Soil sampling: *Bacillus* is a ubiquitous organism and has been isolated from various habitats including soil, water and dead/dying insects in the past (Goldberg and Margalit, 1977; Menon *et al.*, 1982; Travers *et al.*, 1987; Manonmani *et al.*, 1987; Gupta *et al.*, 1991; Orduz *et al.*, 1992; Hastowa *et al.*, 1992). Martin and Travers (1989) reported isolation of *B. thuringiensis* in 785 out of 1185 soil samples which shows that in 70% of the samples, *B. thuringiensis* alone was present. On the other hand, Manonmani *et al.* (1990) found *B. sphaericus* in only 9 (3.37%) of 139 soil samples screened for *Bacillus* species. Since moribund larvae were difficult to obtain from the field in the present study, the upper crust soil in the mosquito-breeding habitats was chosen as the possible source of mosquito-pathogenic bacilli. This choice was influenced by the fact that the spores of these bacilli settle rapidly at the bottom of the water bodies (Mulligan *et al.*, 1980; Ignoffo *et al.*, 1981; Silapanuntakul *et al.*, 1983; Davidson *et al.*, 1984; Orduz *et al.*, 1992). Moreover the soil samples were collected aseptically from the bottom of only those stagnant ponds and paddy fields where the mosquito immature stages were prevalent during the post monsoon months of Oct.-Dec. 1997. In the collection of soil samples monsoons were avoided as it was assumed that the floods and water currents caused by heavy rains in Goa (average rainfall 2500mm from July-September) would wash away the bacilli and prevent their settlement at the bottom of the breeding habitat.

The isolation of 8 promising mosquito-pathogenic isolates from 4 soil samples indicates that soils of Goa are rich source of these strains. One could hope to get large number of mosquito pathogenic strains if extensive surveys are conducted and soil samples are collected from Goa and isolation of bacilli is then attempted in future. Interestingly, from a single mangrove soil sample, three distinct mosquito-pathogenic
isolates were obtained in the current study. Earlier, Manonmani et al. (1991) had also found that the root surface of these hydrophytes favored colonization and harbored mosquito-pathogenic \textit{B. sphaericus} and \textit{B. thuringiensis} strains along with other bacteria. They isolated 109 (78.4\%) strains from 139 samples from mangrove soils showing abundance of these strains in the mangrove ecosystem.

None of the new isolates in the present study was similar to the ones sprayed earlier in Goa. The sites of sampling were so selected that they were not only spread all over Goa but were also sufficiently away from the previous trial sites where the biolarvicides were sprayed for malaria control in Goa (Kumar et al., 1994; 1996 & 1997). This was done to prevent isolation of the same strains of \textit{Bacillus sphaericus} and \textit{B. thuringiensis israelensis} which were used in these trials. In the present study, we therefore intentionally took these earlier sprayed strains as reference strains and compared the new isolates with them for their biochemical characteristics and also dose-mortality response against the three vector species. Subsequently, the new isolates were indeed found distinct from the reference strains of \textit{Bs} (101) and \textit{Bti} (H-14) as became evident not only from their biochemical differences but also toxin profile.

\textbf{Screening and isolation:} The isolation method of Travers et al. (1987) could be considered as a standard with which the heat treatment and acetate selection finally permits selective isolation of bacilli. The advantage of this procedure is that it allows the isolation of those bacilli also which would be otherwise eliminated when the antibiotics are used during the selection process. However, this process is cumbersome and requires plating of all the samples which may include both mosquito pathogenic and non-pathogenic Bacilli strains. If the desired isolates are not found at all or insufficient
numbers there is enormous wastage of media and effort. In the present study therefore, a
new screening technique was devised (protocol 3.2.2.1) which when tested clearly
indicated the presence of mosquito pathogenic bacilli in the soil samples before they were
actually isolated. With this process, 9 out of 10 soil samples were recognised for
containing bacilli pathogenic to either Culicine or Anopheline larvae. With this screening
technique, a lot of time, effort and resources is expected to be saved during the isolation
of bacilli from the soil samples in future.

