Mosquito Control through Plant Extracts

Jackson et al. (1990) studied a laboratory and field reared 2nd and 3rd instar Culex pipiens larvae with the extracts from two varieties of Sorghum bicolor seedlings suggesting a significant (P < 0.05) larvicides with 90% mortality in 2nd instar Culex pipiens larvae at 0.82 ppm and 90% mortality in 3rd instar larvae at 1.12 ppm under laboratory conditions. A preliminary behavioral assessment of late 3rd instar larvae exposed to 1.42 ppm produce 80% mortality after only 4-5 hours of contact. Plant extracts appear stable when stored at up to 32°C in a closed container. Once the extracts are infused in water and exposed to air, however, they biodegrade after 24 h.

Green et al. (1991) reported mosquito adulticidal activity in the extract of Targetes minuta flowers. The essential oil isolated from the plant was very much effective against Anopheles stephensi.

Saxena et al. (1992) discovered growth inhibitory and juvenile hormone mimicking activity in the larvae of Cx. quinquefasciatus treated with acetone extracts of Ageratum conyzoides, Cleome icosandra and Tridax procumbens resulting in larval-pupal intermediates, demelanised pupae, defective egg rafts and adult with deformed flight muscles. Loss of fecundity was also observed in the treated mosquito without any sterilant effect.

Saxena et al. (1993) studied on the larvicidal, growth regulator and chemosterilant activity of the whole plant extract of Annona squamosa (Annonaceae) against An.
The study revealed that alkaloid extracted from the plant have the bioactive potentiality.

Monzon et al. (1994) studied on the larvicidal potentiality of five Philippine plants against *Aedes aegypti* (L) and *Culex quinquefasciatus* (Say). *Annona squamosa* and *Lansium domesticum* showed highest larvicidal potential against *Ae. aegypti* and *Cx. quinquefasciatus* amongst the five plant species.

Perich et al. (1994) compared biocidal activities of the whole plant extracts of three *Targetes* species and showed that *T. minuta* had the greatest biocidal activity on the larvae and adults of *Ae. aegypti* (L)and *An. stephensi* (L). Bioassay of simultaneous steam distilled extracts *T. minuta* flowers showed larval mortality of LC₉₀ at 4 and 8ppm against the adult at 0.4 and 0.45% in *Ae. aegypti* and *An. stephensi* respectively.

Tyagi et al. (1994) compared the repellent activity of *Targetes minuta* (compositae) against the adult of *Cx. quinquefasciatus, Ae. aegypti* and *An. stephensi*. A high degree of repellency (> 90% protection for 2 h and > 50% upto 4 h) was observed in the essential oil fraction.

Ansari et al. (1995) reported on relative efficacy of various oils in repelling mosquitoes. Oils of *Cymbopagon nardus* provided more than 95% protection against *Cx. quinquefasciatus* and *An. culicifacies* in whole night landing collection on human baits.

Dua et al. (1995) studied on the repellency of extract of the methanol *Lantana camara* flowers and mixed with coconut oil against *Aedes* mosquitoes. The mixture provided 94.5% protection against *Ae. albopictus* for two hour. Four fractions viz. MRC – HR1, HR2, HR3 and HR4 were isolated from methanol extract using solvent extraction and chromatographic methods. Of these, MRC – HR2 showed maximum repellency against *Aedes* mosquitoes with a mean fraction time of 2.43 hours Repellent action of MRC – HR2 gave 85% protection for up to 6 hours against *Aedes* sp. in field conditions.
Chatterjee et al. (1997) studied the effect of neem (A. indica) oil on man bait collection of Cx. vishnui at indoor and outdoor locations in Tarekswar, West Bengal. Neem oil saved 92.73% at indoors and 93% bites of Cx. vishnui (group) outdoors.

Jayaprakasha et al. (1997) isolated three limonoids namely limonin, nomilin and obacunone, from the seeds of Cx. reticulata which showed growth inhibition effect on 4th instar larvae of Cx. quinquefasciatus and the EC50 for inhibition of adult emergence was 6.31, 26.61 and 59.57 ppm for obacunone, nomilin and limonin, respectively.

Macedo et al. (1997) studied on the screening of Asteracea (compositae) plant extracts for larvicidal activity against Ae. fluviatilis (Diptera: Culicidae). The extract from T. minuta was found to be most active among 83 plant species belonging to the compositae family. Active compounds have also been identified as triophene derivatives. Ethanol extract of another plant, Eclipta paniculata belonging to family compositae, has also shown significant insecticidal activity, with LC50 of 17.2 mg/l and LC90 of 3.3 mg/l.

Murty et al. (1997) worked on the leaf extract of Polyalthia longifolia (Family: Annonaceae) against larvae and pupae of Cx. quinquefasciatus of different habitats. Significant larvicidal and growth inhibiting effects were recorded. The 250-350 ppm test concentrations produced 64-96% inhibition of adult emergence in tanks and U - drains.

Umerie et al. (1998) used the leaf-extract of Ocimum basilicum (sweet basil) in formulating an aerosol with mosquito coil and their efficacy were tested against adult mosquitoes. The formulations had potencies of 93 ± 4% and 95 ± 5% for the aerosol and coil respectively depending on the duration of the fumigation.

Choochote et al. (1999) investigated four fractions of Kaempferia galanga (hexane fraction, dichloromethane fraction 1, dichloromethane fraction 2 and methanolic fraction) for larvicidal activity toward 4th instar Cx. quinquefasciatus. The hexane fraction was found to exhibit the highest larvicidal effect with the LC50 of 42.33 ppm. Since the hexane fraction did not show any promising adulticidal effect, it possessed
repellency against *Ae. aegypti* (ED$_{50}$ value of 30.73 $\mu$g/ cm$^2$), and provided biting protection for 3 hours. In a field study, it could protect against certain mosquitos, i.e., *Armigeres subalbatus, An. barbirostris, An. aconitus, Mansonia uniformis, Cx. quinquefasciatus, Cx. gelidus, Cx. tritaeniorhynchus* and *Ae. aegypti* without any dermal irritation on human skin.

Eckenbach *et al.* (1999) studied on two isolated acetylenic compounds (Falcarinol and Falcarinidiol) using hot methanol and water mixture from fresh foliage, roots and fruits of *Cryptotaenia canadensis* against 4th instar of *Cx. pipiens* The dried extract was partitioned into chloroform and water, and both phases were bioassayed at concentration between 5 and 50 ppm. Probit analysis revealed LC$_{50}$ values as 3.5 ppm for Falcarinol and 6.5 ppm for Falcarinidiol.

