GENERAL INTRODUCTION
Arthropods, the joint-footed animals, are a highly successful group incomparable by any others in diversity of distribution, structure and function. Among them, the insects include some of the most unique and exciting animals on earth with about 1 million scientifically nomenclatured species. Entomologists do describe hundreds of new species each year. Thus insects represent more than half of the 1.5 million species of living organisms known to science (Marquardt and Kondratieff, 2005). High species richness has been attributed to their small size, short generation time, rather sophisticated nervous system, coevolution with plants, ability to fly and their developmental strategies. They live everywhere from mountain tops in the Himalayas to tide pools at the seashore. Because they dominate all terrestrial environments that support human life, insects are usually our most important competitors for food, fiber and other natural resources. They also have profound impact on the health of humans and domestic animals by causing annoyance, inflicting bites and stings and transmitting disease. On the other hand, insects are beneficial as they pollinate crops, act as natural enemies of damaging pests and produce useful products such as honey, silk and wax for humans.

Four orders of insects which have become extremely diverse are Coleoptera, Lepidoptera, Hymenoptera and Diptera. The latter includes more than 250,000 species belonging to 188 families and some 10,000 genera. Several species are known to be of medical and veterinary importance, the majority of
them belonging to the suborder Nematocera including families such as Culicidae (mosquitoes), Ceratopogonidae (biting midges), Psychodidae (Phlebotominae sub-family, sand flies) and Simuliidae (the blackflies). Among them, mosquitoes are found everywhere in the world, except Antarctica continent (Marquardt and Kondratieff, 2005). Three quarters of all mosquito species live in the humid tropics and subtropics where the warm moist climate has favoured the existence of diversity of habitats permitting the evolution of many species. The family Culicidae is classified in to two medically important subfamilies, the Anophelinae and Culicinae.

Quite a few species of mosquitoes are vectors of pathogens that cause disease in humans and domesticated animals. Many new and re-emerging diseases are transmitted by arthropod vectors (Brogdon and McAllister, 1998). Vector-borne diseases account for around 17% of the estimated global burden of infectious diseases (WHO, 2006b). The pathogens transmitted by mosquitoes include viruses (arboviruses), filarial worms (helminths) and protozoa. Fewer than 150 species, largely refering to genera Anopheles, Aedes and Culex are the indirect cause of more morbidity and mortality among humans than any other group of organisms (Harbach, 2007). Most medical entomologists consider that, about 30-40 species of Anopheles as important malaria vectors of the world. Examples of malaria vectors include Anopheles gambiae and An. funestus in Africa, An. albimanus and An. darlingi in the New World tropics, and An. stephensi and An. culicifacies in Asia. Besides, Aedes aegypti, the yellow fever or Asian tiger mosquito, is one of the most medically important species in the
world. But in addition to its role as the principal urban vector of the yellow fever virus, it is the primary vector of the dengue and chikungunya, the latest scare in India. *Culex quinquefasciatus* is a vector of the nematode worms causing lymphatic filariasis in India and of several arboviral diseases elsewhere (Marquardt and Kondratieff, 2005).

**Malaria epidemiology**

‘Everything about malaria is so moulded and altered by local conditions that it becomes a thousand different diseases and epidemiological puzzles. Like chess, it is played with a few pieces, but is capable of an infinite variety of situations’ (Hackett, 1937).

Malaria (Italian, mala aria means bad air) or Paludism has so many other names such as ague, aestivo-autumnal or harvest fever because in temperate regions epidemic transmission tended to occur in late summer and autumn. Malaria which has got recognition as World’s Day on April 25th, is the ninth most significant cause of death and disability globally and considered as a clear example of re-emerging disease (Krogstad, 1996). In ancient times, malaria and several other infectious diseases were considered as due to God’s punishment for human sins. During the Middle age, the idea that the environment could affect human health by means of *miasms*-unhealthy vapours rising from the ground or other sources- associated with swamps was also applied to malaria. During the period of Renaissance, the Italian physician Girolamo Fracastoro introduced the concept of contagion, which was replaced by that of infectious agents three
centuries later. Therefore, by the end of the 19th century, malaria was considered as an infectious disease caused by plasmodia and transmitted by mosquitoes (Tosta, 2007).