After successful screening, the isolation of bacilli from the selected soil samples
was carried out by the standard acetate selection method of Travers et al. (1987)
[modified by Carozzi et al., 1991]. NYSM (Nutrient Yeast Sporulation Medium) used for
the sporulation of the isolates before testing was found very efficient. It has been reported
that Mn$^{2+}$, Ca$^{2+}$ and Mg$^{2+}$ ions in this medium enhance sporulation (Myers & Yousten,
1980; Yousten & Davidson, 1982; Russel et al., 1989; Manonmani et al., 1991 and Smith
& Couche, 1991). On the basis of preliminary toxicity testing of all the isolates against
Cx. quinquefasciatus and An. stephensi larvae, 8 promising isolates from 4 soil samples
were short listed for further studies and the remaining soil samples were rejected. These
were code-named as KSD-1 to KSD-8. The criterion for this short listing was arbitrary
and only those isolates that produced 50% mortality or above against either Cx.
quinquefasciatus or An. stephensi were considered worth for characterisation and
evaluation.

**Identification and characterization of the new isolates:** Since all the 8 bacterial
isolates were rod shaped, Gram +ve or Gram variable, aerobically form refractile
endospores, therefore they belong to the genus Bacillus according to Bergey’s manual.
To identify the Bacillus species, we used key-2 of Gordon (1973) and that of Berkeley et al. (1984), because the key-2 of Gordon (1973) is not only based on biochemical tests but also takes into consideration morphological differences and the position of the spores in the sporangia, whereas Berkeley’s key (which is basically modified version of key-1 of Gordon) is primarily based on the biochemical characteristics of the species and does not give importance to spore shapes or their position in the sporangia.

It is claimed that these keys help in the tentative identification of the Bacillus species. Using these two keys the species identification of the isolates KSD-1 to KSD-6 tally, only difference being in case of isolates KSD-7 & KSD-8 (Table 4.5). Apparently isolate KSD-7 resembled with *B. thuringiensis israelensis* reference strain in its colony morphology and in producing rhomboidal parasporal crystals of various sizes during sporulation. However, using Berkeley’s key for its species identification, primarily on the basis of biochemical tests, this isolate was identified as *B. popilliae*. It may be mentioned that *B. popilliae* is a well known pathogen of Japanese Beetle and causes 'milky disease' in the grubs of these beetles. *B. popilliae* is known to be catalase –ve, grows slowly and require special media for the growth and observation. Infective spores (after ingestion) abundantly produced in the larvae of beetle, have not however been produced in vitro (Gordon, 1973). Interestingly the isolate KSD-7 not only survived serial transfers on nutrient broth but also readily sporulated and produced parasporal crystals in vitro on Nutrient Yeast Sporulating Medium. Though *B. popilliae* has been commercially used against Japanese beetles, there is no information about its pathogenicity against mosquito larvae available in the literature or on the internet. Therefore, we presume that if the identity of this isolate is finally confirmed as *B. popilliae*, this would be the first report of
pathogenicity of B. popilliae in the mosquito larvae of three medically important species, An. stephensi, Cx. quinquefasciatus and Ae. aegypti. Due to this controversy and pending reconfirmation, we preferred to label this species as Bacillus sp. (MTCC 3673).

The isolate KSD-8 is identified as B. brevis using Berkeley’s key and B. sphaericus by the Gordon’s key. Since this isolate produced terminal spherical spores (Fig.4.3.1.8B) similar to B. sphaericus unlike oval, ellipsoidal spores as in the case B. brevis, we assigned this isolate to B. sphaericus, following Gordon’s key. It may be mentioned that two of our isolates KSD-4 & KSD-7 were sent to IMTECH, Chandigarh and have been assigned the MTCC numbers as B. sphaericus 3672 & Bacillus sp. 3673 respectively by them.

From the biochemical analysis of the isolates we have found that four of our new isolates viz. KSD-5, KSD-6, KSD-7 & KSD-8 were able to reduce nitrate and thus could exist deep inside the soil. Interestingly the last three isolates have been isolated from the mangrove soil. Three of the isolates namely KSD-5, KSD-6 & KSD-7 were able to hydrolyse Tween 80, indicating their sharp protective ability against surfactants. Some of our isolates, viz., KSD-1, KSD-5 & KSD-6 were able to tolerate and grow at 7% NaCl, while one isolate KSD-6 was able to grow at a high temperature of 65°C which is the characteristic of B. stearothermophilus.

**Antibiotic sensitivity:** Our studies have revealed that the three new mosquito-pathogenic B. sphaericus isolates viz. KSD-2, KSD-4, KSD-8 and also B. sphaericus reference strain were sensitive to Chloramphenicol although resistant to Streptomycin. This does not fully conform to the findings of Burke et al. (1983) who reported that the pathogenic strains of
*Bacillus sphaericus* generally exhibit natural resistance to both Streptomycin and Chloramphenicol.