El Hag *et al.* (1999) investigated the toxic and development retarding effects on *Cx. pipiens* mosquito larvae by methanol and ether extracts of *Azadirachta indica, Rhazya stricta* and *Syzygium aromaticum*. *R. stricta* showed marked acute (2d) and chronic (10 d) toxic effects, having an LC$_{50}$ and 95% CL of 251 (209-326) and 140 (110-178) at 467 (416-699) and 211 (198-421) ppm for the methanol and ether extracts respectively. *A. indica* extracts were toxic to *Cx. pipiens* larvae at higher concentrations, showing an acute and chronic LC$_{50}$ and 95% CL of 824 (692-980) and 265 (111-481); 1620 (1380-1892) and 675 (514-887) ppm for the methanol and ether extracts respectively.

Palsson *et al.* (1999) studied the use of plant derived products or methods in 23 rural villages of Guinea Bissau to reduce mosquito biting activity. The results revealed: smoldering *H. suaveolens* (85.4% repellency); fresh *H. suavesolens* (73.2%); burning of the bark of *D. oliveri* (74.7%); smoke of the leaves of *Eucalyptus* (72.7%); smoke of the leaves of *A. indica* (76.0%); smoke of the infructescence of *E. guineensis* (69.07%); fresh *O. canum* (63.6%); and fresh *S. occidentalis* (29.4%).

Ramsewak *et al.* (1999) worked on the larvicidal activity of *Murraya koenigii*. The larvicidal activity was due to the presence of carbazole alkaloids, maharimbine, murrayarol and mahanine.
Ansari et al. (2000) evaluated the oil of *Mentha piperita* L. for larvicidal activity against different mosquito species: *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* by exposing 3rd instar larvae of mosquitoes in enamel trays 6 x 4 inch² size filled to a depth of 3 inch with water. Application of oil at 3 ml/ m² of water surface area resulted in 100% mortality within 24 hour for *Cx. quinquefasciatus*, 90% for the *Ae. aegypti* and 80% for *An. stephensi*. The oil showed strong repellent action against adult mosquitoes when applied on human skin. Percent protection obtained against *An. annularis*, *An. culicifacies* and *Cx. quinquefasciatus* was 100%, 92.3% and 84.5%.

Markouk et al. (2000) tested the larvicidal properties of 16 extracts of four Moroccan medicinal plants: *Calotropis procera* (Wild), *Cotuta cinerea* (L.), *Solanum sodomaeum* (L.) and *Solanum elaeagnifolium* (CAV.) against *An. labranchiae* mosquito larvae. The LC₅₀ (24h) value for nine extracts ranged from 28 to 325ppm.

Tawatsin et al. (2001) evaluated the repellency effect of volatile oils extracted from four plant species, turmeric (*Curcuma longa*), Kaffir lime (*Citrus hystrix*), citronella grass (*Cymbopogon winterianus*) and hairy basil (*Ocimum americanum*) against three mosquito vectors *Ae. aegypti*, *An. dirus* and *Cx. quinquefasciatus*. The oils from turmeric, citronella grass and hairy basil, especially with the addition of 5% vanillin, repelled the three species for upto eight hours in cage conditions, but the oil from Kaffir lime was effective upto three hours.

Chatterjee et al. (2002) investigated on the mortality patterns of fourth stage larvae of *Cx. vishnui* (group) and *An. subpictus* by the application of crude water extract of *Delphinium denudatum* wall leaves in the laboratory. 3% leaf extract brought 100% and 86.6% mortalities of *Cx. vishnui* and *An. subpictus* larvae respectively in 72 hours indicating its efficacy as a strong larval toxicant.

Jang et al. (2002) evaluated the larvicidal activity of methanol extracts of 26 leguminous seeds and 20 grains against early 4th instar larvae of *Ae. aegypti* and *Cx. picipiens pallens*. At 200 ppm of the extracts from *Cassia obtusifolia*, *Cassia tora*, and *Vicia tetrasperma*, more than 90% mortality was obtained in larvae of *Ae. aegypti* and *Cx. picipiens pallens*. Extract of *Cx. tora* gave 86.7 and 100% mortality in the larvae of *Ae.*
*Ae. aegypti* and *Cx. pipiens pallens* at 40 ppm but 59.2 and 78.3% mortality against larvae of *Ae. aegypti* and *Cx. pipiens pallens* at 20 ppm, respectively, where as *Cx. obtusifolia* caused 51.4 and 68.5% mortality respectively.

Jaswanth *et al.* (2002) tested methanolic extract of leaves of *A. squamosa* for mosquitocidal effect against *Cx. quinquefasciatus*. A liquid mosquito insecticide formulation was prepared with the extract (1, 3 and 5% w/w) using deodorized kerosene as solvent and investigated for its knock-down and 24 hr mortality. The extract formulation produced test concentrations dependent activity, exhibited significantly shorter knock down KD50 and KD90 values and produced significant mortality.

Mehra *et al.* (2002) evaluated the efficacy of crude acetone extract of *Cuscuta hyalina* Roth, against pre adult stages of *Cx. quinquefasciatus* in laboratory condition. LC50 value against third and fourth instar larvae and pupae were 303 ppm, 306.44 ppm and 97.66 ppm. The extract completely restrained the adult mosquito emergence at 50 ppm for 3rd instar and 75 ppm for 4th instar larvae. The extract was found to be an effective oviposition deterrent at 80 ppm. The plant was also found to be effective in suppressing the adult emergence when directly sprinkled as dry powder on the water surface.

Moore *et al.* (2002) evaluated the efficacy of three natural repellents (1 eucalyptus based, 1 neem based, and 1 containing several repellent essential oils) against *An. darlingi*. The eucalyptus based repellent containing 30% p-menthane-diol gave 96.89% protection for 4 hour.

Traboulsi *et al.* (2002) studied the insecticidal activities of essential oil extracts from leaves and flowers of some aromatic plants against 4th instar larval form of *Cx. pipiens molestus* Forskal. Extracts of *Myrtus communis* L. were found to be the most effective, followed by those of *Origanum syriacum* L., *Mentha microcorphyla* Koch, *Pistacia lentiscus* L. and *Lavandula stoechas* with LC50 values of 16, 36, 39, 70 and 89 mg litre⁻¹, respectively. Over 20 major components were identified and thymol, carvacrol, (1R) – (+) – alpha – pinene and (1S) – (−) – alpha – pinene were the most
toxic (LC₅₀, 36-49 mg litre⁻¹), while methone, 1, 8-cineole, linalool and terpineol (LC₅₀, 156-194 mg litre⁻¹) were less toxic.

Yang et al. (2002) examined the mosquito larvicidal activity of *Piper longum* fruit-derived materials against the fourth-instar larvae of *Ae. aegypti*. The methanolic extract was found to be active and hexane fraction of methanol extract showed a strong larvicidal activity of 100% mortality. The bioactive compound was identified as pipemonaline with LC₅₀ value was 0.25 mg/l.

Aliero (2003) investigated on the effect of crude extracts of *Azadirachta indica* (neem) against the larvae of *Anopheles* mosquito. Exposure of the larvae to undiluted extracts of seed oil, leaf and bark for 12 hours led to 100, 98 and 48% mortality respectively.