Even now malaria continues to be a major global health problem despite more than 110 years of research since the discovery of malaria parasite in human blood by Laveran in 1880 and establishment of mosquito’s role in the transmission by Ronald Ross in 1898. About 300–500 million people suffer from malaria worldwide each year mainly in Africa and south of Sahara, killing between 1.1 and 2.7 million either with malaria alone or in combination with other diseases and over 2,400 million remain at risk (WHO, 2008a). There were an estimated 247 million malaria cases among 3.3 billion people at risk in 2006, causing nearly a million deaths, mostly of children under 5 years. Out of 109 malaria endemic countries in 2008, 45 were within the African region contributing 91% of global malaria mortality. About 1.2 billion people are living at high risk places (≥1 case per 1,000 population), mostly in Africa (49%) and South-East Asia (37%) regions (WHO, 2008a).

Malaria is a serious public health problem in the South East Asia region as well, where the number of cases reported per year is 2–3 million, which is second only to that reported from sub-Saharan Africa. Out of 11 countries of the South-East Asia region (SEA) 10 countries are malaria endemic. In Maldives, there is no indigenous transmission since 1984 as reported by WHO-SEARO (2008). Around 40% of the global population at risk of malaria resides in SEA region and accounts for 8.5% of the global morbidity and around 4.1% of the
global mortality due to malaria. It is both a disease of poverty and a cause of poverty slowing economic growth by 1.3% per year in endemic areas. WHO estimates that globally 33.96 million disability-adjusted life years (DALYs), as a measure of overall disease burden, due to malaria in which SEA region contributes around 1.34 million. The disease is deeply rooted in the poor communities affecting national development and takes away major share of health budgets. Malaria transmission provides a barrier to national economic growth and poses a constant threat to health, well-being and economic stability to millions of poor people worldwide. In rest of the countries, situation either remain static or no significant change has occurred. About 95% of the population of moderate to high risk of malaria in SEA region are living in India, Indonesia, Myanmar and Thailand (WHO, 2008a). In India around two million malaria cases are being reported annually (NVBDCP, 2009); but much more morbidity and mortality is reported WHO-SEARO (2008).

Malaria is caused by parasites of the genus *Plasmodium*. The parasites are spread to people through the bites of infected female mosquitoes. There are four species causing human malaria: *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*. Among them *P. falciparum* and *P. vivax* are the most common and the former is the most deadly. Malaria transmission rates can differ depending on local factors such as rainfall patterns, the proximity of mosquito breeding sites to people and types of mosquito species in the area. Some regions ‘malaria endemic’, have a fairly constant number of cases throughout the year. In other areas, there are ‘malaria seasons’ usually coinciding with the rainy season. Over
a hundred countries and territories are epidemiologically classified as endemic, or previously endemic with the threat of resurgence. About half of the world’s population (3.3 billion) live in areas that have some risk of malaria transmission and one fifth (1.2 billion) live in areas with a high risk of malaria (more than 1 reported case per 1,000 population per year). Another 2.1 billion live in areas of low risk who produce a relatively small number of malaria cases each year (less than 2 million) and account for less than 3% of cases reported by countries in 2006. There were 247 million cases of malaria in 2006, causing about 880,000 deaths, mostly among African children (WHO, 2008a).

The importance of the ecological context in the epidemiology and management of vector-borne diseases was realised immediately following the discovery of arthropods as vectors of disease in 1877 (Ellis and Wilcox, 2009). Changes in transmission and distribution of vector-borne diseases are related to climate or to one or more of the many other global changes concurrently transforming the world. Abundant evidence indicates that climatic variation can affect the reproduction, development, population dynamics and host-seeking behaviours of arthropod vectors, as well as their abilities to transmit disease agents. Many globally changing factors other than climate that interact to determine the incidence of vector-borne disease in humans, include vector and host ecology, human culture and behaviour, land use, increased economic globalisation, the high speed of international travel and transport of commercial goods, increased population growth, urbanisation, civil unrest, displaced refugee
populations, water availability and management and deforestation (Reiter, 2001; Sutherst, 2004; Gage et al., 2008; Reiter, 2008).

