppm and 81.42 ppm for 2nd and 4th instars larvae respectively after 24 hrs. In addition to its medicinal properties their abundant availability can make it economically applicable for field uses to control mosquito vectors.

6. Vector Biology & Control

6.44 Numerous rearing of *Anopheles stephensi* with human whole blood by artificial feeding

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The purpose of this study was to numerous rearing of *Anopheles stephensi* (Lis.) by artificial feeding with human whole blood in insectary. An artificial membrane, blood-feeding technique was evaluated for *An. stephensi* using insectarium's strain in Iran. Parafilm "M" and human whole blood were satisfactory as feeding membrane and nourishing source, respectively. Comparison of feeding rates and different ages were fluctuated in rearing of 3 generations of *An. stephensi* with human whole blood via membrane Parafilm "M". The maximum feeding rate achieved was 90.9%. *An. stephensi*, a mosquito that normally requires a vertebrate blood meal to produce eggs, has been reared successfully for three generations on this substitute blood meal.

6. Vector Biology & Control

6.45 Insecticidal properties of a plant code MRC Har/04/04

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Studies were carried out to investigate the insecticidal activity of plant code MRC HAR/04/04. Two fractions coded as MRC HAR/04/04/S and MRCHAR/04/04/R have been isolated and tested for adulticidal activity against *Culex quinquefasciatus* by WHO method. Results revealed that fraction code MRC HAR/04/04/S possessed good adulticidal activity against *Culex quinquefasciatus* mosquitoes with its LC₅₀ and LC₉₀ values as 0.05 and 0.097 mg/cm² respectively while fraction code MRC HAR/04/04/R was found inactive. GC-MS analysis of fraction code MRC HAR/04/04/S gave 42 peaks. All peaks were identified using NIST library. 9 Compounds contributed 74% of the total amount.

6. Vector Biology & Control

6.46 Comparative structural analysis of *Anopheles culicifacies* complex eggs by scanning electron microscopy

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An. culicifacies is the most important vector of rural and peri-urban areas, being responsible for 60-70% of the total malaria cases reported in India annually. The discovery of *An. culicifacies* as a species complex added a new dimension to vector control in India and prompted scientists to get a better understanding of biology of different members of this species complex and their relationship to epidemiology of malaria. In present investigations, scanning electron microscopy of eggs of *An. culicifacies* A, B and C was done and the highly magnified view of dorsal surface, lateral surface, ventral surface, micropylar apparatus, anterior end and posterior end were studied to differentiate the three species of *An. culicifacies* complex. The general characters of eggs of *An. culicifacies* appear rather similar under light microscope. However, the measurements of eggs shows that size of *An. culicifacies* C is largest (426.6-470 μ m) and broadest (140-162 μ m), while species A is smallest (322.3-382.5 μ m) and narrowest (110.5-137.1 μ m) and species B is intermediate. The float of species C has maximum number of ridges (20-21), while species A has minimum number of ridges (14-15) and species B has intermediate number of ridges (16-17). Species A, B, and C also significantly differ from each other in structure of tubercle on under float area. The shape and size of tubercle was different in three species studied. The eggs of *An. culicifacies* species A, B, and C could also be distinguished on the basis of micropylar collar and their hexagonal rays. These characters along with different shapes will be discussed to differentiate species A, B and C of *An. culicifacies* complex.

6. Vector Biology & Control

6.47 Biodiversity of mosquitoes from Sangli city of Maharashtra, India

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The Sangli city has climate as moderately hot and dry, with cool weather in the hilly region. It is situated on the bank of river Krishna. The small rivers like Warana, Yerala and Panchaganga joins Krishna in the Sangli region. There are 60 major and minor irrigation projects. Therefore, there are several water bodies in the region. Due to human activities and natural calamities such as excessive rainfall, flood, drought, etc. mosquito borne diseases are increased in this region since last few decades. Hence, biodiversity of mosquitoes have been studied from Sangli city. In all 15 species of mosquitoes have been reported belonging to the genera *Anopheles*, *Culex* and *Aedes*. Their seasonal abundance have been studied.

6. Vector Biology & Control

6.48 Computer based Identification of Indian Anophelines (CIIA)

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The Computer based identification key for Indian Anophelines belonging to subgenera *Anopheles* and *Cellia* has been revised as computer drawn pictures of all the species are incorporated into the main software. As the user proceeds with different menus, morphological features in graphical form are also displayed. The software has been rewritten in Visual Basic 6.0 and main database has been organized in Ms-Access97.