Cheng et al. (2003) studied the bioactivity of 14 essential oils from five plants using *Ae. aegypti* larvicidal assay. The leaf and bark essential oils of *Cryptomeria japonica* demonstrated high larvicidal activity, with a LC₅₀ of 37.6 µg/ml (LC₉₀ = 71.9 µg/ml) for leaf and LC₅₀ of 48.1 µg/ml and 130.3 µg/ml for bark essential oil.

Jayabalan et al. (2003) tested methanol extracts of *Palargonium citrosa* leaf for their biological, larvicidal, pupicidal, adulticidal, antiovipositional activity, repellency and biting deterrency against *An. stephensi*. The 4% plant extract was found to be very much effective.

Kabir et al. (2003) studied the mosquitocide activity of *Bryonopsis laciniosa* L., against larval form of *Cx. quinquefasciatus*. The active ingredient was Goniothalamin and the structure was established through spectroscopic methods using H-1-NMR and C-13-NMR in CDCl₃ from the EL-OAC (CH₃COOC₂H₅) fraction.

Sakthivadivel et al. (2003) showed acetone fraction of the petroleum ether extract of seeds from *Argemone mexicana* L. exhibited larvicidal and growth inhibiting activity against the 2nd instar larvae of *Ae. aegypti* L. This activity occurred at higher concentrations (200, 100, 50 and 25 ppm). Chemosterilant activity, including reduction in blood meal utilization (27.70%), reduction in fecundity (19.00%),
formation of larval pupal intermediates, formation of pupal adult intermediates, adult mortality and sterility of first generation eggs (100%), occurred at a concentration of 10 ppm.

Choochate et al. (2004) investigated a crude seed extract of celery, *Apium graveolens*, for anti-mosquito potential, including larvicidal, adulticidal, and repellent activities against *Ae. aegypti*. The ethanol-extracted *A. graveolens* possessed larvicidal activity against fourth instar larvae of *Ae. aegypti* with LD$_{50}$ and LD$_{95}$ values of 81.0 and 176.8 mg/ l, respectively. In testing for adulticidal activity, this plant extract exhibited a slightly adulticidal potency with LD$_{50}$ and LD$_{95}$ values of 6.6 and 66.4 mg/ cm$^2$, respectively. It showed repellency against adult females with ED$_{50}$ and ED$_{95}$ values of 2.03 and 28.12 mg/ cm$^2$, respectively. It also provided biting protection time of 3 hours when applied at a concentration of 25 g% with out any dermal irritation, any adverse effects on the skin or other parts of the body of human volunteers were observed during 3 mo of the study period or in the following 3 mo, after which time observations ceased.

Chansang et al. (2005) investigated that crude extracts of nine medicinal plants against larvae of *Cx. quinquefasciatus* Say and *Ae. aegypti* (L.). The long pepper, *Piper retrofractum* Vahl (Piperaceae), showed the highest level of activity against mosquito larvae. Extracts of unripe (001/3) and ripe (002/3) was somewhat more active against *Ae. aegypti* than *Cx. quinquefasciatus*. But 001/4 was much more toxic to both mosquito species. Diluted solutions of the solid extract (002/3) in distilled water lost their larvicidal activity upon aging. Loss of activity at 25$^\circ$C was greater than that stored at 4$^\circ$C, and greater in water than in acetone solution.

Choochote et al. (2005) evaluated a crude rhizome extracts and volatile oils of *Curcuma aromatic* for chemical composition and anti-mosquito potential, including larvicidal, adulticidal, and repellent activities against the *Ae. aegypti* mosquito. Volatile oil of *Cu. aromatic* possessed a significantly higher larvicidal activity against the 4th instar larvae of *Ae. aegypti* than that of hexane extracts, with LC$_{50}$ values of 36.30 and 57.15 ppm, respectively. On the other hand, hexane-extract (LC$_{50}$: 1.60 µg/
mg female) was found to be slightly more effective against female *Ae. aegypti* than volatile oil (LC₅₀: 2.86 µg/mg female). When it was applied at a concentration of 25%, a median complete protection time of 1 h (range = 1-1.5 h) appeared to have significantly higher repellency than that of distillate oil (0.5 h, range = 0-0.5 h).

Jang *et al.* (2005) established a *Chamaecyparis obtusa* leaf-derived beta-thujaplicin against the 4th instar larvae of *Ae. aegypti* (L.), *Ochlerotatus togoi* (Theobald), and *Cx. pipiens pallens* (Coquillett) in the laboratory. The hexane fraction of crude methanol extract was found to be active against the 3 species larvae (100% mortality) at 100 ppm. The LC₅₀ values of bioactive component recorded were 2.91, 2.60, and 1.33 ppm against *Ae. aegypti*, *OC. togoi* and *Cx. pipiens pallens* larvae respectively.

Nathan *et al.* (2005) investigated the larvicidal, pupicidal, adulticidal and antiovipositional activity of the neem (*Azadirachta indica* A. Juss) limonoids azadirachtin, salannin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin on *An. stephensi* Liston (Diptera: Culicidae). Azadirachtin, salannin and deacetylgedunin showed high bioactivity at all test concentrations, while the rest of the neem limonoids were less active, and were only biologically active at high test concentrations. Azadirachtin was the most potent in all experiments and produced almost 100% larval mortality at 1 ppm concentration.

Sing *et al.* (2005) evaluated larvicidal properties of fresh and methanolic leaf extract of milkweed (*Calotropis procera*) against mosquito larvae of *An. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti*. Methanolic extracts were, however, recorded as more effective larvicide.

Chaithong *et al.* (2006) studied on the larvicidal effect of ethanolic extracts derived from three *Piper longum* L., *P. ribesoides* Wall., and *P. sarmentosum* Roxb. ex Hunt., against early 4th instar larvae of *Ae. aegypti*. The LC₅₀ values were of 2.23, 4.06, and 8.13 ppm, respectively. Under light microscopy, the internal structures of anal papillae in the treated larvae showed shrinkage, while the external features were normal in appearance. Ultra structural studies, however, clearly demonstrated...
external destruction, with extensive damage and shrunken cuticle of the anal papillae.

Dua et al. (2006) studied on the larvicidal activity of the crude extract of roots of Hibiscus abelmoschus against the larvae of An. stephensi and Cx. quinquefasciatus. The mean median lethal concentrations were recorded as 52.3 and 43.8 ppm, respectively. Fraction code HAM-4 at the rate of 82 ppm showed 91.1% reduction of larval An. stephensi in a tank, whereas 87.4% reduction of larval Cx. quinquefasciatus occurred in a blocked drain 24 hours after application of HAM-4 under field conditions.