Understanding the malaria epidemiology is based on its key elements including biotic and abiotic factors and their interactions. Among them climate change and human related activities have attracted considerable attention during recent years. Climate is a major factor in determining the geographic and temporal distribution of arthropods, characteristics of arthropod life cycles, dispersal patterns and evolution of associated vector-borne diseases and the efficiency with which they transmit pathogens from arthropods to vertebrate hosts (Gould and Higgs, 2009). Since the arthropod vectors are ectothermic (cold-blooded) organisms, globally changing combined factors including climate have a considerable impact on epidemiology of vector-borne diseases and malaria in particular.

The human population has increased from approximately 1 billion at the turn of the 20th century to 6.7 billion in 2007 and it is projected to grow to around 10 billion by 2050 (Sutherst, 2004). That in turn, increases the total number of people at risk. The highest population growth rate happens in developing countries such as Africa where malaria is endemic. A high birth rate supplies a large population of non-immunes and thus of new infections. Clinical studies in some parts of Africa show 998 infections per 1,000 infants (Reiter, 2008). Poverty associated with rapid population growth leads to concentrations of people without the necessary infrastructure for the safe storage and distribution of water and drainage of waste water as opined by Sutherst (2004).
The introduction of agriculture, around 7,000 B.C., led to an increase of population of relatively settled people and favourable conditions for malaria transmission. According to Reiter (2001) the extensive deforestation and ecologic changes along with land cultivation, various water storage and irrigation structures and practices are all the key factors in that regard. The need to intensify agriculture to feed the burgeoning human population especially in the developing world, directly affects the spread and intensification of vector-borne diseases which may invade new areas. Irrigation schemes may significantly increase relative humidity in a large area, favouring vector longevity. The impact of this phenomenon on malaria transmission was clearly demonstrated in the development of irrigated rice production systems in the Ruzizi plains of Burundi (WHO, 1996a). Gratz (1999) has stated that the ecological changes occurring in most malaria endemic regions usually result from water development projects, including dam construction, expanded irrigation, and changing crop practices, such as the shift to irrigated wetland rice. Amerasinghe and Indrajith (1994) have opined that, ecosystem changes concomitant with irrigation development result in long-term changes in the composition of the mosquito fauna, which is characterised by the increasing dominance of species with the potential to transmit human pathogens. Since the vectors such as *Anopheles* use the agricultural land like rice fields as their breading places, land use is often closely associated with an increase in the prevalence of vector-borne disease. There are many examples indicating the relationship between vectors resistance with agricultural related activities. Since
more than 90% of all insecticides produced have been used for agricultural purposes, this has created serious problems in mosquito control programmes (Davari et al., 2007). Likewise, Yadouleton et al. (2009) have reported that, fast development of urban agriculture in many areas of the Republic of Benin and improper use of insecticide to control vegetable pests, exerting a huge selection pressure on mosquito larval population, resulted to the emergence of insecticide resistance in malaria vectors. Over 135 pest and vector anophelinae and culicinae mosquito species have been found in association with riceland habitats (Lacey and Lacey, 1990). Natural biodiversity of hosts can reduce the transmission of vector-borne diseases, indicating the importance of natural enemies in controlling vectors of disease (Sutherst, 2004). On the other hand, nowadays farmers keep large populations of stock in farm buildings rather than in open fields and woodland. These buildings provided attractive sites for adult mosquitoes to rest and feed, diverting them from human habitation as opined by Reiter (2008).

