COMPARABILITY OF TWO COMMERCIAL FORMULATIONS OF *BACILLUS THURINGIENSIS* VAR *ISRAELENSIS* AND *B. SPHAERICUS* AGAINST LARVAE OF *CULEX QUINQUEFASCIATUS* (DIPTERA:CULICIDAE) IN VARIOUS ECOLOGICAL NICHES

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Abstract—A study was initiated in the laboratory to compare the effects of spore dust and sawdust formulations of *Bacillus thuringiensis* var *israelensis* and *B.sphaericus* on immatures of laboratory and field samples of *C.quinquefasciatus*. Various types of ecological niches including cess pits, cement tanks and 'U' drain which form the habitats of the mosquitoes, were selected for this study. *Bacilli* formulations were tested against 2nd, 3rd and 4th instar larvae of laboratory and field collected samples as mentioned above and the LC_{50} values were determined by computer analyses. The LC_{50}s of *B.thuringiensis* were compared with those of *B.sphaericus* and it was concluded from this study that *B.thuringiensis* is highly toxic to all stages of larvae in laboratory and field habitats. Both sawdust formulations produced 80–100% mortality after 24 hrs. in all stages of larvae from three habitats and in laboratory samples at 0.005% concentration. It was concluded from these results that *B.thuringiensis* is a more effective biocide than *B.sphaericus* in moderate and tropical conditions.

INTRODUCTION

Potential problems associated with chemical control of mosquito populations such as persistence of toxic metabolites, deleterious effects on non-target organisms and development of pesticide resistance can often be mitigated by use of microbial insecticides, biocontrol agents and non-toxic natural products (Kerwin and Washino, 1983). Natural products like Vetiver oil (a non-toxic compound) was reported to be highly toxic to immatures of *Culex quinquefasciatus* (Murty and Jamil, 1987). The current interest in development of biocontrol agents for the control of vectors, especially mosquitoes, is an indication of the concern and sheer helplessness felt by the scientific community in the wake of the recurrence of malaria in epidemic proportions which was under control during the 1950s (Rajagopalan, 1981).

Microbial agents form one of the important groups of biological control agents which are now being used in varying degrees to control mosquito larvae in different parts of the world. We have previously demonstrated the successful application of *Bacillus* strains for the biological control of various mosquito vectors and their non-toxic effects to mammals (Narasaiah and Jamil, 1986). Many bacterial strains are now being subjected to intensive studies regarding their mode of action and for their potential use in mosquito control (Balaraman, 1980). Many microbial pathogens of mosquitoes have been recognised and added to the armoury of natural enemies for use as potential weapons to control mosquito vectors of human diseases (USHDHEW, 1975). *Bacillus thuringiensis* production to control agricultural pests is an expanding industry and nearly 12,000 tonnes had been used up to 1980 and much of it on food crops (WHO, 1982). The toxin crystal is formed along side the spore, larval enzymes digest the crystals releasing the toxin within seconds of ingestion and larvae are killed within hours of ingesting a lethal dose.

In the present study *B.thuringiensis* and *B.sphaericus*, as spore dust and sawdust formulations, were evaluated for their efficacy against various instars of *C.quinquefasciatus* from different habitats e.g., cess pits, cement tanks and 'U' drains from in and around the campus of this laboratory i.e., the Tarnaka area of Hyderabad city.

MATERIALS AND METHODS

The following habitats were selected from the Tarnaka area for the present study.

1. *Cess pit:* This is a small drainage pit found in areas with no proper drainage system. They form a highly mosquitogenic habitat for *Culex* species. Algal growth was common in the selected

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pit and it was inhabited by Dragon fly naiads, water bugs, tadpoles and crustaceans which can be considered as associated fauna.

2. *Cement Tank:* The tank selected for this study measured 12'×6'×6' and was situated in the campus. Water hyacinth (*Eichhornia crassipes)* was the predominant flora in the tank which formed a major breeding site for mosquitoes. Decaying organic matter such as leaves, straw green grass and other debris was present and it was observed that *C.quinquefasciatus* was the predominant mosquito species in this tank.