Komalamisra et al. (2005) studied on petroleum ether, methanolic and ethanolic extracts from various parts of 84 Thai plant species for their larvicidal activity against mosquitoes. The ethanolic extracts were tested for their larvicidal activity against Ae. aegypti larvae while petroleum ether (PE) and methanol (MeOH) extracts were tested against against 4 mosquito vector species. The ethanolic extracts from Rhinacanthus nasutus, Derris elliptica, Trigonostemon reidioides, Homalomena aromatica, Stemona tuberosa and Acorus calamus possessed high larvicidal activity with LC50 values between 16.0 and 48.2 mg/1. The PE and methanol extracts of R. nasutus exhibited larvicidal effects against Ae. aegypti, Cx. quinquefasciatus, An. dirus and Mansonia uniformis with LC50 values between 3.9 and 11.5 mg/1, and between 8.1 and 14.7 mg/1 respectively. In case of D. elliptica the LC50 values were recorded between 11.2 and 18.84 mg/1 and between 13.2 and 45.2 mg/1.

Mário Viana et al. (2006) evaluated the toxic effect of the fractionation of the crude latex produced by the green parts of the plant upon egg hatching and larval development of Ae. aegypti. The whole latex and fractions were very toxic to 3rd instars causing 100% mortality within 5 min and 24 hours respectively. Its water-soluble dialyzable (DF) and non-dialyzable (NDF) rubber-free fractions were partially effective to prevent egg hatching and most of individuals were recorded died before reaching 2nd instars or stayed in 1st instars. Both toxic compounds were of
low thermo stable protein in nature. The protease digested NDF lost most of its
toxicity but DF was still strongly active.

Patil et al. (2006) evaluated the larvicidal and pupicidal effect of Clerodendron inerme
against Ae. aegypti. The results showed a test concentrations-dependent larval
mortality and with El50 and EPQ50 of 40.8 mg and 144.8 mg respectively. 40, 50 and
60 mg powder showed pupal mortality after about 18–20 hours of study period. At
the end of 72 h, the mortality was recorded 48, 74 and 96% respectively. The larval
cuticular sclerotization as well as the less sclerotization in dead pupae was found in
compared to untreated ones. The head capsule of the majority of the pupae remained
attached to the pupal head.

Sharma et al. (2006) showed petroleum ether (Pee), carbon tetrachloride (Cte) and
methanol extract (Mee) of Artemisia annua, Chenopodium album and Sonchus oleraceus
as potent larvicide against malaria vector, An. stephensi L.. The Pee of A. annua was
found most effective, with LC50 16.85 ppm after 24 hours and 11.45 ppm after 48
hours of treatment followed by Cte of A. annua and Ch. album, Pee of Ch. album and
Mee of A. annua. The Pee of A. annua was influenced the early life cycle of An.
stephensi by reducing the percentage of hatching, larval, pupal and adult emergence
and also lengthening the larval and pupal periods. The growth index was also
reduced significantly.

Fredros et al. (2007) established larvicidal effects of a neem (Azadirachta indica) oil
formulation on the malaria vector An. gambiae. Neem oil had an LC50 value of 11
ppm after 8 days, which was nearly five times more toxic than the com oil
formulation. Adult emergence was inhibited by 50% at a concentration of 6 ppm.
Significant reductions on growth indices was recorded from 25.8 (in lake water
based medium) to as low as 0.5 and 3.6 when applied at concentrations of 32 ppm
and 16 ppm; 96% and 86% reductions respectively. Prolonged larval development
times were found significantly at concentrations equal to or higher than 16 ppm.
Pupation was significantly inhibited at concentrations higher than 8 ppm (P < 0.05).
Krishnan et al. (2007) screened methanol extracts of four species of Vitex: Vitex negundo, Vitex trifolia, Vitex peduncularis and Vitex altissima for differential larvicidal efficacy against the early 4\textsuperscript{th} instar larvae of Cx. quinquefasciatus larvae. The highest larvicidal activity was found with the extract of V. trifolia (LC\textsubscript{50} = 41.41 ppm) followed by V. peduncularis (LC\textsubscript{50} = 76.28 ppm), V. altissima (LC\textsubscript{50} = 128.04 ppm) and V. negundo (LC\textsubscript{50} = 212.57 ppm).

Murugan et al. (2007) investigated the larval toxicity and smoke repellent potential of Albizia amara and Ocimum basilicum at different concentration (2\%, 4\%, 6\%, 8\% and 10\%) against four instars larvae and pupae of Ae. aegypti. The LC\textsubscript{50} values of A. amara and O. basilicum for I instar larvae was 5.412 and 3.734, II instar 6.480 and 4.154, III instar 7.106 and 4.664, IV instar 7.515 and 5.124, respectively. The LC\textsubscript{50} and LC\textsubscript{90} values of pupae were 6.792\%, 5.449\% and 16.925\%, 15.474\%. The smoke toxicity of A. amara was more effective than the O. basilicum while the combination of two plant powders increased the toxicity of the smoke compared to individual plant powers.

Omena et al. (2007) screened ethanolic extracts of fifty-one species, belonging to 42 genera, of Brazilian medicinal plants for their larvicidal activities against Ae. aegypti. Eleven of the 84 extracts studied showed significant (LC\textsubscript{50} < 100 lg mL\textsuperscript{-1}) activities against larvae, with extracts from Annona crassiflora (root bark, LC\textsubscript{50} = 0.71 lg ml/ 1; root wood, LC\textsubscript{50} = 8.94 lg ml/ 1) and Annona glabra (seed, LC\textsubscript{50} = 0.06 lg ml/ 1) showing the highest activities.

Rahuman et al. (2007) screened ethyl acetate, butanol, and petroleum ether extracts of five plants, Jatropha curcas, Pedilanthus tithymaloides, Phyllanthus amarus, Euphorbia hirta, and Euphorbia tirucalli, for their larvicidal activities against the early 4\textsuperscript{th} instar larvae of Ae. aegypti L. and Cx. quinquefasciatus (Say). The highest larval mortality was found in petroleum ether extract. The LC\textsubscript{50} value of petroleum ether extracts of J. curcas, P. tithymaloides, P. amarus, E. hirta, and E. tirucalli were 8.79, 55.26, 90.92, 272.36, and 4.25 ppm, respectively, against Ae. aegypti and 11.34, 76.61, 113.40, 424.94, and 5.52 ppm, respectively, against Cx. quinquefasciatus. Their LC\textsubscript{90} values were
35.39, 256.77, 384.19, 703.76, and 13.14 ppm respectively, against Ae. aegypti and 46.52, 307.07, 465.28, 1314.01, 25.67 ppm respectively, against Cx. quinquefasciatus.