**Protein analysis:** The protein profiles of the isolates *B. sphaericus* KSD-4 (MTCC 3672), *Bacillus* sp. KSD-7 (MTCC 3673) & *B. sphaericus* KSD-8 highlight their differences from the reference strains. Larvicidal toxins of *Bti* & *Bs* are known to be proteins of different molecular weights (Federici *et al.*, 1990; Davidson & Yousten, 1990). Various authors have studied the protein profiles of *Bt* and *Bs* isolates. According to Poncet *et al.* (1995) some of the lower molecular weight proteins possess toxicity in synergism with larger proteins. Morris *et al.* (1998) related the toxicity of various isolates to proteins present in them.

The protein profile of the isolate *Bacillus* sp. KSD-7 (MTCC3673) showed 10 bands at various positions. Some of the bands at positions just above 68kDa, between 68-97kDa and one above 97kDa are quite similar to those reported by Pfannensteil *et al.* (1984) from the alkali solubilized toxin and native crystals of *Bti*. Chak *et al.* (1994) found that most of the *Bt* isolates produced three typical proteins in 130-140kDa region. They reported that some isolates produced one, while other produced two or three proteins in this vicinity and four of their isolates produced proteins of 130, 65, 40 and 27 kDa like *Bti*. In our studies *Bacillus* sp. KSD-7 (MTCC 3673) produced only one band above 97kDa although in the case of *Bti* reference strain three distinct bands were noticed above 97kDa region. However, there existed some differences in protein profile *Bacillus* sp. KSD-7 (MTCC3673) from those reported by Chak *et al.* (1994).

We compared the protein profiles of *Bacillus* sp. KSD-7 (MTCC3673) with that of *Bti* reference strain as both produce rhomboidal parasporal crystals as stated earlier.
These crystals are known to contain proteins responsible for their toxicity to mosquito larvae (Angus, 1965; Heimpel, 1967; Hoftey & Whiteley, 1989 and Federici et al., 1990). The protein profile of the *Bti* reference strain 164 however showed 11 bands, of which five bands were at the same position as *Bacillus* sp. KSD-7 (MTCC 3673). However the position of the remaining bands did not coincide in the two isolates. This marked differences support the earlier contention that the isolate *Bacillus* sp. KSD-7 (MTCC 3673) is markedly different from the *Bti* reference strain as suggested earlier by the differences in the biochemical characterisation.

According to Broadwell et al. (1990) and Davidson et al. (1990) highly toxic strains of *Bs* used in the fields produce proteins with masses of 51.5 & 41.9kDa which are together responsible for toxicity and accumulate as parasporal inclusions. Since the isolates *B. sphaericus* KSD-4 (MTCC 3672), *B. sphaericus* KSD-8 and *Bs* reference strain were toxic to mosquito larvae and showed only one band at position 51.5 kDa, it may be concluded that either this protein alone was responsible for toxicity or acted synergistically with some protein other than 41.9kDa, which was not detected in our study. Alternatively, some larger protein could lyse upon ingestion in the gut of mosquito larvae and produce this missing component of 41.9kDa which is known to act synergistically with 51.5kDa toxin. There are some reports on production of 100kDa toxin by *B. sphaericus* during vegetative growth (Myers et al., 1979 and Thanabalu et al., 1991). We also observed some proteins in this region in case of the new isolate *B. sphaericus* KSD-4 (MTCC 3672) & the reference *Bs* strain (Fig. 4.8.2). However, other significant differences were also observed in the protein composition of the three new
isolates from the two reference strains that indicate that our isolates are markedly different from the two reference strains.

**Plasmid profiles:** The plasmids of different sizes have been reported for many *Bt* strains by several investigators. Gonzalez and Carlston (1980), Clark & Dean (1983), Ward & Ellar (1983) and Aronson *et al.* (1986) observed that *Bti* had an array of plasmids. Kawalek *et al.* (1995) have reported that a new subspecies of *B. thuringiensis* with 9 plasmids of 135, 120, 105, 87, 85, 26, 17, 15 & 10kb and found that the plasmid profile of *B. thuringiensis jegathesan* was distinctly different from that of *B. t. israelensis*. According to them the 120 or 105 kb plasmid is required for the production of parasporal inclusions, because isolates cured of these plasmids also lost their mosquitocidal activity. Morris *et al.* (1998) have studied the plasmid pattern of the indigenous isolates and found that their six *Bt* isolates could be grouped into 2 classes on this basis.