3. *'U' drain"* : The open drainage system in the Tarnaka area formed a major source of breeding for mosquitoes. The total length of the open drain was 300–350 m. and it contained stagnant water due to the continuous disposal of domestic wastes and this was associated with heavy mosquito breeding. The predominant mosquito species in this habitat was *C.quinquefasciatus.*

Bacterial formulations

Bacillus thuringiensis and *B. sphaericus*: Freeze dried spore formulations and sawdust formulations were dissolved in sterilised water and different concentrations were prepared for the bioassay.

Bioassay: 25 larvae of each instar from each habitat were seeded in 500ml beakers containing 249 ml of water. 1ml of test suspension of each concentration was added to the each beaker and the laboratory bred larvae were used as controls and assayed as above. All the experiments were done in triplicate. Mortality was recorded after 24 hrs and the results were computerised according to Finney's method (1971).

RESULTS AND DISCUSSION

The data obtained from the 4 test strains of *B.thuringiensis* and *B.sphaericus* against all immature stages of *C. quinquefasciatus* are presented in tables (1–8).

Both strains showed significant variability of LC_{50} values. The laboratory bred samples were more susceptible than the field collected strains. The 'U' drain species showed more resistance than those from other habitats. The high organic content and maximum pollution in the drain might have enhanced the resistance in the larvae of *C.quinquefasciatus*. The LC_{50}s of two strains of *Bacilli* varied in the two habitats i.e., cess pit and cement tank. Since the two strains proved effective it is suggested that they can be used in field applications. These strains were reported to be safe to non-target organisms. The larvae from all habitats showed susceptibility towards these biocides.

As there was no clear relationship of LC_{50} values between the laboratory bred samples and field collected samples it was thought that there was a need to study the effect of physico-chemical factors on the breeding status of mosquitoes and the biocide's efficacy. Probably the properties of the individual formulation of the pathogen caused variation of LC_{50} between habitats. Though *B.thuringiensis* is active against mosquito larvae *B.sphaericus* is reported to have recycling capacity particularly in polluted water (TDR/BCV/SWG-7/84.3).

Persistence of biocides is an important factor to be taken into an account when they are evaluated under field conditions. Dry powder formulations of *B.sphaericus* 1593 have been shown to remain active against *C.pipens* for 30 days under natural conditions and cause 50–60% mortality but after 11 weeks mortality decreases to 5–15% (Vankova, 1984). Mulligan and Schafer (1982) described the decreased activity of *B.sphaericus* in nature due to the effect of direct radiation from the sun, higher temperature (25°–30° C) and the presence of mud. Larget-thiery (1983) assumed that the mud function was a competitive factor for mosquito larvae. Pantuwatana (1982) and Davidson *et al* (1984) also discussed the effect of water pollution on the efficacy of *B.sphaericus*.

B.sphaericus and *B.thuringiensis* var *israelensis* have been shown to be very similar in their effect on mosquito larvae (Davidson *et al,* 1981). The persistence of *B.thuringiensis* was tested in nature by Vankova in 1982 and it was found that *B.thuringiensis* retained its efficacy (100% mortality) for 3 months i.e., much longer than *B.sphaericus* which caused 50–60% mortality of *C.pipens* larvae after one month under natural conditions.

B.thuringiensis and *B.sphaericus* also differ in the levels of dosage for the same percent mortality.

Table 1. Effect of spore dust formulation of *B. thuringiensis* on larvae of *Culex quinquefasciatus* from laboratory and cess pit samples.

	Laboratory samples			Cess pit samples		
	II instar	III instar	IV instar	II instar	III instar	IV instar
LC_{50}±SE (Spores/ml)	$0.71\times10^6\pm0.25$	$1.4\times10^6\pm0.55$	$2.18\times10^6\pm1.20$	$2.16\times10^6\pm0.70$	$2.25\times10^6\pm0.70$	$2.83\times10^6\pm0.48$
Regression equation Y=(Y−X)+Xb	Y=(5.31–3.68)+ 1.75×	Y=(4.88–2.60)+ 1.27×	Y=(4.63–6.38)+ 1.38×	Y=(5.12–4.08)+ 1.70×	Y=(5.126–3.960)+ 1.63×	Y=(5.05–6.84) + 2.77×
Chisquare	0.028	0.0072	0.054	0.019	0.044	0.217
Fiducial limits UL	1.193	2.497	4.548	3.420	3.625	3.770
LL	0.224	0.307	0.185	0.893	0.869	1.875

Table 2. Effect of spore dust formulation of *B. thuringiensis* on larvae of *Culex quinquefasciatus* from Cement tank and 'U' drain samples.