Senthil Nathan (2007) studied on secondary metabolites obtained from the leaf of Eucalyptus tereticornis Sm. (Myrtaceae) for its larvaicidal, pupicidal and adulticidal activity against An. stephensi (L.) under laboratory condition. The essential oil extract from the forest redgum, showed strong larvicidal (with LC50 18.3 ppm for 1st instar and 23.8 ppm for 2nd instar larvae where as LC90 values were 51.6 ppm and 63.9 ppm respectively) activity. In general, 1st and 2nd instar larvae were more susceptible to all treatments. No pupal or adult emergence was observed among the treatments as almost 100% mortality occurred within 24 hours. The plant extract evoking almost 100% mortality and maximum oviposition deterrence was observed in 160 ppm and it was significantly different from other lower concentration (except 80 ppm) treatments (F = 24.9205; df = 4; P < .0001).

Hanem F, Afaf AS (2008) screened six commercially available plant oils for their insecticidal effect against 4th larval instars of Cx. pipiens. The LC50 values were 32.42, 47.17, 71.37, 83.36, 86.06, and 152.94 ppm for fenugreek (Trigonella foenum-grecum), earth almond (Cyperus esculentus), mustard (Brassica compestris), olibanum (Boswellia serrata), rocket (Eruca sativa), and parsley (Carum ptroselinum), respectively. The plant oil exhibited various morphological abnormalities on larvae, pupae, and adult stages. The prolongation of larval and pupal durations was resulted from the lowest concentrations of olibanum and fenugreek oils. Where as 1000 ppm test concentration of mustard oil caused a remarkable decrease in pupation rate. Adult emergence was suppressed by earth almond and fenugreek oils at 25 ppm.

Josphat et al. (2008) established the larvicidal activity of leaf extracts from Aloe turkanensis, Aloe ngongensis and Aloe fibrosa against An. gambie. Ground leaves from the three plants were sequentially extracted with hexane, ethyl acetate, chloroform, acetone and methanol. The ethyl acetate soluble extract of A. turkanensis showed very high larvicidal activity with an LC50 of 0.11 mg/ ml. All the extracts of A. ngongensis showed larvicidal activity to An. gambie larvae, with LC50's of 0.84 (0.55 –
1.27), 1.14 (0.72 – 2.28), 0.98 (0.78 – 1.27), 1.08 (0.90 – 1.28), 2.0 (1.85 – 2.36) for the hexane, ethyl acetate, chloroform, acetone and methanol, respectively. The three active fractions of A. fibrosa had very close LC50’s ranging from 1.76 – 1.90 mg/ml.

Kaushik and Saini (2008) tested leaf extract of Millingtonia hortensis (Family: Bignoniaceae) for larvicidal activity against An. stephensi, Cx. quinquefasciatus and A. aegypti. The LC50 and LC90 values for 24 h exposure of Cx. quinquefasciatus were 138 and 269.2, 195 and 302, 208.9 and 316 for 2nd, 3rd and 4th instar respectively. For Ae. aegypti those were 104.70 and 281.8, 162.2 and 363.1, 223.9 and 426.6 and for An. stephensi are 83.18 and 190.5, 147.9 and 257, 138 and 275.4 for 2nd, 3rd and 4th instar of each mosquito respectively.

Nathan et al. (2008) tested secondary metabolites from Dysoxylum malabaricum and Dysoxylum beddomei against mature and immature stage of the mosquito vector An. stephensi under laboratory conditions. The triterpenes 3β; 25-trihydroxycycloartane and beddomeilactone from both of the plants showed strong larvicidal, pupicidal and adulticidal activity. The lower test concentrations treatments inhibited growth and caused mortality in a test concentrations-dependent manner. They have also affected the reproductive potential of adults by acting as oviposition deterrents.

Rao et al. (2008) screened for larvicidal and insecticidal properties of methanol and dichloromethane (1:1) extracts of some marine sponges collected in Palk Bay and Gulf of Mannar against the 4th instar larvae of Ae. aegypti (Linn) and three to four day old of female houseflies, Musca domestica (Linn). Among them, around 40% of test extracts were active against the fourth-instar larvae of Aedes aegypti (Linn) and three to four day old of female houseflies, Musca domestica (Linn) at the concentrations of less than 100 ppm and 100 µg/ insect respectively. However, other extracts of Dendrilla nigra, Petrosia testudinaria, Petrosia similes, Haliclona pigmentifera, Ircinia fusca, Sigmadociafibulata showed LC50 values at <100 ppm. P. purpurea and H. cribricutis having insecticidal properties against female adult M. domestica with LD50 values at <50 µg/ insect.
Elango et al. (2009) studied on the larvicidal efficacy of acetone, chloroform, ethyl acetate, hexane, and methanol extracts of leaves of Aegle marmelos (Linn.) Correa ex Roxb, Andrographis lineata Wallich ex Nees., Andrographis paniculata (Burm.f.) Wall. ex Nees., Cocculus hirsutus (L.) Diels, Eclipta prostrata L., and Tagetes erecta L. against 4th instar larvae of An. subpictus Grassi and Cx. tritaeniorhynchus Giles (Diptera: Culicidae). The highest larval mortality was found in leaf ethyl acetate of A. marmelos, E. prostrate; hexane, methanol of A. paniculata and Cx. hirsutus against An. subpictus (LC50 = 167.00, 78.28, 67.24, 142.83 ppm; LC90 = 588.31, 360.75, 371.91, and 830.01 ppm) and against the larvae of Cx tritaeniorhynchus (LC50 = 99.03, 119.89, 88.50, 105.19 ppm; LC90 = 479.23, 564.85, 416.39, and 507.86 ppm), respectively.

Haward et al. (2009) explored the potential of aqueous extracts of neem Azadirachta indica A. Juss (the neem tree)wood and bark chippings as a larvicide and growth disruptor of An. gambiae s.s. (Diptera: Culicidae) under laboratory conditions. (IE50) of all larval instars was obtained with <0.4 g of neem chippings in 1 liter of distilled water. For pupae, significant mortality occurred at 5 g/liter. Inhibition of pupation was seen with some larvae staying as LIVs for 9 d before dying. In addition to growth retardation, reduced reaction by larvae to visual and mechanical stimuli was observed at higher concentrations. It may make them more susceptible to natural predators. There were no significant differences in the sex ratio of emerged adults or wing length of females compared with the controls. A series of constituents of varying polarity, including the limonoids nimbin and salannin, were quantified from HPLC analysis.

Kamraj et al. (2009) studied on the acetone, chloroform, ethyl acetate, hexane, methanol and petroleum ether extracts of leaf, flower and seed of Cassia auriculata L., Leucas aspera (Willd.), Rhinacanthus nasutus KURZ., Solanum torvum Swartz and Vitex negundo L. against 4th instar larvae of malaria vector, An. subpictus Grassi and Japanese encephalitis vector, Cx. tritaeniorhynchus Giles (Diptera: Culicidae). The highest mortality was found in leaf petroleum ether, flower methanol extracts of Cx. auriculata, flower methanol extracts of L. aspera and R. nasutus, leaf and seed
methanol extracts of *S. torvum* and leaf hexane extract of *V. negundo* against the larvae of *An. subpictus* (LC₅₀ = 44.21, 44.69, 53.16, 41.07, 35.32, 28.90 and 44.40 ppm; LC₉₀ = 187.31, 188.29, 233.18, 142.66, 151.60, 121.05 and 192.11 ppm, respectively) and against the larvae of *Cx. tritaeniorhynchus* (LC₅₀ = 69.83, 51.29, 81.24, 71.79, 44.42, 84.47 and 65.35 ppm; LC₉₀ = 335.26, 245.63, 300.45, 361.83, 185.09, 351.41 and 302.42 ppm, respectively).