The Knowledge base is based on all possible combinations of the morphological characters. For each subgenus the software is divided into four modules. Module 1 is for beginners. The user can identify a mosquito by looking at it under a microscope / hand lens and entering his observations (through computer) menus. Module 2 is for experienced user who can recall the identification characters just by entering the proper code displayed on the monitor. Characters for quick identification are also highlighted. Module 3 is for distant users in remote areas. The user can fill a specially designed proforma and send the coded string to the computer centre where the software is available. As soon as the string is entered the mosquito is identified. Module 4 is based on nested sequence where using successive characters, the mosquitoes are divided into subgroups. This nested sequence of subgroups leads to the identification of the mosquito.

Besides being fast and easily upgradable the added advantage in this electronic key over the earlier couplet keys is that it can also identify the variant species. Also displayed pictures help a beginner in quick learning of the morphological features of the mosquito.

6. Vector Biology & Control

6.49 Comparative immune response of malaria vector *Anopheles culicifacies* to rodent malaria parasite *Plasmodium vinkei petteri*

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Anopheles culicifacies, the most important vector of rural and peri-urban malaria in India exist as a species complex. Various biological differences including differential vectorial capacity has been reported among the five members (i.e., species A, B, C, D and E) of the species complex. Among all these five sibling species, species A is highly susceptible to both human (*Plasmodium vivax*), and rodent (*P. vinkei petteri* and *P. yoelli yoelii*). *Plasmodium* infection followed by species C and B last few years, tremendous progress has been made in understanding the immune response of various mosquito species against their natural and unnatural parasites. In the present study, immune responses of the species A and B of *An. culicifacies* complex was studied against rodent malaria parasite *P. vinkei petteri*. Three groups of mosquitoes i.e., unfed, fed on normal mice and fed on *P. vinkei petteri* gametocyte positive mice were used for the study. Hemolymph was taken from all the three group of mosquitoes for the qualitative analysis of polypeptides which are first conceivable gene product *Plasmodium* challenge. Both species A and B of *An. culicifacies* complex synthesized a number of polypeptides in the hemolymph in response to feeding of normal or *Plasmodium* infected blood. Almost all the polypeptides were same in both the species but they differ in their time of expression, except one approximately 65kDa polypeptide. This particular 65kDa polypeptide was synthesized in species B after 24 hours of infective feeding but not upon normal feeding. Induction of this polypeptide was not seen in species A neither upon normal nor upon infected feeding. It may be mentioned that phenoloxidase, characterized from *Plasmodium*-refractory strain of *An. culicifacies* has molecular weight of nearly 65kDa and is involved in melanotic encapsulation of malaria parasite, the phenomenon also observed in the strain of species B, used in the study after 16-24 hours of infective feeding but not in species A. The inducible polypeptides are candidates for future studies.

6. Vector Biology & Control

6.50 Oviposition attractant behaviour of *Anopheles stephensi*

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Anopheles stephensi is a primary malaria vector in urban areas of India. It breeds predominantly in freshwater bodies. It has a wide distribution in India as well as in some of the West Asian countries. *Anopheles stephensi* has been shown to be directly responsible for about 40-50% of the annual urban malarial incidences.

Laboratory studies on oviposition behaviour of *Anopheles stephensi* were carried out to investigate the role of semiochemicals for selection of site for oviposition. Egg counts were used to assess oviposition preferences. It has been observed that mosquitoes preferred to oviposit in the water containing the larvae of *Anopheles stephensi* (Larval Conditioned Water) rather than the tap water and water with yeast. We observed that the larval habitats in breeding sites have some activity which attract the gravid females to oviposit. In the laboratory, the egg laying was approximately doubled in Larval Conditioned Water as compared to control. These signals, in conjunction with other cues could be used by the female to assess the suitability of potential larval habitats in order to maximize the fitness of offspring. As an effort towards the identification of larval habitat derived semiochemicals for anophelines, we initiated to demonstrate the effect of breeding site on oviposition choices of *Anopheles stephensi*.

6. Vector Biology & Control

6.51 Mosquito larvicidal properties of *Eucalyptus citriodora* leaf extracts

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Hexane extract obtained from leaf of *Eucalyptus citriodora* was tested against larvae of *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti* to assess its toxicity and growth regulating activity. The results showed better activity of Hexane extract against larvae of *Anopheles stephensi* as compared to the larvae of *Culex quinquefasciatus* and *Aedes aegypti*. A concentration of 120 ppm caused 100% mortality of the IV instars larvae of *Anopheles stephensi* within 72 hours, whereas only 84 and 80% mortalities were obtained against *Culex quinquefasciatus* and *Aedes aegypti*. At lower concentration viz. 10ppm, though there was some mortality in larvae of *Anopheles stephensi*, no mortality was found in *Culex quinquefasciatus* and *Aedes aegypti*. However, some morphological abnormalities were observed in pupae which could not moult normally. These results clearly indicated insect growth inhibition effect of the hexane extract of *Eucalyptus* against mosquito larvae, particularly *Anopheles* species. Thus *Eucalyptus citriodora*, which is commonly found in Northern India, has a great potential for the development of an ecofriendly herbal insecticidal product for control of mosquito breeding.