	Cement tank samples			'U' drain samples		
	II instar	III instar	IV instar	II instar	III instar	IV instar
LC_{50}±SE (Spores/ml)	$2.06\times10^6\pm0.23$	$2.23\times10^6\pm0.18$	$2.61\times10^6\pm0.23$	$4.97\times10^6\pm0.44$	$5.48\times10^6\pm0.43$	$5.49\times10^6\pm0.67$
Regression equation Y= Y−X)+Xb	Y=(5.62–16.68)+ 6.95×	Y=(5.47–19.68)+ 8.2×	Y=(5.06–13.38)+ 5.51×	Y=(4.87–15.14)+ 5.67×	Y=(4.88–16.99)+ 6.27×	Y=(4.62–16.32) + 6.09×
Chisquare	0.974	0.933	0.343	0.018	0.014	0.0067
Fiducial limits UL	2.504	2.570	3.061	5.840	6.337	6.825
LL	1.618	1.892	2.168	4.109	4.616	4.167

Table 3. Effect of spore dust formulation of *B. sphaericus* on larvae of *Culex quinquefasciatus* from laboratory and cess pit samples.

	Laboratory samples			Cess pit samples		
	II instar	III instar	IV instar	II instar	III instar	IV instar
LC_{50}±SE (Spores/ml)	$1.11\times10^6\pm0.29$	$1.76\times10^6\pm0.63$	$2.18\times10^6\pm1.20$	$3.12\times10^6\pm0.32$	$3.61\times10^6\pm0.353$	$4.08\times10^6\pm0.413$
Regression equation Y=(Y−X)+Xb	Y=(5.01–3.63)+ 1.77×	Y=(4.67–3.54)+ 1.72×	Y=(4.63–2.85)+ 1.38×	Y=(5.27–13.83)+ 5.44×	Y=(5.36–15.8)+ 6.03×	Y=(5.36–15.62)+ 5.85×
Chisquare	0.269	0.377	0.054	1.37	1.59	0.592
Fiducial limits UL	1.684	2.982	4.548	3.760	3.760	4.892
LL	0.534	0.529	0.185	2.450	2.917	3.271

Table 4. Effect of spore dust formulation of *B. sphaericus* on larvae of *Culex quinquefasciatus* from Cement tank and 'U' drain samples.

	Cement tank samples			'U' drain samples		
	II instar	III instar	IV instar	II instar	III instar	IV instar
LC_{50}±SE (Spores/ml)	$2.91\times10^6\pm0.31$	$3.98\times10^6\pm0.45$	$4.72\times10^6\pm0.41$	$3.96\times10^6\pm0.404$	$4.47\times10^6\pm0.44$	$5.0\times10^6\pm0.69$
Regression equation Y=(Y−X)+Xb	Y=(5.2–12.45)+ 4.98×	Y=(4.9–11.43)+ 4.45×	Y=(4.7–18.147)+ 6.9×	Y=(5.12–13.07)+ 4.99×	Y=(4.87–13.26)+ 5.06×	Y=(4.62–14.26)+ 5.44×
Chisquare	0.301	0.0405	0.145	0.098	0.0243	0.088
Fiducial limits UL	3.517	4.847	5.536	4.754	5.342	6.353
LL	2.311	3.088	3.911	3.170	3.600	3.646

Table 5. Effect of sawdust formulation of *B. thuringiensis* on larvae of *Culex quinquefasciatus* from laboratory and cess pit samples.