Mathew *et al.* (2009) screened few natural products viz., *Saraca indica/asoca*, *Nyctanthes arbor-tristis*, and *Clitoria ternatea* for mosquito larvicidal activity against three major mosquito vectors *Ae. aegypti*, *Cx. quinquefasciatus*, and *An. stephensi*. *C. ternatea* was showing the most promising mosquito larvicidal activity among all the plants studied. The petroleum ether extract of the leaves and the chloroform extract of the bark of *S. indica/asoca* were effective against the larvae of *Cx. quinquefasciatus* with respective LC₅₀ values 228.9 and 291.5 ppm. The LC₅₀ values of chloroform extract of *N. arbor-tristis* leaves and methanol extracts of *C. ternatea* seed against *Ae. aegypti* were 303.2, 65.2 ppm; *An. stephensi* 518.2, 154.5 ppm and against *Cx. quinquefasciatus* 420.2 and 54.4 ppm, respectively. The methanol and chloroform extracts of flowers of *N. arbor-tristis* showed larvicidal activity against larvae of *An. stephensi* with the respective LC₅₀ values of 244.4 and 747.7 ppm. The phytochemical analysis of the promising methanolic extract of the seed extract was positive for carbohydrates, saponins, terpenoids, tannins, and proteins.

Rahumann *et al.* (2009) studied on the effects of crude leaf acetone, chloroform, hot water, methanol, petroleum ether (60-80°C), and water extracts of *Calotropis procera* (Ait) R. Br., *Canna indica* L., *Hibiscus rosa-sinensis* Linn., *Ipomoea carnea* Jacq. spp. *fistulosa* Choisy, and *Sarcostemma brevistigma* against 2nd and 4th instar larvae of the laboratory-reared *Cx. quinquefasciatus* Say. The highest larval mortality was found in leaf acetone, chloroform, methanol, and petroleum ether of *CX indica* (LC₅₀ = 29.62, 59.18, 40.77, and 44.38 ppm; LC₉₀ = 148.55, 267.87, 165.00, and 171.91 ppm) against 2nd instar larvae and against 4th instar larvae (LC₅₀ = 121.88, 118.25, 69.76, and 56.31 ppm; LC₉₀ = 624.35, 573.93, 304.27, and 248.24 ppm). Where as acetone, hot water,
methanol, and petroleum ether extracts of *I. carnea* (LC$_{50}$ = 61.17, 41.07, 41.82, and 39.32 ppm; LC$_{90}$ = 252.91, 142.67, 423.76, and 176.39 ppm) against 2$^{nd}$ instar larvae and against 4$^{th}$ instar larvae (LC$_{50}$ = 145.37, 58.00, 163.81, and 41.75 ppm; LC$_{90}$ = 573.30, 181.10, 627.38, and 162.63 ppm) of *Cx. quinquefasciatus*, respectively.

Rajkumar and Jabensan (2009) studied on the ethanolic leaf extract of *Cassia obtusifolia* for the larvicidal and oviposition deterrence effects against *An. stephensi*. The larvicidal effect was recorded with LC$_{50}$ and LC$_{90}$ values of 52.2 and 108.7 mg/l, respectively against late 3$^{rd}$ instar larvae. In oviposition behaviour study, four different concentrations ranging from 100 to 400 mg/l were studied against gravid female mosquitoes indicating a concentration dependent oviposition deterrent activity. At higher concentration (400 mg/l) showed 92.5% effective repellency against oviposition, followed by 300, 200 and 100 mg/l showed 87.2%, 83.0% and 75.5%, respectively.

Santhilkumar *et al.* (2009) studied on the larvicidal and mosquitocidal efficacy of 11 commonly available medicinal plants against *An. stephensi* (L). The lethality varied in adults and plant extracts of mixture; *Eucalyptus globulus*, *Cymbopogan citratus*, *Artemisia annua*, *Justicia gendarussa*, *Myristica fragrans*, *Annona squamosa*, and *Centella asiatica* were found to be most effective. Larval mortality between 80% and 100% was observed in mixture treatment, *Cx. asiatica* and *E. globules*. The adults that emerged from all the treatments were malformed. Further, the treated larvae showed significant decrement in the levels of protein, carbohydrate, and lipids and affect negatively the presence of certain amino acids.

**Phytochemical Screening and Seasonal Distribution of Primary and Secondary Biochemicals**

Planas *et al.* (1981) reported that shoots of *Myriophyllum spicatum* contained 7% phenolic compounds on an average. Expressed in a similar way, values range from 6.7 to 0.8% for *P. gramineus* winter buds and monoecious *H. veriticillata* tubers respectively.
Harrison and Duronce (1989) reported that phenolic content of *Zostera marina* L. shoots varied seasonally from 0.65 to 1.54% of dry weight.

Buchsbaum *et al.* (1990) reported that leaf nitrogen content and phenolic content were inversely related for *Zostera marina* growing in running sea water mesocosms with either mud or sand substratum.

Lavola (1998) demonstrated the soluble carbohydrates and secondary phytochemical in *Betula*. The study revealed that the amount of primary and secondary biochemicals undergo marked variation in the SO2 pollution.

Durand *et al.* (1999) studied the pathological effects of *Cestrum diurnum* suggesting toxic plant intoxication. Blood examination (haemogram, albumin, total protein, calcium and phosphorus) and determination of bone calcium of bovine were done. Observed metabolic, pathological and clinical alternations were compatible with intoxication from *Cestrum diurnum*.

Haraguchi *et al.* (1999) worked on the polyhydroxylated spirostanol sapogenin and saponin in the leaves of *Cestrum senatenerianum*. Their structures were determined to be spirosta-5, 25(27)-diene-1β, 2α, 3β, 12β-tetrol and spirosta-5, 25(27)-diene-1α, 2α, 3β, 12β-tetrol 3-O-D-galactopyranoside respectively on the basis of spectroscopic analysis, including two-dimensional NMR techniques, and the result of hydrolytic cleavage.

Wang *et al.* (1999) studied carbohydrate content and composition in white spruce [*Picca glauca* (Moench) voss] seedlings growing in nursery beds. The study revealed a constant increase in starch content but total carbohydrate content was relatively constant. The composition of soluble carbohydrates changed in early spring with an increase in sucrose accompanied by a decrease in raffinose and other soluble carbohydrates.