6. Vector Biology & Control

6.52 Larvicidal activity of crude aqueous extract of *Trianthema portulacastrum* (Family: Aizoaceae)

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Many species of mosquitoes in genus *Anopheles*, *Culex* and *Aedes* are vectors of malaria, filaria and dengue fever, and have been traditionally controlled with synthetic insecticides. This type of control can be hazardous to human, result is insecticide resistance in mosquitoes, damage fauna and flora; contaminate the air, water and soil, and be expensive. In spite of the continuous use of synthetic insecticide, mosquito population have not been reduced significantly, and vector species retain their potential to support the out break and development of vector born diseases in endemic areas. Therefore, it is necessary to evaluate other control methods. Larvicidal effect of crude aqueous extract of the leaf of a medicinally important plant *Trianthema portulacastrum* (Family: Aizoaceae) was tested against *Anopheles culicifacies* species A, *An. culicifacies* C, *Anopheles stephensi*, *Culex quinquefasciatus* and *Aedes aegypti*. III/IV instars larvae were used for bioassays following standard W.H.O method for a range of concentrations (0.0025% to 0.3% water). The calculated LC₅₀ (lethal concentrations for killing 50% of treated larvae) for different species were respectively. *An. culicifacies* A-800ppm, *An. culicifacies* C-850ppm, *An. stephensi* - 750ppm, *Cx. quinquefasciatus*-900ppm and *Ae. aegypti* - 1100ppm.

6. Vector Biology & Control

6.53 GIS for habitat mapping of malaria vector *An. minimus* in forest fringe areas in India

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In India, malaria is transmitted by six major vectors of malaria namely *An. culicifacies*, *An. stephensi*, *An. minimus*, *An. fluviatilis*, *An. dirus* and *An. sundaicus* in the area of their distribution. These vector species differ greatly in their biology, behaviour, breeding and distribution habitats. It is therefore, mandatory to know the distribution of each vector species for effective control of malaria. GIS was applied to map distribution of malaria vector of forest fringe areas namely, *An. minimus* using ecological parameters namely, soil type, altitude, rainfall, temperature and forest. Thematic maps in the scale of 1: 6,000,000, published by National Thematic Mapping Organization (NATMO), Govt. of India, on ecological parameters were digitized. Favourable conditions for different parameters for the species were identified from reported distribution. These conditions were extracted from each thematic map for each species and integrated to predict the distribution of vector species using ESRI Arc View 3.2.

For validation of *An. minimus*, precision field surveys in nine locations of four states were conducted. Interestingly *An. minimus* could be found in all GIS predicted locations. In two districts viz. Banbasa, distt. Nainital, and Dhubri distt., Assam, in the former, the species was reported to have disappeared after 1950s, and in latter, it was not reported in earlier surveys. Amazingly, GIS predicted precisely the location in these districts to conduct entomological surveys and the species could be found there.

The authors intend to emphasize that this unique technique identifies the favourable location for species prevalence and also identifies the areas which do not carry the breeding potential for the species. This approach can help in stratifying the areas into endemic and non-endemic areas of species for building up cost effective and sustainable control strategy. The technique can delineate the areas favourable for any species and is good specially to cover vast and inaccessible areas.

6. Vector Biology & Control

6.54 Effect of leaf extracts of *Leucas aspera* and *Azadirachta indica* individually and synergistically on survival and development of mosquito larvae *Anopheles stephensi* (Diptera: Anophelinae)

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Among the insect group, mosquitoes have been reported to be vectors of many diseases. Mosquitoes are controlled by so many methods. Both adult and larval forms are effectively controlled by chemical methods. Chemical control methods using synthetic insecticides have been favoured so far because of their speedy action and easy application. In recent times, sustained efforts have been made to reduce the population of insect vectors, particularly mosquitoes by using biological means which have advantages over chemical pesticides. Chemicals from plants exhibiting antifeedant, repellent and other properties against insects have been isolated and identified. They help in the development of new effective synthetic insecticides and don't appear to pose hazards of toxicity to humans (or) domestic stock. In the present study, the two plant extracts such as *L. aspera* and *A. indica* were tested individually and synergistically on survival and development of mosquito *Anopheles stephensi*. The LC_{50} and LC_{90} values were found to be high in the case of individual test solution and very low in the case of synergistic mixtures. The larval, pupal and adult mortality were greater in the synergistic mixture treatment. Prolongation of larval and pupal period and reduction of mosquitoes during synergistic mixture was highly effective than the individual effect. Among the treated plant extracts, *A. indica* was more effective when compared to *L. aspera*.