	Laboratory samples			Cess pit samples		
	II instar	III instar	IV instar	II instar	III instar	IV instar
LC_{50}±SE (Spores/ml)	0.0017± 0.07	0.0024±0.04	0.0042±0.04	0.0023±0.03	0.003±0.05	0.0045±0.07
Regression equation Y=(Y−X)+Xb	Y=(0.052–0.0016)+ 1.24×	Y=(0.052–0.043)+ 3.16×	Y=(0.047–0.091)+ 6.08×	Y=(0.053–0.062)+ 4.43×	Y=(0.054–0.07)+ 4.66×	Y=(0.046–0.077) + 4.78×
Chisquare	0.093	0.02	0.149	0.0273	0.031	0.009
Fiducial limits UL	0.0031	0.0032	0.005	0.0028	0.004	0.0059
LL	0.0034	0.0017	0.0034	0.0016	0.002	0.0031

Table 6. Effect of sawdust formulation of *B. thuringiensis* on larvae of *Culex quinquefasciatus* from Cement tank and 'U' drain samples.

	Cement tank samples			'U' drain samples		
	II instar	III instar	IV instar	II instar	III instar	IV instar
LC_{50}±SE (Spores/ml)	0.0027±0.02	0.0032±0.03	0.0043±0.03	0.0032±0.03	0.0037±0.03	0.0047±0.02
Regression equation Y=(Y−X)+Xb	Y=(0.05–0.0063)+ 4.5×	Y=(0.053–0.0976)+ 6.1×	Y=(0.053–0.012)+ 7.78×	Y=(0.055–0.014)+ 8.6×	Y=(0.053–0.112)+ 6.95×	Y=(0.05–0.127) + 7.95×
Chisquare	0.107	0.0385	0.0473	0.548	0.0436	0.086
Fiducial limits UL	0.0032	0.0039	0.0049	0.0036	0.0044	0.0053
LL	0.0021	0.0026	0.0036	0.0027	0.0031	0.0042

Table 7. Effect of sawdust formulation of *B. sphaericus* on larvae of *Culex quinquefasciatus* from laboratory and cess pit samples.

	Laboratory samples			Cess pit samples		
	II instar	III instar	IV instar	II instar	III instar	IV instar
LC_{50}±SE (Spores/ml)	0.0021±0.02	0.0027±0.03	0.0043±0.03	0.0026±0.03	0.0033±0.03	0.0047±0.02
Regression equation Y= Y−X)+Xb	Y=(0.054–0.087)+ 6.22×	Y=(0.053–0.079)+ 5.3×	Y=(0.054–0.125)+ 7.8×	Y=(0.055–0.088)+ 6.26×	Y=(0.053–0.093)+ 6.12×	Y=(0.05–0.126)+ 7.95×
Chisquare	0.126	0.034	0.047	0.222	0.038	0.087
Fiducial limits UL	0.0026	0.0034	0.0049	0.0032	0.0039	0.0052
LL	0.0017	0.0021	0.0036	0.0019	0.0026	0.0041

Table 8. Effect of sawdust formulation of *B. thuringiensis* on larvae of *Culex quinquefasciatus* from Cement tank and 'U' drain samples.

	Cement tank samples			'U' drain samples		
	II instar	III instar	IV instar	II instar	III instar	IV instar
LC_{50}±SE (Spores/ml)	0.0034 0.03	0.0044±0.03	0.0048±0.03	0.0039±0.03	0.005x±0.03	0.0056±0.03
Regression equation Y=(Y−X)+Xb	Y=(0.052–0.096)+ 6.42×	Y=(0.052–0.131)+ 8.2×	Y=(0.05–0.126)+ 7.9×	Y=(0.055–0.144)+ 8.99×	Y=(0.053–0.159)+ 9.45×	Y=(0.05–0.153)+ 9.0×
Chisquare	0.121	0.109	0.037	0.0299	0.0525	0.0867
Fiducial limits UL	0.0039	0.0049	0.0053	0.0047	0.0033	0.0062
LL	0.0028	0.0038	0.0042	0.0059	0.0046	0.0051

B. thuringiensis causes mortality of larvae at low doses whereas the treatment with *B. sphaericus* preparations resulted in the same degree of mortality at higher doses. From the persistence and effectiveness point of view *B. thuringiensis* is highly recommended as a biocide which can be successfully employed under the field conditions found in different habitats.

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