Palaniswamy (2001) worked on the stage of harvest and polyunsaturated essential fatty acid concentrations in purslane (*Portulaca oleracea*). The study revealed a
significant change of unsaturation in the fatty acid concentration before harvesting and during the time of harvesting.

Francis et al. (2002) reviewed the biological action of steroidal saponins in animal system. These structurally diverse compounds had been observed to kill protozoans and molasses, impaired the digestion of protein and the uptake of vitamins and minerals in the gut and act as antifungal and antiviral agents.

Hendry (2002) classified the seeds on the presence of phenol content. Phenol contents around 25μM gdw⁻¹ were described as low and those with phenol contents of 32 or 45 μM gdw⁻¹ were described as intermediate. Values of 98 to 96 μM gdw⁻¹ characterized seeds with high phenolic contents.

Hoch et al. (2002) worked on low temperature driven carbon shortage to explain slow growth rate and tree line formation at high elevation. Concentrations of non-structural carbohydrates (NSC) in needles, branches, stems and roots as well as lipids (acylglycerols) were measured throughout the growing season in Pinus cembra. Starch was the most prominent non-structural carbon compound in needles, whereas lipids represented 50-75% of the mobile carbon compounds in wood.

Din et al. (2002) investigated on phytochemical screening of alkaloids, steroids or triterpenes and saponins was carried out on 103 leaf samples from 102 plant species representing 78 genera and 41 families. All plant materials were collected during the Crocker Range Scientific Expedition. From the samples screened, a total of 4, 19 and 53 leaf samples were found to give positive results for alkaloids, steroids/triterpenes and saponin, respectively.

Li et al. (2002) examined the responsiveness of non-structural carbohydrate (NSC) concentrations in tissues of trees which were defoliated (removal of sources), debudded (removal of sinks) or pruned (removal of both source and sinks) in naturally grown Pinus cembra L. at the upper treeline in the Swiss Central Alps. Complete defoliation and pruning of 66% of all branches in late winter caused a massive reduction of NSC (glucose, fructose, sucrose and starch) in all tissues during
and after the following growing season, whereas 100% debudding led to a small increase of NSC, except in new buds.

Mimaki et al. (2002) analyzed the steroidal glycoside constituents of the leaves of *Cestrum nocturnum* and resulted in the isolation of eight new steroidal glycosides, which were classified into a spirostanal saponin, a furostanol saponins, a pseudofurostanol saponin, two pregnane glycosides, two cholesterol glycosides and pregnane-carboxylic acid gamma-lactone glycoside and two known spirostanol glycoside. The structures of the new compounds were elucidated on the basis of chemical and spectroscopic evidences.

Miyazaki et al. (2002) worked on the allocation of resources to reproductive organs from reproductive shoots, from non-reproductive shoots and from the main trunk. In both the main trunk and non-reproductive shoots the pattern of seasonal variation in the amount of starch did not differ but the amount of starch in the reproductive shoots was less than that in non-reproductive shoots during the growing season.

Newell (2002) concluded that seasonal pattern of carbohydrate storage was related to seasonal variation in water and light variability and to foliar and reproductive phenology. The seasonal variation in non-structural carbohydrate (TNC) concentrations was determined in branch, trunk and root tissues of *Anacardium excelsum, Luehea seemannii, Cecropia longipes* and *Urera caracasana* and predicted that maximum carbon supply would occur when canopies were at their fullest and that maximum carbon demand would occur when leaves, flowers and fruits were produced.

Rippi et al. (2002) worked on seasonal pattern in allocation to growth and defense compounds by monitoring several chemical and physical traits in the leaves of mountain birch from early June (bud burst) to late September (leaf senescence), changes were very rapid in the spring, slow in the middle of the season, and there was another period of fast changes in the senescing leaves. Concentrations of protein and free amino acids declined through the growing season whereas individual
sugars showed variable seasonal pattern and the phenolics increased throughout the season.

Soumela et al. (2002) worked on the variation among and within mountain birch trees in foliage phenol, carbohydrates and amino acids during the growth of *Epirrita autunnata* larvae.

Tsialtas et al. (2002) worked on leaf ash content and concentrations were evaluated as surrogates of carbon isotope discrimination (Delta) in *Poa pratensis*, *Lolium perenne*, *Festuca valida* and *Taraxacum officinale* in an upland grassland ecosystem. The study established a curvilinear relationship between Delta and ash content.

Jayasankar et al. (2003) analyzed the biochemical constituents such as protein, carbohydrate and lipid of three different species viz., *Gracilaria edulis*, *G. corticata* and *G. crassa* and compound with the growth of the plant in different months. Carbohydrate content showed significant positive correlation with the growth of the plant but a reciprocal relationship was obtained with protein and lipid content in different species of *Gracilaria*.

Mello JRB (2003) reported the pathological and biochemical effects of calcinogenic plants viz., *Cestrum diurnum* in laboratory animals such as cattle, sheep, goats, pigs, horses and buffalo. The chemical nature of the toxic agents and the precise mechanism has been defined. The active principle present as steroidal glycoside is 1, 25 (OH) (2) D-3. The excess of vitamin D stimulates CaBP synthesis and calcium and phosphate absorption, producing hypercalcemia and/or hyper phosphatemia. The deposition of excess Vit-D results in calcinosis.

Anderson et al. (2005) investigated on crown buds of field-grown leafy spurge (*Euphorbia esula* L.) to determine relationships between carbohydrate metabolism and gene expression during the transition from summer, autumn, and winter, respectively. An inverse relationship developed between starch and soluble sugar (mainly sucrose) content in buds during the shift from para- to endodormancy, which continued through eco-dormancy. Unlike starch content, soluble sugars were
lowest in crown buds during para-dormancy but increased over two- to three-fold during the transition to endo-dormancy. Several genes (AGPase, HK, SPS, SuSy, and UGPase) coding for proteins involved in sugar metabolism were differentially regulated in conjunction with well defined phases of dormancy in crown buds.

Okwo (2005) analysed two Nigerian medicinal plants (Garcinia Kola Heckel) and (Aframomum melegued) for its phytochemicals, vitamins and minerals constituents. He has recorded the presence of bioactive constituents comprising: flavonoids (5.76-1.98 mg 100_lg), phenols (0.09-0.11 mg 100"g), saponins (1.24-11.48 mg 100"g), tannins, (0.26-0.38 mg/100g); vitamins like ascorbic acid (12.32-23.10 mg 100"g), niacin (0.05-1.60 mg 100"g), riboflavin (0.22-0.26 mg 100_lg) and thiamin (0.24-0.45 mg 100_lg) and minerals such as: Ca, P, K, Mg, Na, Fe, Zn, Mn and Cu.

