INTRODUCTION

Malaria is a disease of antiquity which has been proved to be a formidable deterrent to the cultural and socio-economic progress of man in tropical, subtropical and monsoon prone zones of the world. It is one of the major public health problems in developing countries. About 3.3 billion people - half of the world's population - are at risk of malaria (WHO, 2009). Every year, this leads to about 250 million malaria cases and nearly one million deaths. People living in the poorest countries are the most vulnerable.

India recorded 1.53 million cases of malaria and 1, 100 deaths in 2009. Majority of the cases and deaths are in the under-30 age group. Out of theses 90% of the deaths are in rural areas, and 86% do not occur in any healthcare facility. Researchers had based their estimate on interviews with families of more than 1.22 lakh people (NVBDCP, 2009).

There are about 400 species of Anopheline mosquitoes throughout the world, but only 60 species are vectors of malaria. In India 58 Anopheline species are reported (Nagpal & Sharma, 1995), out of which 10 have been incriminated as malaria vectors (Rao, 1981). Among these, six species viz., An. culicifacies, An. stephensi, An. fluviatilis, An. minimus, An. dirus and An. sundicus are the major vectors while other four species viz., annularis, philippinensis, jeyprieonis and varuna are minor or secondary vectors in transmission of malaria. The principal vector urban malaria in India is An. stephensi and secondary vector is An. culicifacies (Hati, 1997).

Under the continued assault of insecticides, vector mosquitoes have developed resistance. Since, insecticides remain one of the main tools for mosquito control, the ability to detect the emergence of resistance and to predict its spread throughout mosquito populations is important. The malaria parasite become drug resistant and till now there is no vaccine for the control of malaria. Multi-disciplinary approaches using complimentary strategies appear to be the most promising for the control of this disease. Vector control remains the most generally effective measure to prevent malaria transmission and therefore it is one of best way of the Global Malaria Control Strategy (WHO, 2011). 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.

Malaria vaccines could be one of the most cost-effective interventions to reduce the enormous burden of the disease in the poorest countries of the world. Though a large research effort has been directed toward the interaction of the malaria parasite with its human host, there is currently neither a malaria vaccine nor a vaccine candidate ready for market. Transmission blocking vaccines (TBV) consists of antibodies that are ingested by mosquitoes in blood meal and interfere with the parasite development. The mosquito antigens that are not normally introduced in vertebrate host during blood feeding are not exposed to immune selective pressure and in consequence antigenic diversity and poor immunogenicity should not be a problem. The antibodies against these concealed antigens (Midgut, salivary gland, haemolymph and ovaries) proved to be more effective. Hence, development of vector based malaria transmission blocking vaccines remains one such pragmatic approach that can complement or replace existing control methods.

Earlier studies indicated that vector internal organs (concealed antigens) can induce an artificial immune response in various species viz., An. stephensi (Alger and Cabrera, 1972; Gulai and Gakhar, 2001, 2003; Gulia et al., 2002, 2011; Jacobs-Lorena and Lemos, 1995; Chugh et al., 2011; Willadsen and Billingsley, 1996). Logically any control intervention should target the parasite at the most vulnerable stages in its life cycle. Within Anopheles mosquitoes, the two epithelial interfaces – the midgut and the salivary glands represent major survival bottlenecks for the parasite.

Midgut plays a central role in the transmission of malaria and is home for parasite during the major events of its life cycle like fertilization, ookinetes to oocyst transition. Moreover, several studies with rodent and human malaria parasites have revealed that the ookinetes to oocyst transition is most vulnerable link in sporogonic development of malaria parasites (Vaughan et al., 1992; Lal et al., 2001). Anti-midgut antibodies have already been postulated to cause deleterious effects on the reproductive capacity of mosquitoes and they simultaneously block parasite development in the mosquito midgut (Suneja et al., 2003; Lal et al., 2001). Dinglasan et al., 2003 reported the dose dependent blocking effect on the P. yoelli development in the midgut of An. stephensi.
Another pragmatic approach is vector transgenesis i.e. to introduce foreign gene (effector molecule) into the vector for genetic transformation, so that the vector become unable to support parasite development. Vector transgenesis requires, a technology to transfer foreign gene in to the vector, an ideal sex and tissue-specific promoter to drive the expression of foreign gene in the organ where maximum interaction with the parasite take place. Therefore, the present investigations have been carried out on Indian malaria vector *Anopheles culicifacies*, which is responsible for about 65-70% of malaria in India. During this study an attempt has been made to develop the indigenous techniques for mosquito transgenesis and to identify an effector/ antiparasitic molecule for transmission blocking and for anti-mosquito immunity.

For this, the ultra structure of *An. culicifacies* eggs has been observed with the help of scanning electron microscopy so as to prepare the eggs for microinjection/transgenesis.

*An. culicifacies*, the most important malaria vector of India is responsible for approximately 65-70% of the total malaria. The evidence of the existence of two biologically distinct species, species A and species B within the taxon *Anopheles culicifacies* Giles, reported by Green and Miles in 1980. Subsequently three more species, species C (Subbarao *et al.*, 1983), species D (Vasantha *et al.*, 1991) and species E (Kaur *et al.*, 1999) have been reported. Members of *An. culicifacies* complex are reported to have differential disease transmission potential.

In addition, midgut protein pattern was analyzed during different developmental stages of *An. culicifacies* and in different species of *Anopheline*, in sibling species complex of *An. culicifacies* (Type – A, B & C). The low molecular weight polypeptides were N-terminally sequenced. Antiserum was raised against cecropin like protein of glucose fed *An. culicifacies*. Antibodies raised against cecropin like protein were screened for their influence on *P. vivax* development and reproductive performance of *An. culicifacies*. Molecular analysis of effector gene cecropin A has been also done.

The results from the present study assertively implicated that the cecropin like protein as the likely source for candidate antigen for transmission blocking vaccines and/ or effector / antiparasitic molecule for the use in mosquito transgenesis to block the development of parasite within the host.