Chapter 6: SUMMARY
Bacilli based commercial formulations are available as bio-insecticides against various insect pest and disease vector species. Among these *Bacillus sphaericus* and *Bacillus thuringiensis israelensis* have emerged as important bio-control alternative to the chemical insecticides for the control of mosquito vectors. In order to isolate indigenous pathogenic bacilli from Goa, we selected ten distantly located sites for the sampling of soils. A new screening protocol was devised to screen these soil samples for the presence of mosquito-pathogenic bacilli before processing them for isolation. This method was found to be economical and time saving as it indicated the presence of pathogenic bacteria in the samples before the isolation process. After having tested these soil samples for the presence of pathogenic bacilli by this new screening technique, we short-listed three soil samples for the bacilli isolation. An additional soil sample from the mangrove soils that are known to harbour bacilli, was also processed for selective isolation of bacilli. The isolates so obtained were purified and tested for toxicity against *Cx. quinquefasciatus* and *An. stephensi* larvae. From the preliminary bioassays we obtained 8 mosquito pathogenic bacilli isolates which were coded named as KSD-1 to KSD-8. These isolates were morphologically and biochemically characterized along with the *Bs H5a5b* (B-101) and *Bti H-15* (164) strains isolated from commercial powders. They were identified up to species level by using keys of Gordon (1973) and Berkeley *et al.* (1984). Surprisingly, two of the isolates, KSD-7 and KSD-8 were identified differently by these two keys. Two of these isolates *B. sphaericus* KSD-4 & *Bacillus sp.* KSD-7 were deposited at IMTECH, Chandigarh and have been assigned MTCC numbers as *B. sphaericus* 3672 & *Bacillus sp.* 3673 respectively. Large parasporal crystals of various sizes, released from the sporangia were observed in the isolate *Bacillus sp.* KSD-7 (MTCC 3673) as well as in
the reference strain *Bti* 164(H-14) when the cultures were observed under Light as well as Scanning Electron Microscope. All the isolates were found sensitive to Chloramphenicol. Out of these four *B. sphaericus* isolates KSD-2, KSD-4, KSD-6 & KSD-8 were found resistant to streptomycin while the others were sensitive to this antibiotic.

Range finding bioassays were performed with all the isolates against III instar larvae of three mosquito species, to get a narrow range of concentrations within which the LC$_{50}$ value of each fell. Some of the isolates lost or showed decline in toxicity over a period of time. The main bioassays were carried out according to standard procedure with the three isolates *B.sphaericus* KSD-4 (MTCC 3672), *Bacillus* sp. KSD-7 & *B.sphaericus*KSD-8 and the two reference strains, *B. sphaericus* 101(H5a5b) & *B. thuringiensis israelensis*164(H-14). Their LC$_{50}$ values were calculated from the dose-mortality data using MS Probit package along with the chi-square values within lower and upper 95% Confidence Interval Limits.

All the three new isolates *B. sphaericus* KSD-4 (MTCC 3672), *Bacillus* sp.KSD-7 (MTCC 3673) and *B. sphaericus* KSD-8 were found relatively more effective than *Bti* reference strain against Anopheline and Culicine larvae although against *Aedes aegypti* larvae they were less effective than this reference strain. Against Culicine larvae, the isolate *B. sphaericus*KSD-8 and against Aedine larvae both *B. sphaericus* KSD-4 (MTCC 3672) & *Bacillus* sp. KSD-7 (MTCC 3673) were found more effective than *Bs* reference strain. However, these three new isolates were relatively less effective than *Bs* reference strain against *Aedes aegypti* larvae.

From the comparison of the efficacy of these isolates against three vector species, we observed that for both *B. sphaericus*KSD-4 (MTCC3672) and *Bacillus sp. KSD-7*
(MTCC 3673) the efficacy was in the order of Cx. > Ae. > An. But for the isolate
B. sphaericus KSD-8 this order was as Cx. > An. > Ae.

Successful attempts were also made to prepare working dry powder formulation
of two isolates B. sphaericus KSD-4 (MTCC 3672) & Bacillus sp. KSD-7 (MTCC
3673). These primary powder formulations were tested for toxicity against the III instar
larvae of Cx. quinquefasciatus, An. stephensi and Ae. aegypti. These powder formulations
produced up to 100% mortality against these mosquito species at various doses ranging
from 0.01g/ml to 4.0g/ml.

The comparisons were also made amongst these isolates for their efficacy against
three vector species. Against Culicine larvae B. sphaericus KSD-8 was the most effective
and Bacillus sp. KSD-7 (MTCC 3673) was the least effective. Against Anopheline larvae
B. sphaericus KSD-4 (MTCC 3672) was found to be most effective and B. sphaericus
KSD-8 the least effective. However, against Aedine larvae the most efficient was the
Bacillus sp. KSD-7 (MTCC 3673) although quite similar to B. sphaericus KSD-4 (MTCC
3672), whereas the least effective was B. sphaericus KSD-8.

From the protein analysis of these three new isolates and the two reference strains
using SDS-PAGE, we found significant differences in their protein composition amongst
them, as well as with two reference strains. This difference could account for their
variable toxicity against the vector species and also show that our isolates are either
different strains or serotypes of the pathogenic bacilli species.

Plasmid analysis of these new isolates and the two reference strains was carried
out on agarose gel. Although no plasmid could be detected in B. sphaericus KSD-4
(MTCC 3672), and B. sphaericus KSD-8 and the Bs reference strain two plasmids were
detected in *Bti* reference strain and five plasmid bands were observed in *Bacillus* sp. KSD-7 (MTCC 3673). Apart from this the mode of action of the bacilli toxins was also briefly studied in mosquito larvae.

During this research, we have obtained three indigenous and very promising bacilli strains for future bio-control for mosquito vector species. These could be formulated and commercialized after small and large-scale field trials. This study has also revealed that the soils of Goa are a potential source for the isolation of new mosquito-pathogenic bacilli strains.