The world’s urban population increased from 1.7 billion (39%) in 1980 to 2.7 billion (46%) in 1997 and are expected to reach 5 billion (60%) by 2030. Rural populations are declining as industrialisation and other developments draw people to urban areas. Dense urban settlements with poor infrastructure are widespread in the developing world which lead to increases in the incidence of human diseases (Sutherst, 2004). Many tropical cities are surrounded by large satellite settlements (slums) that retain rural characteristics. Their dense populations promote conditions that are ideal for malaria transmission.
absence of cattle can promote stable transmission by forcing zoophilic species to feed on people. Infection rates in these semi-rural habitats are often higher than the cities themselves (Reiter, 2001). According to Norris (2004), urban development in many parts of the world occurs without the consultation of entomologists, epidemiologists and healthcare specialists to address issues such as storm water management, urban refugia and vector control.

Globalisation of trade and movement of people to the emerging megacities with inadequate sanitation and public health infrastructures could accelerate vector-borne diseases (Sutherst, 2004). One million people are reported to travel internationally each day and another million travel from developed to developing countries (and vice versa) each week. Each of these types of movements has the potential to spread disease pathogens and their vectors over long distances. The latest pandemic of swine flu is the best example. People can either act as carriers of pathogens into new environments or accidentally translocate vectors in transport vehicles. The worldwide migration of insecticide resistant genotypes of mosquitoes is an excellent example of the extent to which vectors have been spread around the world (Hemingway and Ranson, 2000). Each year, 20 million people visit malarious areas and there are 10,000 cases of imported malaria in the European Community alone, most of which are caused by the most virulent P. falciparum. Malaria cases in European travelers are increasing steadily due both to an increase in the volume of international tourism to endemic areas and to migration of people from these areas to Europe. For example, in 2004, United Kingdom residents made a total
of 1.2 million visits to the Indian sub-continent alone (Reiter, 2008). According to Gratz (2006) a total of 1,337 cases of malaria, including 8 deaths, were reported for the year 2002 in the USA, while of all of these 1,337 cases but 5 were imported. Infected mosquitoes introduced through airports are also likely to survive longer in the future if there is increased rainfall and will therefore enhance the problem known as airport malaria (Sutherst, 2004).

Social disturbances such as wars, civil unrest and natural disaster also cause public health problems, with resultant outbreaks and spread of infectious and vector-borne diseases, particularly malaria (Sutherst, 2004). Mass movements of people, e.g., soldiers and refugees, often promote malaria transmission. The breakdown of public health services, damage to water distribution and drainage systems and the destruction of homes often exacerbate the situation. High concentrations of people in camps for displaced persons can also be disastrous. To date, no approach has been successful without social stability and maintenance of an effective public health system. It is worth noting that challenges such as civil unrest, tribal wars and lack of political goodwill need to be addressed, because effective malaria control is only possible under a stable civil setting (Muturi et al., 2008). Rapid economic decline combined with political instability has already brought back epidemic typhus, malaria, and other infectious diseases to several countries of the former Soviet Union. For example, in the 1990s epidemic malaria has made a dramatic reappearance in Armenia, Azerbaijan, Tajikistan and Turkmenistan (Reiter, 2001). Based on such problems, Slutsker and Newman (2009) state that focus must be maintained on
improving and sustaining scale-up in high-burden countries. At the same time, the strategies and planning for strengthening surveillance, health systems, human-resource capacity and regional coordination mechanisms must proceed.

To sum up it is better to say that combinations of global change drivers tend to increase the suitability of the environment compared with the effects of each driver separately. These include combinations of climate change, increased trade and travel, increased urbanisation in poor countries and intensification of agriculture with irrigation, deforestation and loss of biodiversity (Sutherst, 2004).

Beyond the human toll, malaria causes significant economic loss in endemic areas, decreasing Gross Domestic Product (GDP) by as much as 1.3% in countries with high levels of transmission (Gallup and Sachs, 2001). Over the long-term, these aggregated annual losses have resulted in substantial differences in GDP between countries with and without malaria (particularly in Africa). In some heavy-burden countries, the disease accounts for up to 40% of public health expenditures, 30 to 50% of inpatient hospital admissions and up to 60% of outpatient health clinic visits.

