2. REVIEW OF LITERATURE

2.1. Dengue vector, *Aedes aegypti*

Mosquitoes are transmitters of diseases like Malaria, Filariasis, Dengue, Dengue haemorrhagic fever, Chikungunya and Japanese Encephalitis and are among the most serious vector borne diseases that contribute significantly to poverty and social debility in tropical countries (Jang *et al.*, 2002). They are about 90 genera and 2500 species of mosquitoes all over the world. Mosquitoes are known to perceive visual, thermal and olfactory stimuli which enable them to detect light source, odour and several other volatile chemicals emanating from the skin, breath and waste products of their hosts (Davis and Bowen, 1994). Mosquitoes breed in varied habitats such as ponds, marshes, ditches, pools, drains; water containers and other similar water collections (Rozendaal, 1997). Eggs are laid by adult females on wet surfaces, just above the water level. In their original environment, the breeding sites corresponded to tree-holes; however, exotic populations in urban habitats rely on small artificial containers such as cans, buckets, flower pots, and bottles placed in yards, gardens, and even inside houses. Eggs can resist low temperatures and they hatch when they mature and get wet, generating the larvae (Christophers, 1960).

The yellow fever mosquito, *Aedes aegypti*, is responsible for dengue fever in India where the number of dengue fever cases has increased significantly in recent years. Dengue viruses cause a broad range of diseases, including clinically asymptomatic forms, classic dengue fever, and the more severe forms such as dengue hemorrhagic fever and dengue shock syndrome. Hemorrhagic complications causing encephalopathy is rare but fatal (Kumar *et al.*, 2009). Agarwal *et al.*, (2009) observed a 4-year-old female child from north eastern India with dengue encephalitis. This is supposed to be the first reported case of dengue encephalitis from this region. Chikungunya fever has been originally distributed in several parts of Africa, South Asia and Southeast Asia. The disease is caused by, an enveloped single-stranded ribonucleic acid virus of the alpha virus genus (family Togaviridae). In Asia, virus transmission to humans occur predominantly by the bite of the female *Aedes aegypti* or *Aedes albopictus* mosquito. The mosquito can be recognized by white markings on
legs and a marking of a form of a lyre on the thorax. *Aedes* mosquito originated from Africa.

Initially spectacular success was noticed in controlling the mosquitoes with DDT during the 1950s and 1960s. It was not sustainable because the mosquitoes evolved resistance to DDT. Other pesticides have gone into use, but there has been no overall reduction in the disease. Nearly one hundred million people in tropical Asia and Latin America are infected with dengue each year, about one-half million children are afflicted with life-threatening Dengue Hemorrhagic Fever, and thousands die. The pesticide-based strategy of the last fifty years has been a failure. There are many causes that explain the re-emerge of dengue, such as the natural selection of strains resistant to insecticides, as well as global changes, such as an increase in international flight, the migration of people from rural to urban areas and uncontrolled re-urbanization (Guzman and Kouri, 2002). The peak of the plastics industry around the world could be another factor associated with the re-emergence of dengue, because of the huge quantity of discarded plastic containers that provide an infinite number of artificial breeding grounds for the mosquitoes. At present, no effective vaccine is available for dengue; therefore, the only way of reducing the incidence of the disease is by mosquito control, which is frequently dependent on applications of conventional synthetic insecticides. In most urban and rural areas of the country, mosquito populations are menacing throughout the year, except for some attenuation during summer and winter (Sharma, 2001).

The incidence of mosquito-borne diseases such as malaria, dengue, and chikungunya is rising in India due to climate change. These diseases are no longer restricted to old areas and spread to new areas due to rising temperature. Studies have found that while intensified rainfall favours mosquito breeding, rising temperature along with humidity increases its survival period during the adult phase and hence the disease transmission capacity of each mosquito magnifies several times. There are reports of a rising number of vector-borne diseases in traditionally low endemic areas, such as Himachal Pradesh, Uttarakhand and Jammu and Kashmir (Atanu Sarkar, 2008; Mahendale *et al.*, 1991).
Dengue virus spreads in tropical areas of the world. It is horizontally transmitted to humans by infected Aedes mosquitoes, but it is also able to be vertically or transovarially transmitted to insect progeny. Günther et al. (2007) analyzed the vertical transmission of dengue virus in Aedes aegypti mosquitoes collected in two endemic localities in the state of Oaxaca, Mexico. Dengue viruses (DV) belong to family Flaviviridae and have four serotypes (Abhyankar et al., 2006., Agarwal et al., 1998, 1999a, b.). They are transmitted mainly by the Aedes aegypti mosquito and also by Aedes albopictus. Biologically, DV are highly adapted to the mosquito and are maintained by vertical transmission. DV produces from a subclinical infection to a mild self limiting disease, the dengue fever (DF) and a severe disease that may be fatal are dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS). The mosquito vectors are present in tropical and subtropical regions of the earth that determines the prevalence of DV in a region. Prior to 1970, only 9 countries had experienced cases of DHF; since then the number has increased more than 4-fold and continues to rise. The WHO published a global map of the distribution of the dengue epidemic activity during the year 2006 that shows whole India in red colour. A comparison with similar maps prepared in earlier years shows that the activity of both the vector and the virus has spread to newer areas, acquiring global public health importance. An estimated 2.5 billion people in more than 100 countries are at risk of acquiring dengue viral infection with more than 50 million new infections being projected annually, 500000 cases of DHF that must be hospitalized and 20000–25000 deaths, mainly in children (Halstead, 2000; Chaturvedi and Shrivastava, 2004). Dengue has been an urban disease but now has spread to rural areas of India as well (Kumar et al., 2009).