6. Vector Biology & Control

6.55 Toxocological study of *Solanum xanthocarpum* extracts against malaria and dengue vectors

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Plants have provided mankind with lots of valuable compounds and also answers and remedies to lots of medical problems faced by humans. In the quest for answer to the increasing resistance in mosquitoes against available synthetic chemical pesticides, scientists are focussing on plants for the development of new plant based pesticides. Therefore, in this endeavour we have studied the extracts of *Solanum xanthocarpum* against larvae of *Anopheles stephensi* and *Aedes aegypti*. The extraction was carried out in the sequential manner with the solvents of increasing polarity i.e. non-polar to polar. Of the four solvent extracts studied, the petroleum ether extract was found most effective. The bioassay results revealed 100 % mortality against I/II instar larvae of *Anopheles stephensi* and *Aedes aegypti* with Petroleum ether extract at concentrations below 100 ppm, whereas the LC₅₀ values against III and IV instar larvae of *A. stephensi* and *Aedes aegypti* were 82ppm and 126ppm after 72 hours of observation. Further observations did not yield any adult emergence at a dose of 250ppm. These observations indicate growth inhibition activity of petroleum ether extracts of *S. xanthocarpum* against mosquitoes.

6. Vector Biology & Control

6.56 Investigation on insecticidal properties of a plant code MRCHAR 03/05 from Garhwal region of North-West Himalaya

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A new plant code MRCHAR03/05 has been identified from the Garhwal region of North-West Himalaya. Different fractions were isolated from this plant and tested for their insecticidal properties. Studies showed that the fraction code MRCHAR03/05P1 isolated from this plant has excellent adulticidal activity against *An. stephensi*, *An. culicifacies*, *Ae. albopictus* and *Culex quinquefasciatus* using WHO test method by impregnating paper with test compound. This fraction was further tested for different sibling species complexes of *An. culicifacies* and was found effective against species A, B and C. Efforts are being made to isolate most active fraction from the plant code MRCHAR/03/05. Steam distillate fraction MRCHAR03/05S along with other semi purified fraction MRCHAR03/05P1 (solvent extraction methods) were tested for their adulticidal activity against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes. Results revealed that steam distillate fraction showed more adulticidal activity than others. KDT₅₀ value of steam distillate was 15 min and 20 min on 1% impregnated paper for *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes respectively. Steam distillate fraction MRCHAR03/05S also showed larvicidal activity against *Aedes aegypti* with their LC₅₀ and LC₉₀ values of 53 and 91 ppm respectively. GC- MS analysis of fraction MRCHAR03/05S gave 37 peaks. All peaks were identified using NIST library.

6. Vector Biology & Control

6.57 Development of mosquito transgenesis in *Anopheles stephensi* to control malaria

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The burden of malaria cases is rising due to drug resistance parasite and insecticide resistant mosquitoes. Moreover, an effective vaccine has not been developed. An alternative strategy for malaria control, namely the genetic modification of mosquitoes to make them inefficient vectors for parasite will be discussed. The mosquito is a potential weak link in malaria transmission and generating mosquito populations that are refractory to the parasite is a potential means of controlling disease.

Transgenic technology has been developed for the malaria vector *Anopheles stephensi*. Successful integration of exogenous DNA into the germ line of this mosquito has been achieved by class II transposable elements. A number of marker genes including cinnabar+ and fluorescent proteins genes can be used to monitor the insertion of these elements. These tools have proved the ability to engineer a refractory mosquito vector. With the ideal mosquito on the horizon, the most important task is to begin laying foundation for the introduction into the wild.

Different strategies for interfering with the *Plasmodium* development in mosquito together with issues and strategies related to the transfer of laboratory acquired knowledge to the field such as minimizing transgenic fitness load, driving mechanism through population, and how to produce and release population of male only will be discussed. In addition, ethical and political issues for the release of genetically modified organism will also be discussed. However, the reasons to be optimistic to add transgenic mosquitoes to our arsenal in the fight against malaria will also be presented.