In our studies though no plasmids could be detected in the case of *B. sphaericus* KSD-4 (MTCC 3672), *B. sphaericus* KSD-8 and *Bs* reference strain, one isolate *Bacillus* sp. KSD-7(MTCC 3673) and the *Bti* reference strain exhibited complex plasmid patterns. *Bacillus* sp. KSD-7 (MTCC 3673) had 4 plasmids of molecular weights ranging from very low molecular weight to 9.4kB, whereas the *Bti* reference strain possessed two plasmid bands. Lonc *et al.* (1997) have reported a similiar complex large plasmid pattern in their new serovar *Bt wratislaviensis* H-47 and also for *Bt kurstaki* H3a3b3c. Although there are clear differences in the plasmid contents of *Bti* reference strain and *Bacillus* sp. KSD-7 (MTCC 3673), it is difficult to suggest that whether this difference is on account of the presence of different plasmids or due to polymerisation of the covalently closed forms of the same plasmid.
One 75 mDa plasmid has been reported to play a special role in crystal toxin production by Ward & Ellar (1983) and Himeno et al. (1985). Donovan et al. (1988) reported the isolation of a gene encoding 72-kDa crystal proteins from a native 75-MDa plasmid by the use of a gene specific oligonucleotide probe. Also in our study the isolate *Bacillus* sp. KSD-7 (MTCC 3673) exhibited the presence of a plasmid larger than 23kB in some of the experiments (not seen in the Fig 4.9) that was absent in the reference *Bs* or *Bti* strains.

**Mode of action of the mosquito-pathogenic bacilli:** We have made visual attempts to study larvicidal action of *Bs* and *Bt* on the mosquito larvae (Fig. 4.10.5.1-to 4.10.5.3). Our observations on the mode of action of mosquito-pathogenic bacilli show that after ingestion of the toxin, the larval gut is lysed which is followed by paralysis of the body and death. The larvae generally turn black and swell upon death as reported earlier by Kellen et al. (1965), Davidson (1981), Davidson & Titus (1987), Chilcott et al. (1990) and Gupta et al. (1991). They eventually turn whitish on prolonged action of the bacilli, which has been reported to happen due to septicaemia.

**Bioassays:** After the preliminary toxicity testing, identification and characterisation of the eight new isolates, we performed several range finding bioassays against the three test mosquito species. These isolate wise range finding bioassays not only gave narrow range of effective doses but also pointed to the fact that whether these isolates retained their mosquito pathogenicity upon storage and repeated subculturing prior to the conducting of main bioassays. This prevented wastage of both resources as well as time. For these tests the fully sporulated cultures were centrifuged, washed and resuspended in distilled water to obtain the desired doses (OD's). Subsequently, the dose determination in terms of dry
weights corresponding to the various OD's was done on the basis of dry weight-OD curves (Fig. 4.10.1.1 & 4.10.1.2). During this study the alternative method of counting the viable cells corresponding to different OD's of the isolate suspensions was not applied because the clumping did not permit proper cell-spore count and this would have led to erroneous results. Moreover the crystals produced in the case of Bacillus sp. KSD-7 (MTCC 3673) and Bti 164(H-14) reference strain could have also altered the Optical Density values thus influencing the OD-spore count relationship.

Range finding bioassays showed that the three isolates B. sphaericus KSD-4 (MTCC 3672), Bacillus sp. KSD-7 (MTCC 3673) and B. sphaericus KSD-8 were quite stable over a period of time in terms of toxicity and produced good mortality in the larvae of three mosquito species. Hence these were short-listed for the main bioassay studies. The larvicidal efficacy of the other isolates however, declined on storage and repeated subculturing (Fig. 4.10.2.1 to 10). There are earlier reports on the loss of plasmids containing endotoxin genes with the passage of time (Clark & Dean, 1983; Sekar, 1986 and Sekar, 1990). The decline in the activity of our isolates KSD-1, KSD-2, KSD-3, KSD-5 and KSD-6 during their repeated bioassays after a time gap could be due to a similar phenomenon though it could not be confirmed. In one case the mortality actually increased when the bioassay was repeated after a few months (Fig. 4.10.2.6)

