5. DISCUSSION

An understanding of the biology of any species is important before evolving any control strategy (Mattingly, 1963). The early detection and effective monitoring of vector breeding habitats is paramount importance (Hill et al., 1981 and Crane and Crampton, 1987). All mosquitoes breed in water. As bacteria, algae, yeasts, etc., are utilized by mosquito as food materials, certain breeding places are richer in one or another type of food. So, this factor along with saline condition influence the suitability of a breeding place for a given species (Merritt et al., 1992).

The filarial vector mosquito, Culex quinquefasciatus is said to be an ubiquitous breeder since it can breed in any type of habitat which contains water. However, this species prefers the water with decayed organic matter (Srinivanakaran, 1976 and Rajagopalan, 1990). When an attempt was made in the present study to assess the chemical parameters of the sewage water, wherein the mosquito species usually breeds, it shows higher values in all parameters when compared to tap water, particularly in salt content.

The presence of high salt may be the reason for the high total hardness, alkalinity and pH when compared to tap water. Sinha (1976) also recorded high salt content in the breeding sites of the
mosquito. Thereby, it seems that higher salt content of the medium favours the larval life. But, in the present study, when a tray containing tap water and another tray containing tap water with yeast material were placed together (adjoining to each other) in open condition, the wild mosquitoes preferred the water with yeast for their breeding purpose. No one animal preferred the tap water. It indicates that the mosquitoes are assumed to be having some sense of chemoreception to detect food materials from where it is available. In this regard, it may be pointed out that odour plays a role in differential oviposition site behaviour (Strickman, 1982). Almost the same view has been given by Benzon and Apperson (1988) that cues by the presence of microflora and their metabolites change the oviposition behaviour.

The other experiment of the present study also indicates that the mosquito species prefers water which is having higher amount of food materials for their oviposition. It is evident by higher number of egg-raft collection in water medium having higher amount of yeast material when compared to other medium having less amount of yeast present in the same volume of water.

The attraction by the yeast medium may be attributed for the change in chemical nature of the tap water towards the chemical nature of the sewage water (Tables 2 and 3). This observation is getting support by the report of Merritt et al.(1992) who stated that
high percentage of ammonia, organic carbon, nitrates and high concentration of salts with slightly alkaline pH seems to attract the gravid mosquitoes for oviposition. Krida et al. (1997) and Wotton et al. (1997) have also expressed their view that mosquito respond favourably to increased amount of organic matter in water. These authors have expressed their view based on the positive correlation of presence of higher number of eggs and larvae in highly polluted natural water bodies. The present study gives an experimental proof that only the tap water with yeast attract the gravid mosquitoes for oviposition whereas the tap water alone is not attracting the mosquitoes. Moreover, the high salt content seems to play a role for the attraction of gravid mosquitoes as evidenced by the observation of higher number of eggs in tap water having the concentration of yeast when compared to distilled water having the same concentration of yeast. (Table -5).

It is also interesting to note that the addition of yeast material to tap water displaces the oxygen content from the medium. It is reported from the present study by an attempt to measure the dissolved oxygen content of the sewage water, tap water and the yeast medium prepared with yeast pellets. The yeast medium and sewage water showed absence of dissolved oxygen whereas the tap water showed the dissolved oxygen to 3.37ml/litre. Generally, it is said that polluted water is free from dissolved oxygen (Michael, 1984). Based
on this view, it may be said that the tap water because of the addition of yeast is now converted to polluted medium and thereby it might have attracted the wild mosquitoes for their oviposition purpose.

With regard to the nutritive value of a food for the proper growth of mosquito larvae has been reported by Singh and Brown (1957). These authors have reported that the larvae of *Aedes aegypti* grew on a complete synthetic diet consisting of 17 amino acids, 12 vitamins, glutathione, 8 inorganic elements, 3 lipids, RNA and glucose and adult so obtained laid viable eggs. In this connection, it is appropriate to point out that all the essential vitamins and other ingredients for the larval growth are present in baking yeast body of *Saccharomyces cervisiae* (Pelczar *et al.*, 1983). From ecological point of view, the above authors have stated that a few surveys including fresh, salt and estuarine waters have shown widespread incidence of yeast in all events studied. If this is so, then the presence of any kind of yeast which can survive in aqueous condition may be expected to be present in any polluted water bodies such as sewage water and this condition may be reason for the preference of polluted water by the mosquito species for their oviposition.