2.2. Bio pesticides for mosquito control

In the absence of effective vaccine and drugs, dengue prevention and control programs have depended on vector control. Management of this disease vector using synthetic organic chemical insecticides has failed because of insecticide resistance developed by the Aedes mosquitoes (Liu et al., 2003; Lee et al., 1982). The harmful effects of chemicals on non-target populations and the development of resistance to these chemicals in mosquitoes along with the recent resurgence of different mosquito-
borne diseases (Chung and Shin, 1989), have prompted us to explore alternative, simple, sustainable methods of mosquito control. Major drawback with the use of chemical insecticides is that they are non-selective and could be harmful to other organisms in the environment. It has also provoked undesirable effects, including toxicity to non target organisms, and fostered environmental and human health concerns (Lee et al., 2001; Moore et al., 2002). The toxicity problem, together with the growing incidence of insect resistance, has called attention to the need for novel insecticides (Macedo et al., 1997; Perich et al., 1994) and for more detailed studies of naturally occurring insecticides (Ansari et al., 2000). In the last few years, there was an increase of public concern on the safety of many chemical products that instigated a renewed interest on the use of natural products from plant origin for vector management. New botanical natural products are effective, environment-friendly, easily biodegradable, inexpensive, readily available in many areas of the world, no ill effect on non-target organisms and have novel modes of action (Mulla and Su, 1999). It has been proved that larvicidal measures contain mosquito population for a short period and require repeated applications of chemicals and eventually develop resistance against that chemical (Gratz NG., 1999; Rameswak, 1999).

The use of plants for medicinal and insecticidal purposes dates back to antiquity (Sofowora, 1984; Goshi, 1996; Govere et al., 2000). Recent studies have focused on natural plant products as alternatives for disease control. Majority of rural dwellers in developing countries still depend on medicinal plants to prevent or eliminate diseases (Parekha and Chanda, 2008). Co-evolution has equipped plants with a plethora of chemical defences against insect predators. Aware of this effect, mankind has used plant parts or extracts to control insects since ancient times. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticide properties (Balandrin 1985; Rawls 1986; Sukumar et al., 1991). Natural insecticides such as pyrethrum, rotenone and nicotine, among others, have been extensively used until recently for insect control (Balandrin, 1985). Limonoids such as azadirachtin and gedunin, present in species form the Meliaceae and Rutaceae are recognized for their toxic effects on insects and are used in several insecticide formulations in many parts of the world (Dua et al., 1995; Nagpal et al., 1995). Recently, the discovery of insecticide activity of
phyto toxins present in Asteraceae species has stimulated the interest in this plant family as part of the search for new plant derived insecticides (Rawls, 1986).

Larvicidal effect of neem (Azadirachta indica) and karanja (Pongamia glabra) oil cakes (individuals and combination) was studied against mosquito species. Both the oil cakes showed larvicidal activity against the mosquito species tested. The combination of neem and karanja oil cakes in equal proportion proved to have better effect than the individual treatments (Shanmugasundaram et al., 2008). Phytochemicals obtained from plants with proven mosquito control potential can be used as an alternative to synthetic insecticides or along with other insecticides under the integrated vector control (Kamaraj et al., 2010). Plant products can be used, either as insecticides for killing larvae or adult mosquitoes or as repellents for protection against mosquito bites, depending on the type of activity they possess. A large number of plant extracts have been reported to have mosquitocidal or repellent activity against mosquito vectors (Sukumar et al., 1991; Singh et al., 2007), but very few plant products have shown practical utility for mosquito control.

Zhu et al., (2006) assessed the larvicidal activity and repellency of 5 plant essential oils--thyme oil, catnip oil, amyris oil, eucalyptus oil, and cinnamon oil--were tested against 3 mosquito species: Aedes albopictus, Ae.aegypti, and Culex pipiens pallens. Larvicidal activity of these essentials oils was evaluated in the laboratory against 4th instars of each of the 3 mosquito species, and amyris oil demonstrated the greatest inhibitory effect with LC$_{50}$ values in 24 h of 58 microg/ml (LC$_{90}$ = 72 microg/ml) for Ae. aegypti, 78 microg/ml (LC$_{90}$ = 130 microg/ml) for Ae.albopictus, and 77 microg/ml (LC$_{90}$ = 123 microg/ml) for Cx. p. Pallens.

Okigbo et al., (2010) tested the potency of petroleum ether leaf extracts of A.indica (Juss), O.gratissimum (L.) and H.suaveolens (L.) as mosquito larvicides under laboratory conditions. 100% mortality was achieved by A. indica at concentrations 40% and 35% and O. gratissimum at concentration of 50% after 24hrs while H.suaveolens at 60% which showed no significant effect as mosquito larvicide (P≤0.05). O.gratissimum and A.indica petroleum ether extracts to have potential utilization as a larvicide to control Culex mosquito and its borne diseases.
Mohsen and Ali (2009) studied the composition of the essential oil hydrodistilled from the aerial parts of 18 individual *Artemisia herba-alba* Asso. The persistency of larvicidal effects of 13 oils (camphor, thyme, amyris, lemon, cedarwood, frankincense, dill, myrtle, juniper, black pepper, verbena, helichrysum, and sandalwood) was examined by storage of 50-ppm solutions under different conditions (open, closed, in the light, and in the dark) for one month after the preparation of the solutions.