From the analysis of dose mortality data of the main bioassays of three isolates viz., B. sphaericus KSD-4 (MTCC 3672), Bacillus sp. (MTCC 3673) and B. sphaericus KSD-8, the LC\textsubscript{50} values were obtained using MS probit package together with Chi-square values of differences between observed and expected values of the mortality. Also the upper and lower limits of 95% Confidence Interval were calculated (Table 4.10.3.1).
In the case of isolates *B. sphaericus* KSD-4 (MTCC3672), *B. sphaericus* KSD-8 and the reference *Bti* strain when tested against *Ae. aegypti* larvae, we observed a linear relationship between various doses and their corresponding mortality as indicated by $\chi^2$ values with $p > 0.05$ which means that the observed values were not significantly different from the expected mortality that increased as the dose was increased. However, in most of the cases, the relationship was non linear showing thereby that per unit increase in dose did not result in corresponding increase in the larval mortality, and the observed and the expected values of mortality differed significantly from each other. This relationship is expected because unlike chemical insecticides in this case the mortality of larvae depends upon ingestion of bacilli cells and endospores or endotoxins and also virulence of the strain. The ingestion in turn depends upon the state of hunger of larvae. Further in a natural habitat other factors such as availability of alternative food/microflora and detritus are known to influence the active intake of the bacilli and its endotoxins by the larvae. It is also known that at low temperatures as the metabolic activity of the larvae is lowered, the ingestion of food is significantly reduced. These factors play an important role in the overall success of bacilli based control programmes and should therefore be taken into consideration while planning vector control activities in different types of breeding habitats.

Relative efficacy of these isolates (Fig.4.10.3.2A & B) have revealed that the isolates *B. sphaericus* KSD-4 (MTCC 3672) & *Bacillus* sp. KSD-7 (MTCC 3673) are more active than *Bti* reference strain against *An. stephensi* and *Cx. quinquefasciatus*. Although *B. sphaericus* KSD-8 was more active than *Bti* reference strain against *Anopheles* and *Culex* larvae it was comparatively less effective than this reference strain
against *Aedes* larvae. On the other hand, the comparison of the activity values w.r.t *Bs* reference strain showed that all the three isolates were less effective against *An. stephensi* larvae. In the case of *Cx. quinquefasciatus*, the activity of only *B. sphaericus* KSD-8 was more than *Bs* reference strain, whereas in the case of *Ae. aegypti* the activity of both *B. sphaericus* KSD-4 (MTCC 3672) & *Bacillus* sp. KSD-7 (MTCC 3673) was higher than *Bs* reference strain. This data suggests that three isolates are very good candidates for selective vector control and they have the potential for suitable formulation and commercialization after their effectiveness has been confirmed in the small and large scale field trials.

The comparative efficacy of these new isolates has been worked out individually against the three test species of mosquitoes (Fig.4.10.3.3). It was seen that the isolate *B. sphaericus* KSD-4 (MTCC 3672) and *B. sphaericus* KSD-8 were most effective against *Culex* larvae than the other two test species. This was also true in the case of *Bacillus* sp. KSD-7 (MTCC 3673) and the two reference strains. Our findings are in close agreement with those of Singer (1973), Lacey & Singer (1982) and Mittal *et al.* (1985) who also found that *Culex* spp. is most susceptible to *B. sphaericus*. Ansari *et al.* (1989) have also reported that *Cx. quinquefasciatus* larvae are more susceptible to *B. sphaericus* than *An. culicifacies* larvae. The bioassays results of *B. sphaericus* KSD-4 (MTCC 3672) where the efficacy is in the order of *Cx.* > *Ae.* > *An.* do not however, agree with the findings of de Barjac (1990) & Mittal *et al.* (1990) who found the order of activity of *B. sphaericus* for the three species of mosquitoes as *Cx.* > *An.* > *Ae.* However, the order of activity against these three mosquito genera was the same for *B. sphaericus* KSD-8. On the other hand, Mulla (1990) reported the order of *Bti* toxicity against three species was *Ae.* > *Cx.* > *An.*
But we found that the order of activity for the isolate *Bacillus* sp. KSD-7 (MTCC 3673) was Cx. > Ae. > An. The difference in our findings could be due to the fact that our isolate may be a different species or strain. It was also seen in our experiments that the order of activity for the *Bti* reference strain was the same as that for *Bacillus* sp. KSD-7 (MTCC 3673).