There are 444 formally named and 40 unnamed members of species complexes recognised as distinct morphological and/or genetic species of *Anopheles* all over the world (Dash *et al.*, 2007). The current global malaria strategy includes the selective control of malaria transmission as one of its four main elements. According to Najera and Zaim (2001) vector control is the most
generally effective way of prevention and control of malaria transmission. The principal objective of vector control is the reduction of malaria morbidity and mortality by reducing the levels of transmission. Vector control methods vary considerably in their applicability, cost and sustainability of their results. The choice of the method will depend on the magnitude of the malaria burden and the possibility of sustaining the resulting modified epidemiological situation. WHO (2006a) recommends a systematic approach to vector control based on evidence and knowledge of the local situation. This approach is called integrated vector management (IVM) mainly include indoor residual spraying (IRS) of insecticides, personal protection measures, larval control, biological control and environmental management.

Continuous and indiscriminate application of insecticides can lead to reduced susceptibility, tolerance and finally development of resistance by the vector species. A vector population becomes resistant because the insecticide progressively eliminates the majority of the susceptible individuals while allowing the survival and reproduction of those possessing some mechanism whereby its toxic effect is avoided (Najera and Zaim, 2001). Resistance to pesticides and other xenobiotics is an evolutionary response to stress that will remain a persistent problem. The chemical control subjects the population to Darwinian selection and survival of the fittest. Attempts to kill the tolerant individuals lead to ever increasing doses and eventually emergence of resistant vector populations as opined by Casida and Quistad (1998). Pyrethroids act on the nervous system by modifying the gating kinetics of voltage-sensitive
sodium channels. This mechanism confers resistance not only to DDT but also to pyrethroids, which shares a similar mode of action (Gayathri and Murthy, 2006). Pyrethroid resistance is emerging despite early optimism because of its rapid toxicologic action, that this new class of insecticides would not produce resistance. According to Brogdon and McAllister (1998) a far more threatening development in pyrethroid resistance is the appearance of target-site resistance (also termed knockdown resistance) in several important vectors in multiple locations.

More than 500 species of arthropods are reported as resistant to insecticides (Shelton et al., 2007) including about 100 mosquito species to one or more insecticides and more than 50 of these are Anophelines (Hemingway and Ranson, 2000). Malaria, which accounts for around 90% of deaths caused by vector-borne diseases, has been worsening during the past decades, particularly in sub-Saharan Africa, because of spread of resistance to antimalarial drugs and insecticides, general weakening of health systems and a systemic lack of resources, as opined by van den Berg and Takken (2007).

With an ever increasing public interest and awareness about the environment, in both developed and developing countries, positive public perception of natural pesticides is an added incentive in vector control programmes. A large number of plant species representing different geographical areas around the world have been shown to possess phytochemicals that are capable of causing a range of acute and chronic toxic effects against insects. Mosquitoes are susceptible to a greater or lesser extent to some
phytochemicals. It should be mentioned however, that the high degree of biodegradation exhibited by most phytochemicals is what makes them eco-friendly and attractive as replacements of synthetic chemicals in the first place.

A wide selection of trees and shrubs has been found to contain phytochemicals that may be of use in the bio-control of mosquitoes. Studies have ranged in focus from ornamentals to fruit trees and from leguminous to eucalyptus and other timber trees. According to Shaalan et al. (2005a) the susceptibility of *Anopheles* larvae can vary since they can be more or less susceptible than *Culex* and *Aedes* larvae to botanical derivatives and insecticides. Phytochemicals offer not only effective mosquito control agents, but also are biorational alternatives to organic synthetic pesticides. The fact that these chemicals are from natural sources, with a high degree of biodegradation, makes them environmentally sound control agents (Sukumar et al., 1991).