Earlier researchers have reported the bioactivity of essential oils or on the effectiveness of plant extracts from various plants against the larvae of mosquitoes (Mehlhorn *et al*., 2005; Amer and Mehlhorn 2006b; Rahuman *et al*., 2009a, b). The use of different parts of locally available plants and their various products in the control of mosquitoes has been well established. The larvicidal properties of indigenous plants have also been documented in many parts of our country along with the repellent and anti-juvenile hormones activities (Singh and Bansal, 2003). Products of secondary plant metabolisms may be responsible for the chemical communication between plants and insects. Volatile plant constitutions have attractive or repellent properties to insects or microorganisms. Allelochemicals have been considered as potential natural insecticides and can be used for insect management in integrated control (Jilani and Su, 1983; Qureshi *et al*., 1986).

Beserra and Castro (2008) aimed at comparing the life cycle and estimating the patterns of fertility of population of *A. aegypti* (L.). The life cycles were studied at the temperature of 26 +/- 2 degrees C, and 12h photoperiod. The development period, egg viability and larval and pupal survival were evaluated daily as well as adult longevity and fecundity. The durations of egg, larva and pupa stages varied from 3, 9 to 4, 5 days, from 6, 4 to 8, 3 days and from 2,0 to 2,5 days, respectively.

The chemical composition of the leaf essential oil of *Croton regelianus* collected from wild plants growing in two different sites at Ceará State (Brazil) was analyzed by GC/MS and GC-FID. Twenty monoterpenoids, representing more than 96% of the chemical composition of the oils, were identified and quantified (Torres *et al*., 2008). Waliwitiya *et al*., in 2009 assessed the acute toxicities of 14
monoterpenoids, trans-anethole and the essential oil of rosemary against different larval stages of *Ae.aegypti*.

The potential for piperonyl butoxide (PBO) to act as a synergist for these compounds to increase larvicidal activity was also examined. The ovipositional response of gravid *Ae.aegypti* females to substrates containing these compounds was evaluated in behavioral bioassays. The bioactivity of four flavonoid compounds, namely poncirin, rhoifolin, naringin and marmesin, from *Poncirus trifoliata* against the *Aedes aegypti* was studied by Rajkumar and Jebanesan in 2008.

Bio-larvicides like *Azadirachta indica* (neem) could repel adult mosquitoes from laying their eggs in the treated larval habitats. Howard et al. (2011) observed the response of *Anopheles gambiae* s.s. mosquitoes towards varying doses of crude aqueous neem extracts. Non-choice oviposition tests were used to measure the proportion of mosquitoes laying on the first or second night, or not laying at all, when compared to the control. For each individual mosquito, the number of eggs laid and/or retained in the ovary was counted to determine the relationship between wing length and egg production. Larger female mosquitoes produced larger egg batches. The results showed that a dose of 0.1 g/l, was found to be effective at controlling mosquito larvae. The results indicate that these simple neem extracts were effective and potentially sustainable.

A study with *Anopheles stephensi* Liston and *Anopheles culicifacies* Giles using a range of neem extracts found that 7 day old gravid mosquitoes exposed to neem volatiles for 90 min exhibited oviposition suppression, with neem-exposed females retaining significantly more eggs than control mosquitoes. Females that were exposed to neem-derived volatiles immediately after mating and were left exposed to these volatiles for several days did not fully develop eggs either in that or successive GCs (Dhar et al., 1996). Similarly, when neem was fed to *A.stephensi* mosquitoes either before or during a blood meal, egg maturation and oviposition were adversely affected (Lucantoni et al., 2006).

Three commercial repellents marketed in Tanzania: Zero Bite (a blend of microcrystalline waxes, mineral oils, natural flavours, Olibanum oil, Eucalyptus oil,
Geranium oil, Citronella oil and Isopropyl myristate; X-pel (a petroleum jelly formulation containing diethyl toluamide (DEET) and dimethyl phthalate); No Bite (a spray formulation with diethyl toluamide, 2 methyl 2,4 pentondiol and phthalic ester acids) were tested and compared for their repellency effect against wild anthropophilic mosquito populations (Magesa and Kamugisha, 2006). Amer and Mehlhorn (2006), used 41 plant extracts and 11 oil mixtures on the skin of human volunteers to find out the protection time and repellency against the yellow fever mosquito, *Aedes aegypti* (Linnaeus), the malaria vector, *Anopheles stephensi* (Liston), and the filariasis and encephalitis vector, *Culex quinquefasciatus* (Say) (Diptera: Culicidae).

Anees A. M., (2008) studied on the acetone, chloroform, ethyl acetate, hexane, and methanol leaf and flower extracts of *Ocimum sanctum* against fourth instar larvae of *Aedes aegypti* and *Culex quinquefasciatus*. Singh, *et al.*, (2007) tested the Hexane extract obtained from leaves of Eucalyptus citriodora Hook against larvae of *Anopheles stephensi* Liston, *Culex quinquefasciatus* Say and *Aedes aegypti* Linn to assess its toxicity and growth inhibiting activity. Laboratory studies were carried out to observe the oviposition responses of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) to several C21 fatty acid esters by Sharma *et al.*, in 2008.

*Aedes albopictus* were reared in different containers: a tree hole, a bamboo stump and an auto tire. The total times from egg hatching to adult emergence were of 19.6, 27.3 and 37.5 days, respectively, according to the container. The first, second and third-instar larvae presented growth periods with highly similar durations. The fourth-instar larvae were longer than the others stages. The pupation time was longer than the fourth-instar larvae growth period (Gomes, 1995).

*Aedes aegypti* larvae go through four stages (instars) in a period of a few days, depending on the temperature, and the last instar culminates in the pupal stage (pupation). The larval stage is the only immature form of the mosquito that eats and it is supposed that the natural regulation of *Aedes aegypti* populations occur due to intraspecific competition for food and other resources in this stage. While adults, which also eat, are capable of flying in search of food, larvae cannot and are restricted
to live and eat in the container where they were born (Southwood et al., 1972; Gleiser et al., 2000).