From this study it is understood that yeast material as food is preferred by the action of its detection, perhaps, by chemoreception. The preference of yeast medium which is prepared by the tap water
for the oviposition by neglecting the tap water medium gives the strength for the above assumption. At the same time it could not be considered that yeast only is the food material for mosquito larvae since it has been already reported by Merritt et al. (1992) that microorganisms and particulate organic detritus generally constitute the major part of the larval mosquito diets. Earlier to this report De Meillon et al. (1945) have pointed out that *Aedes aegypti* larvae can complete its life cycle from egg to adult in a medium of yeast.

As sterol is needed for growth and metamorphosis in larval development (Robbins et al., 1971) and in all cases cholesterol can fulfill this need (Dadd, 1960), it may be expected that yeast is also providing some sterol nutrient for the growing larvae. Moreover, the larvae were fed only with the Baker’s yeast in the present study from the time of hatching (first instar) to fourth instar which in turn enter to pupal stage (non-feeding stage) and then to adult. The emerged adults were observed to be healthy and laid viable eggs after blood feeding. It indicates that proper sterol component should have been derived from the yeast material for their metamorphosis. The above said assumption is based on the general view that the insect group is lacking its sterol biosynthetic mechanism and hence it needs a dietary or exogenous source of sterol.

It is also assumed from the present study that better growth of larvae depends upon the amount of available nutrients in the
surrounding medium. It is evident from the experiment wherein equal number of egg-rafts (2 rafts) were placed in different trays having equal volume of tap water with different amount of yeast material like 100, 250, 500 and 1000 mg/litre. Better growth in terms of reaching pupal stage and adult emergence was observed only in the highest concentration of the experimental set up whereas the larvae in other trays were observed to be in second stage to fourth staged larvae. This kind of observation is in full agreement with the report of Krida et al. (1997) that mosquito respond favourably to increased amount of organic matter in water.

As far as the yeast food material of mosquito larvae are concerned, it has been reported that there are 18 yeast species belonging to 7 genera. It has also been reported that Brewer’s yeast is the richest from nutritional stand point. The Brewer’s yeast is said to be having all the vitamins of B complex, mineral salts, 45% protein and 35% carbohydrates. The yeast in addition to supplying calories also supplies ample material for tissue building (Zucoloto, 1987). In the present study also it has been observed a difference in total carbohydrate, protein and cholesterol between the Baker’s yeast and Brewer’s yeast. When the larval growth rate of the mosquito larvae of Culex quinquefasciatus, was assessed by treating the larvae with equal amount of these two yeast materials separately in equal volume of water, difference in larval growth rate is not observed. Thereby, it
may be assumed that Baker’s yeast is also functioning as a good diet for the mosquito larvae equal to the Brewer’s yeast.

From ecological point of view it has been reported that out of the large pool of various types of water body, mosquitoes select small bodies of stagnant water, shallow, partially isolated, rich in vegetation and organic matter (Dabrowska, 1979). According to Srinivanakaran (1976), the filarial vector mosquito *Culex quinquefasciatus* is the most common domestic species occurring abundantly in houses and in practically all types of human and animal shelters in urban communities throughout South East Asia. The success of its survival is mainly because of its breeding habit. It breeds in any type of habitat as pointed out by Dabrowska (1979) particularly it prefers ditch water (Rajagopalan, 1980), which is polluted with organic matter. Such polluted area is very common because of the rapid unplanned urbanization and hence these polluted areas are functioning as ideal breeding grounds for the filarial vector mosquito, *Culex quinquefasciatus* (Hettiarratchi et al., 2000).

From mosquito control point of view, it has been suggested that anti-larval method are more important, for once mosquitoes take flight only 25 percent can be eliminated (India Today, 1988). Based on such views, attempts are continuing in search of new compounds which are more effective against target species and safe to non-target organisms.
Although some authors (Stage, 1952; Das and Rajagopalan, 1979 and Mariappan et al., 1985) are of the opinion that chemical larvicides may have to be used to bring about drastic reduction of the mosquito population, the research workers may take advantage of the habitat of the mosquito larvae for their control measures. Since the filarial vector mosquito *Culex quinquefasciatus* and some other mosquitoes like *Armigeres* and others prefer the depthless ditches and stagnant sewage water in and around the urbanized area, the nature of the useless living medium of the larvae could be easily changed by the application of even the common salt. Any water living organism has to face the vital physiological process of osmo-ionic regulation other than the dissolved oxygen condition. In such a condition there is a limit of tolerance either for hypo or hyper osmotic condition. When a change in the salt content of the environmental medium in which an animal has to live, it would be subjected for a stress and finally it will lead to death.