6. Vector Biology & Control

6.58 Genetic engineering of cry toxin genes of *Bacillus thuringiensis* effective against mosquito larvae

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Bacillus thuringiensis is a ubiquitous Gram positive, sporulating bacterium that forms parasporal crystal inclusions (δ - endotoxins) which are toxic to insects belonging to order Diptera, Lepidoptera and Coleoptera. With a view to identify and characterize novel/unique cry genes in Bt isolates obtained from soil/sewage samples collected from different geographical locations from India as many as 825 isolates were randomly selected. There existed wide variations in their spectrum of toxicity against III instar larvae of *A. aegypti*, *An. stephensi* and *C. quinquefasciatus*. The isolate PBT-602 that exhibited maximum larvicidal activity was used in our studies for cloning and expression of *cry4b* gene in *E. coli*. The recombinant (PDBT2) was analyzed for presence of insert *cry4B* gene by PCR analysis.

Since Bt is known to be susceptible to UV radiation in sunlight, the field application of Bt in mosquito abatement programme is very much hampered. Hence *Deinococcus radiodurans* was desired to be used because of its resistance to UV radiation and desiccation for cloning *cry4b* gene from Bt (PBT-602) for applicative purpose in the field. Further to sub clone, the *cry4b* gene from PDBT2 *D. radiodurans* shuttle vector pRAD1 was employed and the recombinant (RADN7) was found to produce 130kDa protein when run on SDS page. However, when the recombinant was tested against III instar larvae of *C. quinquefasciatus* no larvicidal activity was observed. The possible mechanism for inaction of *D. radiodurans* recombinant (RADN7) will be discussed.

6. Vector Biology & Control

6.59 Field evaluation of Pyriproxifen (0.5G) against larvae of mosquito vectors in the district Shahjahanpur, Uttar Pradesh, India

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Pyriproxifen (S-71639) is a juvenile hormone mimics (JHM). The mode of action on pest insects is quite different from those of neuro-toxic insecticides and has low toxicity to beneficial insects. This JHM affects the morphogenesis, reproduction and embryogenesis of insects. Two mosquito vector species namely, *Culex quinquefasciatus* and *Anopheles culicifacies* were tested against Pyriproxifen (0.5G) in three dosages of 0.01 ppm, 0.02 ppm and 0.05 ppm in different breeding habitats.

Cx. quinquefasciatus was found to be susceptible species against the compound. In polluted pits, the compound produced 100 percent emergence inhibition (EI) from 2-42 days at the dosages ranging from 0.01 - 0.05 ppm whereas, in polluted pools, stagnant drains and slow moving drains the effective EI was obtained from 2-35, 2-42 and 7-21 days respectively at dosages ranging from 0.01-0.05 ppm. *An. culicifacies* was also found to be susceptible species against the compound. In river bed pools and pits the effective EI was obtained from 2-28 days at dosages ranging from 0.01-0.05 ppm. Control was achieved through a characteristics break down of pupa-adult metamorphosis within the puparium.

6. Vector Biology & Control

6.60 Habitate Preference of *Aedes aegypti* Breeding in Delhi

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Studies were carried out in five areas in Delhi to know the breeding potential of *Aedes aegypti*, the principal vector of dengue/DHF in different wet receptacles and its seasonal prevalence during 2004. Result revealed that Average House Index has been above 3% from July to October 2004 with maximum in September (4.5%) and October (3.5%). It ranged between 2.4 to 4.5%. Average CI was reported between 2.2 to 3.1%. The average Breteau index for all five sentinel sites ranged between 3.2 to 5.71. It was observed that the annual house index and container index were high in North (3.1% & 2.57%) and Central (3.61% & 3.60%) Delhi areas. The breteau index was found to be 4.19 and 5.88 respectively.

It was found that the proportion of wet receptacles & positive containers has been in North Delhi - 21.98% (33.9%), South Delhi - 24.79% (10.06%), East - 19.55% (9.4%), West - 17.09 (10.69%) & Central - 16.60% (35.85%). Therefore, the container positivity was quite high in North & Central Delhi as compared to proportion of wet containers received. In Delhi, the proportion of wet receptacles such as metallic, plastic, earthen, rubber, cement & others have been 31.0%, 52.74%, 0.74%, 2.5% & 0.45% respectively. The proportion of positive containers with *Aedes* breeding detected in metallic, plastic, earthen, rubber, cement was found to be 61.6%, 16.35%, 14.46%, 1.25% & 6.3% respectively.



Inauguration of the Laveran square in front of the church of the Val-de-Grâce military hospital in Paris, was named after Laveran on November 6, 1930