Field studies were carried out to determine the relative efficacy of repellant action of vegetable, essential and chemical base oils against vector mosquitoes. Results revealed that essential oils viz. Cymbopogan martinii martinii var. Sofia (palmarosa), Cymbopogan citratus (lemon grass) and Cymbopogan nardus (citronella) oils are as effective as chemical base oil namely mylol. These oils provide almost complete protection against Anopheles culicifacies and other anopheline species. Per cent protection against Culex quinquefasciatus ranged between 95-96%. Camphor (C.camphora) oil also showed repellent action and provided 97.6% protection against An.culicifacies and 80.7% against Cx.quinquefasciatus (Ansari and Razdan, 1995).

2.2.1. *Azadiracta indica* (Neem) as a bio pesticide

Neem products containing azadirachtin and other ingredients have anti-feedant, ovipositional deterrence, repellency, growth disruption, sterility and larvicidal action against insects. Neem oil and other commercial preparations of neem have been found as potential mosquito larvicide (Mittal et al., 1995). Dhar et al., (1996) demonstrated the effect of neem oil volatiles on gonodotropic cycle and inhibition of oviposition in An.stephensi and An. culicifacies. Control of mosquito breeding has also been demonstrated in the field in some confined habitats using indigenous methods of application of neem oil in water and neem oil coated on wooden scraps (Ipek et al., 2004 ; Nagpal et al, 1995). Neem cake powder and urea coated with neem cake powder were evaluated for the control of mosquito breeding in rice fields (Rao et al., 1992). Zebitz (1984) suggested that azadirachtin acts as an anti-ecdysteroid and thus kills the larvae by growth inhibition effect. This, along with other delayed effects of neem products (Dhar et al., 1996) provides an alternative approach to chemical larvicides in mosquito control.

The neem plant (*Azadirachta indica*) and its derived products have shown a variety of insecticidal properties on a broad range of insect species (Isman, 2006; Schmutterer, 1992, 2002). Neem products have been shown to exhibit a wide range of effects that are potentially useful for malaria control and include antifeedancy
(Lucantoni *et al.*, 2006), ovicidal activity, fecundity suppression (Su and Mulla, 1998), insect growth regulation (Mordue, 1993) and repellency (Singh *et al.*, 1996; Sharma *et al.*, 1993). These effects are frequently attributed to the azadirachtin contents of the products (Isman, 2006; Mordue, 1993). Recent studies have also demonstrated neem-induced effects on vitellogenesis and severe degeneration of follicle cells during oogenesis in mosquitoes (Lucantoni *et al.*, 2006). It has been argued that the pesticidal efficacy, environmental safety, and public acceptability of neem and its products for control of crop pests would ensure its adoption into mosquito control programmes (Su and Mulla, 1998).

Neem seeds contain approximately 99 biologically active compounds of which azadirachtin, nimbin, nimbidin and nimbolides are major molecules. Many of these derived products have antifeedancy, ovicidal activity, fecundity suppression besides insect growth regulation and repellency against insects (Lucantoni *et al.*, 2006) and can play a vital role in mosquito control measures (Sukumar *et al.*, 1991). Although intensive work on neem as natural insecticides in Nigeria began in 1981 in the crop protection Unit of the Department of Agricultural Extension Services, University of Ibadan (Ivbijaro, 1987), little attention has been given to the potential of neem in mosquito control in Nigeria (Aleiro, 2003). Extracts of different parts of Neem tree have been effective at killing mosquito larvae both in the laboratory and field (Okumu *et al.*, 2007; Howard *et al.*, 2009). This technique is environmental friendly, biodegradable, less expensive, and locally available in mosquito endemic areas.

Azadirachtin, a limonoid, has been reported to have adverse effect on endocrine system of a bean beetle, Epilachna varivestis, and to cause sterility in the female insects (Schluter and Schulz, 1983). They also reported that azadirachtin, compound caused degradation in larval epidermis prevented the larvae from moulting. The effect of crude aqueous extracts of *Azadirachta indica* (neem) against the larvae of *Anopheles* mosquito was investigated by Aliero in 2003. Exposure of the larvae to undiluted extracts of seed oil, leaf and bark for 12 hours led to 100, 98, and 48% mortality, respectively. Dilution of these extracts also resulted in mortality of the larvae. It was suggested that the seed oil and leaf extract of neem contain properties that could be developed and used in the control of mosquitoes in the tropics.
Ethno botanical survey in 2 communities in western Kenya revealed that the most commonly known repellent plants were *Ocimum americanum* L. (64.1%), *Lantana camara* L. (17.9%), *Tagetes minuta* L. (11.3%) and *Azadirachta indica* A. Juss (8.7%) on Rusinga Island, and *Hyptis suaveolens* Poit. (49.2%), *L.camara* (30.9%) and *O.basilicum* L. (30.4%) in Rambira. Direct burning of plants is the most common method of application for *O.americanum* (68.8%), *L.camara* (100%) and *O. basilicum* (58.8%). Placing branches or whole plants inside houses is most common for *H.suaveolens* (33.3 and 57.8% for the respective locations), *A.indica* (66.7 and 100%), and *T.minuta* (54.8 and 56.0%). The repellency of plants suggested by the ethnobotanical survey and other empirical information was evaluated against the malaria vector *Anopheles gambiae s.s.* Giles in experimental huts within a screen walled greenhouse. Thermal expulsion and direct burning were tested as alternative application methods for the selected plants (Seyoum *et al.*, 2002).