It has been pointed out from the present study that by the addition of 1.5 gram of common salt to 100 ml of the tap water medium in which the mosquito larvae thrive well, leads to 100 percent mortality of the larvae. When 1.0 g of the salt is added to 100 ml of the water 50 percent mortality occurs on the third day in the mosquito population. Such mortality effect is only because of the disturbance in osmo-ionic regulation of the mosquito larvae as evidenced by the
shrinking effect of the anal-papillae, which are reported to be the main organ for ionic regulation (Meredith and Phillips, 1973). As no other reports are available in the osmo-ionic regulatory process of the mosquito larvae and in the control measure using salt, comparison is not made here. However, it may be suggested from the present study that the useless water in an unused area called sewage and ditch waters which are functioning as an ideal breeding ground for the filarial vector mosquito could be subjected for spraying the salt (common salt). Enhancement of salt condition to 1.5 percent level of the medium may control the mosquito population to certain extent without any drastic pollution effect.

When an attempt has been made in the present study to reduce the salt content with the addition of neem seed kernels, the additive effect is observed to certain extent but not to 100 percent. Although Attri and Prasad (1980) have reported that Azadirachtin of neem seed does the function as an effective larvicide. However, the increased mortality effect of neem kernel in an enhanced 1.0 percent salt medium (from 50 percent to 70 percent mortality) may be due to its effect as antifeedant, growth disruption, larvicidal action, hormonal imbalance (Schmutterer, 1990 and Vijayalakshmi et al., 1995). However, the mortality effect even upto 70 percent within 3 days should not be encouraged, since the remaining 30 percent population may develop resisting character in due course. So, enhancement of
salt content to 1.5 percent than to the original or normal level of salt condition may be encouraged because of its effect to 100 percent mortality of the mosquito population within 24 hours, thereby there will not be any chance for raising or developing resisting character. Moreover, this kind of approach is not only economical but also it is practically feasible to control mosquito population.

As an alternative to larval control, mosquito population, the other method is adult control. As said by Ramasamy et al. (1988) that prospects for the control of vector-borne diseases depend to a large extent on the ability to control vector populations. Due to the limitations of conventional methods of vector control, for example, insecticide resistance, logistic problems, costs etc., new methods may be supplemented with molecular, biological and immunological techniques. According to Kay and Kemp (1994) a vaccine method is suggested against ectoparasite as an alternative to chemical control.

Suspensions of micro-organisms employed for artificial active immunization are referred to as vaccines. Such preparations usually contain several different antigens. Other immunizing agents such as protein solutions are also used for certain types of artificial active immunization. However, the vaccine must be effective, that is, it must contain antigens that will stimulate the production of protective antibodies. At the same time, it must also be made safe by rendering the substances non-toxic or avirulent in immunizing doses.
When an attempt has been given with this basic principle, earlier workers like Alger and Cabrera (1972), Sutherland and Ewen (1974), Ramasamy et al. (1988) and Hatfield (1988a) have observed increased mortality and fecundity in mosquitoes fed on rabbits immunizing with mosquito antigens. The later author (Hatfield, 1988b) is of the opinion that anti-mosquito antibodies ingested with a blood-meal bind specifically *in vivo* to tissues, both inside and outside the mosquito gut. In so doing they may play an important role in mediating the detrimental effects seen in mosquito mortality and fecundity after blood feeding on immunized hosts.

Ramasamy et al. (1988) have observed a reduction in number of eggs laid and they are of the opinion that it may be a direct result of the presence of antibodies in the vitellogenic oocytes. This reaction might have occurred by immunization of the host with the antigen from 24 hours post engorged mosquitoes. This view has been strengthened by the report given by Hatfield (1988b) that there is no such reaction (antigen - antibody complex) in the ovarian tissues of the mosquitoes when they feed on blood from a host immunized with sugar-fed mosquitoes, but, other tissues like nervous tissue, muscle and fat body have been found to contain antibodies. This kind of immunological specificity of particular antibody has been reported earlier by Dufour et al. (1970) by using the antibody prepared against
the female sexual protein selectively give toxic effect to the cells of females both in vitro and in vivo by injection.