According to National Vector Borne Disease Control Programme (NVBDCP, 2009), in India 1.67 million cases of malaria (including 0.77 million *P. falciparum* cases) and 1487 deaths have been reported in 2006, whereas in 2007 with a slight decrease in statistics, 1.50 million cases (including 0.75 million *P. falciparum* cases) and 1274 deaths are reported. Provisional data for the year 2006 reveals the largest number of cases in the country were from Orissa, followed by Jharkhand, West Bengal, Assam, Chhattisgarh, Rajasthan, Gujarat & Uttar Pradesh and the largest numbers of deaths were reported in Assam followed by Orissa, West Bengal, Arunachal Pradesh, Meghalaya, Maharashtra, Mizoram, Gujarat & Karnataka. In Karnataka State 1,32,000 cases
and 33 deaths have been reported in 2002, compared to 49,355 cases and 18 deaths in 2007. Despite decreasing trend in morbidity because of all control programmes, even now about half lakh of people suffer malaria. WHO-SEARO (2008) has estimated 15 million cases and 20,000 deaths in India, so she contributes about 77% of the total malaria in South-East Asia (Kumar et al., 2007). Retrospective analyses of burden of malaria in India showed that disability adjusted life years lost due to malaria were 1.86 million years. Cost–benefit analysis suggested that each Rupee invested by the National Malaria Control Programme pays a rich dividend of 19.7 Rupees (Kumar et al., 2007).

In India, 58 species of *Anopheles* have been described, six of which are implicated to be the main malaria vectors, namely *An. culicifacies*, *An. dirus*, *An. fluviatilis*, *An. minimus*, *An. sundaicus* and *An. stephensi*. Besides, some are of local importance, viz *An. philippinensis-nivipes*, *An. varuna*, *An. annularis* and *An. jeyporiensis*. *Anopheles culicifacies* contributes to about 60–65% of all malaria cases of India mainly from rural and periurban areas and is widely distributed throughout the country.

Sharma (1999) states that of the six principal malaria vectors in India, major vectors *i.e.* *An. culicifacies* and *An. stephensi* have shown wide spread resistance, while other vectors are more or less susceptible to all the insecticides. Presently, *An. culicifacies* has been reported to be resistant to DDT in 286 districts and to both DDT and malathion in 182 districts of India (Dash et al., 2007).
Anopheles stephensi is a sub-tropical species and distributed throughout the Middle East and South Asia region (Afghanistan, Bahrain, Bangladesh, China, Egypt, India, Iran, Iraq, Oman, Pakistan, Saudi Arabia and Thailand) and is considered as an important vector in India, Pakistan and Iran. So far there is no description of sibling species in An. stephensi. However three races, ‘type form’, ‘type mysoriensis’ and ‘type intermediate’ have been described based on egg-dimension and number of ridges present on the floats (Subbarao et al., 1987; Alam et al., 2008). All these three forms are found in India and Iran. According to Dash et al. (2007), An. stephensi is primarily a zoophilic species but considerable variability in human blood index (HBI) has been reported. In Kolkata, an urban area, as high as 100% HBI has been reported. This is mainly an urban vector and majority of breeding sites are in urban areas, man-made and limited. It breeds in wells, water storage tanks, discarded tins, containers, coconut shells, leaking water supply, rain water collections etc. (Sharma, 1999).

In the light of the said information and the existing endemicity of several mosquito-borne diseases and in particular malaria in Karnataka state, India, the study of ‘Efficacy of bioactive extracts of two plant species and detection of insecticide resistance in Anopheles stephensi, a malaria vector’ was chosen. Detailed investigations were undertaken in particular on An. stephensi with respect to phytochemical efficacy, insecticide bioassay, resistance mechanisms related to biochemical and isozyme differentiation. As malaria is endemic in local situation, the present investigation will add more knowledge on current and future measures in management of the disease.
The present investigations were undertaken with the objectives to:

1. Analyse the insecticidal efficacy of extracts of two plants (Indigenous and exotic) after detailed solvent extraction and isolation of bioactive fractions employing *An. stephensi* as the test organism.

2. Study the resistance/tolerance development if any, with cypermethrin (a pyrethroid insecticide) in *An. stephensi*.

3. Study the biochemical mechanisms of resistance through qualitative and quantitative assays with target enzymes such as α-esterase, β-esterase, Cytochrome P450, G6PD and ADH.