Abba *et al.*, (2006) conducted laboratory bioassays to evaluate the pupicidal activity of neem (*Azadirachta indica*) seed kernel extracts (NSKE) on *Aedes aegypti*. The neem seed kernel powder was sequentially extracted with hexane, benzene, ethyl acetate, acetone, DMSO, 2-propanol, ethanol, methanol and distilled water. Ten concentrations (0.0, 0.25, 0.5, 1.0, 2.0, 4.0, 5.0, 10.0, 15.0 and 20.0%) of the neem extracts were used for the bioassays. The ability of some neem extracts to kill Aedes pupae at relatively low concentrations presents an alternative to the use of synthetic pesticides for control of mosquitoes.

Neem oil has good larvicidal properties for *An.gambiae s.s.* and suppresses successful adult emergence at very low concentrations. Considering the wide distribution and availability of this tree and its products along the East African coast, this may prove a readily available and cheap alternative to conventional larvicides (Okumu *et al.*, 2007). The combination of neem and karanja oil cakes in equal proportion proved to have better effect than the individual treatments (Shanmugasundaram, *et al.*, 2008).

Crude neem products have earlier shown considerable promise for control of culicine mosquito vectors in rice fields as a by-product of their agricultural use as
fertilizers, but suffer from disadvantages of bulkiness and lack of stability in storage. Relatively stable lipid-rich fractions of neem were shown to be as effective as good-quality crude neem products in control of breeding of culicine vectors of Japanese encephalitis, and also produced a slight but significant reduction in populations of anopheline pupae (Rao et al., 1995). Howard et al., in 2011 studied the oviposition deterrent activity of neem in Anopheles gambiae mosquito.

2.2.2. Strychnos nux-vomica as a bio pesticide

*Strychnos nux-vomica* is the name of an evergreen tree native to Southeast Asia, especially India and Myanmar, and cultivated elsewhere. Its dried seeds or beans, and sometimes its bark (called *nux vomica*) are used in herbal remedies. The seeds contain organic substances, strychnine and brucine, that are used in herbal remedies. *Strychnos nux-vomica* is one of the ingredients used, in small amounts, in traditional Chinese herbal treatments for liver cancer and numerous other health problems. Alkaloids are the main bioactive chemicals in nux-vomica (Bisset and Phillipson, 1976), responsible for the pharmacological and toxic effects exerted by nux-vomica to a great extent. 16 alkaloids have been separated and identified from the crude nux-vomica, among which strychnine and brucine take up 80% (Cai et al., 1990). Based on the principles of Chinese medicine, nux vomica is commonly prescribed in clinical practice after sand processing, the purpose of which is not only to clean its fine hairs which causes throat irritation (Wu Yin et al., 2003) but also reduce its toxic side effects because after processing, the intrinsic alkaloids such as brucine and strychnine transform into their isofoms or nitrogen oxidative derivatives with less toxicity (Cai et al., 1990).

*Strychnos nux-vomica* L. (Loganiaceae), widely used in Chinese folk medicine, is grown extensively in southern Asian countries (Bisset and Phillipson, 1976). The dried seeds of this plant have been claimed to improve blood circulation and relieve rheumatic pain (Guizhi, 1996). Historically, this plant has been widely used in treating diseases, such as tumor and rheumatic arthritis. Phytochemical analysis has identified alkaloids as the major components of this species (Bisset and Phillipson, 1976). A few related studies also showed that these alkaloids are to a great
extent, responsible for the pharmacological properties of *Strychnos nux-vomica*, such as cytoprotective and antitussive activities.

*Strychnos nux-vomica* L. (Loganiaceae) seeds have been used in activating the channels, alleviating pain, reducing swelling, and moving the blood in oriental medicine (Bensky and Gamble, 1986). The pharmacological effects of this plant have also been known to increase spinal reflexes and stimulate respiratory and sensory centers of the cerebral cortex. In oriental medicine, *Strychnos nux-vomica* seeds have been used in combination with aconite roots to treat spasms, numbness, or weaknesses associated with wind damp painful obstructions, and with myrrh to treat trauma-induced pain, swelling, fractures and sprains topically, and with sophora roots to treat severe and painful swelling of the throat.

Larger doses of strychnine are known to be a deadly poison, which leads to violent muscular convulsions (Han, 1988). In addition, strychnine is known to be severely toxic in human beings, although small doses of this compound can give subjective feelings of stimulation.

Native tribes in Central and South America have also used extracts from this plant for centuries as a medicine to inhibit muscle contractions and as a poison for the tips of arrows. Some physicians used *Strychnos nux-vomica* in the treatment of stomach cancer in the late nineteenth century. It was given to patients to induce vomiting, which was felt to help relieve the patient's discomfort. Phytochemically the plant has been reported to contain alkaloids like strychnine, brucine, and strychnicine and glycosides like loganin, caffeotannic acid and also traces of copper (Wu et al., 2003; Yang et al., 1993). In pharmacology, only few activities such as analgesic (Yin et al., 2003), apoptotic effect, antidepressant (Yarnell and Abascal, 2001), antidote for snake poisoning (Grieve, 2007) antitumor have been proved so far.

Wu Yin et al. (2003) developed various pain and inflammatory models to investigate the pharmacological profiles and to further understand the purpose of the traditional processing method of the seeds of *Strychnos nux-vomica* L. (Loganiaceae) as well as analgesic and anti-inflammatory activities of brucine and brucine N-oxide.
Both brucine and brucine N-oxide revealed significant protective effects against thermic and chemical stimuli in hot-plate test and writhing test. However, on different phases they exerted analgesic activities in formalin test. Brucine N-oxide showed stronger inhibitory effect than brucine. These results suggest that central and peripheral mechanism are involved in the pain modulation and anti-inflammation effects of brucine and brucine N-oxide, biochemical mechanisms of brucine and brucine N-oxide are different even though they are similar in chemical structure. Zhang et al. (2011) isolated three iridoids, 6-O-acetylloganic acid, 4-O-acetylloganic acid and 3-O-acetylloganic acid together with two known iridoid glucosides, loganic acid and 7-O-acetylloganic acid from seeds of Strychnos nux-vomica.