Large proteins are generally considered as better antigens because they have more potential determinants. Moreover, better immune response will be obtained by using more foreign antigen (Roitt, 1988). As vitellin (yolk protein) has been considered as female-specific-protein (Ziegler et al., 1996) composed by two subunits 200 and 65 kd in mosquitoes (Raikhel and Bose, 1988). In the present study, when vitellogenic process of the filarial vector mosquito has been studied, sufficient amount of yolk granules have been observed to be accumulated in each primary follicle of the newly emerged mosquito, when the mosquitoes feed on untreated host (chick). The yolk protein (vitellin) as evidenced by the two bands in the ovarian sample in the electrophoretic run of the present study may be functioning as an ‘immunogenic antigen(s)’ for producing antibodies in the body of a host subjected for immunization. With the above assumption, the present study has been started by administering the antigen to rabbits through the oral route instead of giving the antigens by the conventional parenteral administration like intramuscular, intradermal, intraperitoneal, intravenous and subcutaneous methods. The trial and error methods of giving antigens through oral route for immunization has given good results near to intramuscular approach.
in chick and rabbit, although it is generally assumed that digestive enzymes often hydrolyze and destroy the antigenic quality.

The mosquito which fed on orally immunized rabbits with different antigens prepared from whole homogenate of newly emerged, gravid and ovaries and freshly-laid egg-rafts of the mosquito, showed higher mortality for gravid antigens when compared to other antigens from other sources. The gravid antigen not only gave higher mortality to 60 percent but also it has given its influence for producing the membranous eggs with incomplete chorion formation. Such an interesting observation gives an encouragement and high hopes that administering the mosquitogenic antigens through oral route may be practiced as that of oral polio drops. In this instance the virus Polio myelitis which is taken in an attenuated condition orally invades the cells lining the intestinal tract and reproduces itself. Because of this reproduction a larger antigenic dose is produced than in fact administered. It has been suggested by Barrett (1978) that it would hold true for other antigenic materials that actually reproduce in the digestive tract.

In this regard it may be pointed out that micro-organisms like Escherichia coli (E. coli) in the intestinal parts of man and higher animals (Sinnott et al., 1958) and the acid-tolerant bacteria can colonize in the alimentary canal (Sharma, 1995). Recombinant DNA Technology is a recent innovation in the field of genetic engineering.
which has revolutionized the science of immunology. This technology is used for production of required antigens (parasite protein) in large amounts. Once the required antigen is identified, a fast growing bacterium like *E. coli* is induced to make the antigen in large quantities (Srivastava, 1991). So, this organism may be treated as bio-vector. By the Recombinant DNA Technology, the recombinants of *E. coli* may be produced and introduced into man and other domesticated animals as that of polio drops and thereafter it will work inside the animal bodies with the principle as described earlier pertinent to *Polio myelitis*.

It is rather a suggestive statement from the observation of the present study. As the present study has been dealt with very crude antigen preparation with different parts of mosquito and administered them orally without any adjuvant, it is premature to give any conclusive statement with regard to practical feasibility in this connection. But, it may be suggested that the 'vitellogenic process' thereby the reproductive capacity of the mosquitoes could be disturbed at least, since the vitellogenin or vitellin of different species of mosquitoes seem to be similar to certain extent (Raikhel, 1992). It is appropriate, in this context, to point out that the antibody induced against *Boophilus microphilus* cross-reacted with the extracts of the stable fly, *Stomoxys calcitrans* (Brown, 1985). So, a suggestion could be made here from the present study that an elaborative study in an
immunological laboratory will give a clear picture with regard to a common antigen to bring out a broad immune response against haematophagous arthropods.

From practical point of view, it may be suggested by the present study that the recombinant bacteria either *E. coli* or some other microbes, if it is standardized as that of *Polio myelitis*, may be administered orally or in the form of medical pills or capsules. Thereby, it may be easy for distribution to the public and other animals which are used as host for the blood-meal by mosquitoes.