The efficacy of the three new isolates has also been compared amongst themselves for individual test species (Fig. 4.10.3.3). It was observed that against *Cx. quinquefasciatus*, *B. sphaericus* KSD-8 was the most effective and *Bacillus* sp. KSD-7 (MTCC 3673) the least effective. On the other hand, against *An. stephensi* larvae *B. sphaericus* KSD-4 (MTCC 3672) was found to be slightly more efficient than *Bacillus* sp. KSD-7 (MTCC 3673), whereas *B. sphaericus* KSD-8 was found to be the least effective against this species. The isolates *B. sphaericus* KSD-4 (MTCC 3672) and *Bacillus* sp. KSD-7 (MTCC 3673) showed almost same activity but were more active than *B. sphaericus* KSD-8 (MTCC 3672) against *Ae. aegypti* larvae.

**Powder formulations:** Powder formulations are generally known to have better shelf-life than the liquid ones. However, there are very few reports on how the powder formulations of *Bti & Bs* products which are commercially available, were actually prepared. Freeze-drying technique has been the primary method for obtaining dry and stable but crude concentrate of spore-crystal complex in laboratory. However, significant losses of spores and crystals occur in this process. Dulmage *et al.* (1970) reported co-precipitation with lactose as a means of recovering spore–crystal complex of *Bacillus thuringiensis*. Acetone used in this technique is a protein precipitant and lactose helps partially to protect the biological material. Asimeng & Mutinga (1992) have also
prepared powders from *Bacilli* culture by this method and used them in toxicity testing. We used two of our isolates *B. sphaericus* KSD-4 and *Bacillus* sp. KSD-7 (MTCC3673) to obtain their powder formulations by this technique and used them in the lab trials at different concentrations. Though these powders do not easily dissolve in water, they were very efficient. *B. sphaericus* KSD-4 (MTCC 3672) produced absolute mortality in all the three test species of mosquitoes at the doses ranging from 0.1g/l to 4g/l. In the case of *An. stephensi* absolute mortality was obtained with this powder at a low dosage of 0.1g/l although the mortality at the higher doses of 0.5 and 1g/l was less i.e. 80 & 90% respectively (Fig 4.10.4A & Appendix III). Powder formulation of *Bacillus* sp. KSD-7 (MTCC 3673) at the dosage ranging from 0.1 to 4.0g/l produced 44-100%, 76.5-88.2% & 70-90% mortality against *Cx. quinquefasciatus*, *An. stephensi* & *Ae. aegypti* larvae. In case of Anopheline and Aedine larvae 65% & 30% mortality was observed at a low dose of 0.05g/l with this formulation (Fig. 4.10.4; Appendix III).

This was a successful attempt to prepare a working powder formulation of the two of the mosquito-pathogenic isolates obtained during this study. This formulation should be preferred due ease of storage and longer shelf life as compared to liquid formulation.

**Conclusion:** Mosquito control using chemical insecticides has serious limitations owing to various reasons cited earlier, most important being development of resistance, environmental pollution and increasing costs. Bio-control with bacilli has emerged as a major thrust area of research in the past few decades. A keen interest evinced in these pathogenic bacilli by the WHO has led to the worldwide searches for the new pathogenic strains. In the current study, we isolated 3 indigenous bacilli strains from Goa highly effective against vectors of malaria, filaria and dengue in their larval stages. From their
protein analysis it is apparent that they possess different toxins and if used in combination in the field could prevent the development of resistance in the vectors. Their relative activity ratio to the known mosquito-pathogenic strains of Bs and Bti strains has revealed that they are attractive candidates for the biological control of mosquitoes if they are selectively used against vectors. Moreover, they will also form a part of the genetic pool for future genetic manipulations i.e. their toxin genes could be introduced into other bacteria or algae in order to increase their efficacy further.

There is a need to suitably formulate these isolates as biolarvicides and their small and large- scale field trials should be conducted to study their effectiveness against different vector species in the environment. It is recommended that the impact of abiotic and biotic factors upon which the efficacy of a biolarvicide depends should be well analysed in the application sites before recommending their use. Also their safety profile should be studied before they are commercialized.

In the end it would be nice to remember the golden words of a grand lady who lived thousands of years ago who said

"What we have learnt, is like a handful of earth.
While what we have yet to learn is like the whole world."

-Saint Avvaiya (Tamil Poetess)