The content of strychnine from Strychnos nux-vomica seeds was analyzed and compared to processed seeds by the HPLC–ESI/MS method. Using this technique, levels as low as 1 ng of strychnine were detected. In contrast to conventional UV detectors, this method also made it possible to discriminate brucine. The content of strychnine in detoxified seeds to be one tenth of unprocessed Strychnos nux-vomica seeds. Promsiri et al., (2006) reported that Strychnos nux-vomica, Knema globularia, Stemona tuberosa, Samanea saman, Annona muricata, Abutilon indicum at a concentration of 100 μg/mL showed moderate percentage mortality of larvae (93%, 88%, 80%, 78%, 69% and 57%, respectively), with their LC_{90} after 48 hours < 200 μg/mL, 82.6-130.3 μg/mL respectively. They also stated that based on the 96 h lethal concentration, Ricinus communis, Datura alba and Strychnos nux vomica showed the strongest piscicidal activity to fish. During exposure, fish exhibited discoloration, gulping for air, erratic swimming, loss of reflexes, slow opercular movement and ultimately settling at the bottom motionless. The trial toxicity tests showed that locally available plants have the potential to be used as piscicides, which can be an alternate to an expensive and scarcely available imported rotenone for eradication of undesirable fish species present in fish ponds.

Yin et al.,(2003) studied the anti-tumor effects of alkaloids from the seeds of Strychnos nux-vomica and reported that among the four alkaloids, strychnine and brucine were selected to represent the alkaloids stemmed from crude nux- vomica,
whereas isostrychnine and brucine N-oxide stood for the alkaloids from processed nux-vomica. The results of their investigation might provide a scientific explanation for the traditional application of this herbal medicine in liver cancer therapy. In addition to its anti-tumor effects, nux-vomica is also claimed to have an ability of improving circulatory system and relieving pains in patients with rheumatic disorders. However, the mechanism underlying the anti-tumor property of the nux-vomica remains largely unknown.

Chitra et al., (2010) studied the anti-diabetic and antioxidant effects of the methanolic extract of Strychnos nux-vomica in alloxan induced diabetic model. The antidiabetic activity of the methanolic extract of Strychnos nux-vomica was evaluated in normal and alloxan induced diabetic rats. Increased body weight and decreased blood glucose level of the test animals show that the extract exhibited significant antidiabetic activity when compared to diabetic control group.

2.2.3. Bacillus thuringiensis as a biological pesticide

Microbial larvicides such as Bacillus thuringiensis israelensis (Bti) and Bacillus sphaericus (B. sphaericus) are gram-positive, aerobic (facultative anaerobic) entomopathogenic soil bacteria (Johnson et al., 1995., de Maagd et al., 1999, Bobrowski et al., 2002). Moreover, vector control is an essential and effective means for controlling transmission of vector-borne diseases, especially in areas where resistance in parasite to drugs have been proven (Jekow et al.,1998). Unlike insecticides, bio-control agents are host specific, safer to the environment, find easy application in the field, are cost-effective in production, lack infectivity and pathogenicity in mammals including man and has little evidence of resistance development in target mosquito species (WHO, 1981).

Bacillus thuringiensis subspecies israelensis is widely accepted as a biological pesticide because of its highly specific activity against dipteran insects without adverse effects on other organisms and because no resistance has been developed by the target mosquito larvae (Georghiou and Wirth 1997). Foo and Yap (1982) reported that the LC$_{50}$ values for A.aegypti, C.quinquefasciatus, Anopheles balabacensis, and Mansonia indiana after a 48-h exposure to B. thuringiensis H-14 (San 402-I)
formulation were 54.6, 223.1, 405.1 and 177.6 ITU A.aegypti/L and to B.thuringiensis H-14 (Bactimos) formulation were 57.2, 175.7, 35.6 and 514.5 ITU A.aegypti / L, respectively. The decreased mortality (by 25%) of Aedes taeniorhynchus larvae is observed when the same weight of B. thuringiensis subsp. israelensis preparation has been applied to pans of 9cm depth than of 3cm (Nayer et al., 1999). They have also reported that the insecticidal activity and antifeedant effect of a new type biocides GCSC-BtA and its two reactants B.thuringiensis (B.ti) crystal and Abamectin against third-instar larvae of Plutella xylostella showed significantly higher toxicity with LC\textsubscript{50} of 0.021 mg/ml than B.t crystal with 0.060 mg/ml and Abamectin with 0.139mg/ml in leaf dip bioassay and the net leaf-dip test gave LC\textsubscript{s} of 0.073, 0.071 and 0.670 mg/ml for GCSC-BtA, B.t crystal and Abamectin, respectively. The LC\textsubscript{s} of FCSC-BtA B.t formulation (Dipel, 16,000IU) and diazion to P.xylostella collected from vegetable field were 0.3445, 0.8404 and 1.3160 mg/ml respectively.

Murugan \textit{et al}. (1999) found that B.t var. \textit{Kurstaki} considerable inhibited food consumption of \textit{H. armigera} due to which the efficiency of conversion of ingested and digested food was significantly reduced and the profiles of digestive enzyme. Espinel (1981) observed that third-instar larvae of \textit{Mamestra brassicae} avoided \textit{B.var thuringiensis}-treated cabbage leaves. \textit{B. thuringiensis} isolates were examined for oral toxicities against larvae of the silkworm, \textit{Bombyx mori}, and the dengue fever mosquito, \textit{A.aegypti} and reported that an isolate of sero var israelensis (H14) exhibited strong larvicidal activities against both lepidopteran and dipteran insects. Haq \textit{et al} (2004) have reported that the two bacterial larvicide (bio-larvicide) formulations, Bacticide ® and VectoBac ® containing viable endospores and delta endotoxin of \textit{B. thuringiensis var israelensis} H-14 were evaluated and VectoBac produced reduction in the density of third and fourth-instar larvae of \textit{A.stephensi} (98-100%) and \textit{A.aegypti} (100%) in the first week of application whereas Bacticide produced 71-100% reduction in \textit{A.stephensi} and 100% in \textit{A.aegypti}. Decrease adult densities of these mosquito species is to treat the larval sites in ephemeral wetlands with the crystalline pre-toxins produced by the bacteria \textit{B.thuringiensis israelensis} (bti) and the treatment is highly efficient against mosquito larvae, causing almost 100% mortality, and has been shown to have very limited direct effect on other
organisms when applied at the appropriate dose for control of mosquito larvae (Boisvert and Boisvert, 2000).

Sreshty et al. (2011) reported that the toxicity of the wild-type *B. thuringiensis* subsp *israelensis* H 14 (Bti) and *Bacillus sphaericus* -2362 (Bs) was determined towards *Ae. aegypti* larvae, the LC50s were estimated to be 0.094 and 1.8 µg/ml and LC90s to be 0.179 and 2.12 µg/ml, respectively and the Bti and Bs spore-crystal toxins were assayed in six different proportions that resulted in LC50 and LC90s varying from 0.018 to 1.51 µg/ml and 0.090 to 2.88 µg/ml, respectively. The strain improvement by combining five major toxins in to Bti/Bs recombinant has been more effective for delaying mosquito resistance (Zahiri et al., 2004; Park et al., 2005), but the risk of conjectural hazards associated with the deliberate release in field conditions has failed to warrant the commercial development of recombinants as bio larvicides (WHO, 1996; Rao et al., 1995).

Zahiri and Mulla (2005) observed that lower numbers of egg rafts have been recorded in ovitraps treated with Bti and *B. sphaericus* than in controls, and furthermore, an adverse relationship was found between the *Bacillus* product concentration and oviposition of *Aedes aegypti*. Earlier, Murugan et al., (2002, 2003 & 2007) investigated the interactive effect of botanicals and bacterial insecticides (*Bacillus thuringiensis* Subsp. *israelensis* and *Bacillus sphaericus*) on different species of mosquito species.

### 2.3. Copepods as a bio control agent

Copepods are widespread in ponds, lakes, streams, and small reservoirs in tropical and subtropical regions. The first description of copepods in Costa Rica was reported by Picado in 1913 in the water puddles caught in bromeliads, that he called “aerial swamps”. The ability of certain cyclopoid copepods to destroy larval mosquitoes was first noted in 1938 by Hurlbut. Collado et al., (1984) reported a systematic description of copepods from fresh-water bodies in Costa Rica and indicated that the genus *Mesocyclops* (Harada) was endemic. Thirteen years later, this species was selected as a means of biological control for the Dengue-fever-moquito *Aedes aegypti* in Costa Rica (Hernandez et al, 1999).
The exceptional potential of copepods for mosquito control was first recognized by Riviere and Thirel in 1981, who observed in Tahiti that the number of *Ae.aegypti* and *Ae. polynesiensis* larvae was greatly reduced in ovitraps that contained *Mesocyclops aspericornis* accidentally introduced with creek water. Marten (1984) independently discovered the same for *M.aspericornis* with *Ae.albopictus* larvae in artificial containers in Hawaii, and Suarez et al. (1992) did the same for *Ae.aegypti* larvae in water storage tanks in Colombia. Some of the larger species of cyclopoid copepods, as predators of first and second-instar mosquito larvae, are now in operational use to eliminate *Aedes* larvae from container habitats such as tires, water storage tanks, and wells (Marten et al. 1994a, Nam et al. 1998).

Since then, various species of predacious copepods have been tested for their potential to control mosquitoes. Most of them are omnivorous and prey on immature mosquitoes, especially first-instar larvae, but rarely on later stages (Williamson 1999; Marten et al., 1989). Several species of copepods, including *Mesocyclops aspericornis*, *M. thermocyclopoides*, *M.guangxiensis*, and *M.longisetus*, have been reported as potential biological control agents of *Ae.aegypti*. (Locantoni et al., 2006; Manrique-Saide et al., 1998; Schaper 1999; Rawlins et al., 1997). *Mesocyclops longisetus*(Thiebaud), *Mesocyclops thermocyclopoides* Harada, *Mesocyclops venezolanus* Dussart, and *Macrocyclops albidus* (Jurine ) were tested for their effectiveness in controlling *Aedes aegypti* (L.) larvae in a variety of containers around homes in El Progreso, Honduras. All four cyclopoid species killed >20 larvae per cyclopoid per day under container conditions (Marten et al., 1990a,1994;Nam et al., 1998).

Cyclopoids are more effective for biological control than other predatory invertebrates because it is common for cyclopoids to be numerically abundant even when mosquitoes are not present (Marten et al., 1994). The potential of cyclopoid crustaceans for the control of mosquitoes has been undergoing evaluation since the early 1980's (Marten, 1984; Riviere & Thirel, 1981; Suarez, 1992). Field trials and even cyclopoid-based programs for mosquito control have been carried out in many countries including Australia (Brown, 1991), the United States (Marten et al., 1994), Honduras (Marten et al., 1994), Colombia (Marten et al., 1989) and Brazil.
The first large-scale field trials of cyclooids for mosquito control were with *Mesocyclops aspericornis* (Daday) in crab holes that served as habitat for larvae of *Aedes polynesiensis* Marks (Riviere et al., 1987b; Lardeux et al., 1992). *M.aspericornis* has also been field tested in tyres and domestic containers where *Aedes aegypti* (L.) breeds (Riviere et al., 1987b; Lardeux, 1992; Suarez, 1992). Other species of *Mesocyclops* have been observed to prey on *Ae.aegypti* or *Anopheles* larvae in the laboratory (Marten, 1989; Marten et al., 1989; Brown et al., 1991; Kay et al., 1992). *Mesocyclops* spp. was observed to have a pronounced negative association with larvae of *Anopheles albimanus* Wiedemann in ponds and other small bodies of water (Marten et al., 1989). In Australia, six species of *Mesocyclops* were evaluated as biological control agents of *Aedes aegypti* (Brown et al., 1991).

*Mesocyclops aspericornis* is a dominant species found in artesian wells and peridomestic containers in eastern Thailand. It has been shown to be an effective predator of *Ae aegypti* larvae in both laboratory experiments and field trials (Russell et al., 1996; Kay et al., 1992). Larvivorous copepods such as *Macrocyclops albidus*, *Mesocyclops longisetus* and *Mesocyclops aspericornis* are highly effective for controlling *Aedes* sp. larvae in discarded tyres (Marten 1990a, Riviere et al., 1987). They may also have considerable potential for controlling a variety of species of mosquito larvae in groundwater habitats (Marten 1990b).

Many species of *Mesocyclops* with biocontrol potential against mosquito larvae are relatively easy to culture, to maintain, and to deliver to the target areas (Kumar and Rao, 2003; Kumar and Rao, 1999b; Marten et al., 1994b, 2000; Rey et al., 2004). Some of the common container dwelling mosquito species, such as *Ochlerotatus notoscriptus*, prefer containers that are not exposed to direct sunlight, and this is only rarely found in containers such as stock troughs (Lee et al., 1982; Laird 1990, 1995). Cyclopoid copepods can survive in and colonize such habitats whereas other predators are more likely to find larger habitat patches.

Cyclopoid copepods are abundant in eutrophic water bodies and play important roles in their trophodynamics. Like many predators in aquatic environments, cyclopoid copepods are known to strongly influence the structural and functional organization of prey communities on which they feed. Cyclooids are
predominantly carnivorous but with an ability to utilize plants as well (Kumar and Rao 1999b; Kumar and Hwang, 2005). Cyclopoids have a wide spectrum of potential food items available in their habitat, including algae, ciliates, rotifers, cladocerans, and mosquito larvae. The patterns of prey selection and feeding rates in this group of invertebrate predators have been the focus of considerable research (Williamson, 1986; Rao and Kumar, 2002). Some cyclopoids have long been known to have the ability to utilize mosquito larvae as food (Hurlbut, 1938; Fryer, 1957; Brown et al., 1991; Marten 1990a; Marten et al., 1994a; Kay et al., 1992). Kumar and Rao (2003) conducted a series of behavioural observations of the cyclopoid *M. thermocyclopoides* in handling and predating on mosquito larvae. Cyclopoids now appear to offer high promise as biological control agents for *Ae. aegypti* (Marten et al., 1994a), *An. stephensi* (Kumar and Rao, 2003) and *Ae. albopictus* mosquitoes (Rey et al., 2004).

Williamson and Reid (2001) studied the ecology of copepods and their abundance in different seasons. They conducted the laboratory and field studies of *Macrocyclops albidus* (Crustacea: Copepoda) for biological control of mosquitoes in artificial containers in a subtropical environment. To understand the predatory capacity of *Mesocyclops thermocyclopoides*, the damage caused by them was evaluated using scanning electron microscopy by Schaper et al., 2006. Planktonic copepods are key organisms in aquatic ecosystems, because they are the most important link between the primary productivity of micro algae and the production of many fish species in the oceans (Irigoien et al., 2002; Tseng et al., 2008). Ramanibai and Kanniga (2008) tested the predatory capacity of *M. aspericornis* against *Aedes aegypti* mosquitoes.

A pilot community-based intervention using integrated physical and biological control, including a combination of copepods and Bti, in eastern Thailand was successful and showed potential for expansion into other areas (Kittayapong et al., 2006). Several species of copepods, including *Mesocyclops aspericornis*, *M. thermocyclopoides*, *M. guangxiensis* and *M. longisetus*, have been reported as potential biological control agents of *Ae. aegypti* (Rawlins et al., 1997; Manrique-Saide et al., 1998; Schaper 1999; Lucantoni et al., 2006). The efficacy of a local Thai-strain of the copepod, *Mesocyclops thermocyclopoides* and the larvicide, *Bacillus*
thuringiensis var. israelensis (Bti), used jointly and singly studied against Aedes aegypti in water containers (Chansang et al., 2004). Murugan et al., (2011) studied the efficacy of plant extracts (neem tree, Azadirachta indica A. Juss.; Meliaceae) and copepods [Mesocyclops aspericornis (Daday)] for the control of the dengue vector Aedes aegypti L.

The seeds of Strychnos nux-vomica, selected for the present study has been already used as rodenticide and fungicide but, its mosquitocidal activity has not been explored so far. Even though, information is glut for neem and Bti as bio pesticides, as far as our literature survey could ascertain, no information was available on the mosquitocidal activities of, Azadirachta indica and Bacillus thuringiensis in combination with Strychnos nux-vomica. The aim of the present work is to explore the potential of these bio pesticides in combination. Mosquito control using copepod, Mesocyclops species is also not common in India. So, the present investigation throws light on the efficacy of copepods with these biological products to control Aedes aegypti population.