Afolabi et al. (2007) screened the crude and methanolic extracts of Chromolaena odorata for phytochemical constituents. Tests for tannins, steroids, terpenoids, flavonoids and cardiac glycosides were positive in both methanolic and crude extracts. Alkaloids were detected only in the methanolic extract. The total phenolic content, reducing power and percent DPPH scavenging effect were 0.01 and0.00 mg/g GAE, 0.22 and 0.01 and 28.85 and0.99%, respectively.

Akubugwo et al. (2007) investigated the nutritional and chemical value of Amaranthus hybridus using standard analytical methods showed the percentage moisture content, ash content, crude protein, crude lipid, crude fibre and carbohydrate of the leaves as 84.48, 13.80, 17.92, 4.62, 8.61 and 52.18%, respectively while its calorific value is 268.92 Kcal/100 g. The chemical composition in mg/100 g (DW) for alkaloid, flavonoid, saponin, tannins, phenols, hydrocyanic acid and phytic acid were 3.54, 0.83, 1.68, 0.49, 0.35, 16.99 and 1.32, respectively with the major elements in high quantity, an appreciable amount of nutrients, minerals, vitamins, amino acids and low levels of toxicants.

Idu et al. (2007) screened a preliminary phytochemicals on extracts of water, methanol, chloroform and petroleum ether, of Senna alata flowers showed the presence of phenols, tannins, anthraquinoes, saponins, and flavonoids. Those
extracts were examined for antimicrobial properties against clinical isolates of *Staphylococcus aureus, Candida albicans, Escherichia coli, Proteus vulgaris, Pseudomonans aureginosa* and *Bacillus subtilis* and their zones of inhibitions showed that those phytochemicals are responsible for the bioactivity.

Ahmed *et al.* (2008) characterized leguminous plant species of Soone Valley (Salt Range) in Pakistan for carbohydrates, starch and total sugar contents in pods and leaves as well. They showed that carbohydrates ranged from 54.37 to 64.43; starch contents varied from 30.85 to 40.55 mg g\(^{-1}\) dry weight in leaves while it ranged from 61.65 to 69.09 and 32.55 to 36.11 mg g\(^{-1}\) in pods respectively. Overall values of soluble sugars range from 23.88 to 31.42 in leaves and 35.65 to 40.70 in pods in species under investigation in different pastures with a sufficient amount of total soluble sugars in forages.

Obog *et al.* (2008) carried out phytochemical investigation on the leaves of *Lecaniodiscus cupanoides* Planch (Sapindaceae) employing standard procedures revealing the presence of flavonoids, tannins, saponins and cardiac glycosides in the crude extract. The 90% ethanol and crude extracts of the leaves were against of *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and a standard strain of *Staphylococcus aureus* (NCTC 10788) to verify its claimed ethno medicinal use in the treatment of microbial infections. The minimum inhibitory concentration (MIC) values indicting the bioactive quality of the phytochemicals.

Pagter *et al.* (2008) studied on cold acclimation and deacclimation and associated physiological adaptations of shrub *Hydrangea macrophylla* and *H. macrophylla* from late September 2006 to early May 2007 indicating species-specific differences in carbohydrate metabolism during winter. Protein profiles differed between *H. macrophylla* and *H. paniculata*, but distinct seasonal patterns associated with winter acclimation were observed in both species. More over species-specific differences in cold hardiness was found to be related to differences in abscisic acid.
Sezai et al. (2008) analyzed the seasonal variations of total phenolic, antioxidant activity, PNE (Plant Nutrient Elements), and fatty acids in fresh tea leaves grown in Turkey and compared during the three commercial harvest seasons (May 15, July 15, and September 15) in both 2005 and 2006. The levels of total phenolics and antioxidant activity was higher at 2nd harvest time (62.88 µg/ mg and 89.27%). The seasonal variations of the individual fatty acids were significant (P < 0.05) between the three harvest seasons. The amount of N and P in tea leaves was the highest at 1st harvest; however K, Ca, Mg, S, and Mn were highest at 2nd harvest time.

Iniagie et al. (2009) investigated proximate composition and phytochemical constituents of leaves of Acalypha hispida, Acalypha marginata and Acalypha racemosa. They have recorded moisture (11.02%), crude fat (6.15%), ash (10.32%), crude protein (13.78%), crude fibre (10.25%) and carbohydrate (44.48%) contents in Acalypha hispida. Similarly, Acalypha marginata contained moisture (10.83%), crude fat (5.60%), ash (15.68%), crude protein (18.15%), crude fibre (11.50%) and carbohydrate (38.24%); while Acalypha racemosa contained moisture (11.91%), crude fat (6.30%), ash (13.14%), crude protein (16.19%), crude fibre (7.20%) and carbohydrate (45.26%). The phytochemicals detected in both crude and methanolic extracts of each of the different species of leaves were the same and are phenolics, flavonoids, hydroxyanthraquinones and saponins. Steroids and phlobatannins were detected in Acalypha hispida and Acalypha racemosa, while glycoside was detected only in Acalypha hispida.

Kumar et al. (2009) carried out phytochemicals Investigation on ethyl acetate and methanol extracts of Syzygium cumini seed used in this study revealed that the crude extracts contained alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids.

**Mosquito Control through Bacteria**

Majori et al. (1987) evaluated two wettable powders (Bactimos and Vectobac) and one flowable concentrate (Teknar) of Bacillus thuringiensis var. israelensis (Bti) and
primary powders of *B. sphaericus* isolates 1593 and 2362 (laboratory) against field-collected larvae of *An. gambiae* s.l. and *Cx. quinquefasciatus* in. Both wettable powders of B.t.i. showed superior activity than the flowable concentrate formulation against *An. gambiae* s.l. in the laboratory where as *Cx. quinquefasciatus* was more susceptible (3-4X) to B.t.i. (Bactimos) than *An. gambiae* s.l. The isolates of *B. sphaericus* were more effective (2-3X) against both mosquito species than Bactimos.

Arredondo *et al.* (1990) carried out the efficacy of 2 formulations of *B. sphaericus* (strain 2362) against *An. albimanus* and culicine (mostly *Cx. coronator* and *Cx. quinquefasciatus*) mosquito larvae of southern Mexico. The optimum concentrations of each formulation for effective control of larval populations was over a periods of 3-4 months were 0.125 ml/ m² of liquid product for *Culex* spp. and 2 g/m² of granular product for *An. albimanus*.

Jones *et al.* (1990) showed four application rates of *B. sphaericus* strain 2362 for efficacy in septic ditches against *Cx. quinquefasciatus* 2nd-4th instar larvae for over a 2-year period. The rate of larval reduction as well as residual effect was found higher in domestic sewage effluent ditches than that of dairy effluent ditches. A rate of 0.9 l/ha provided good to excellent control (greater than 88%) for a period of 10 days in domestic sewage effluent ditches.

Perich *et al.* (1990) evaluated four biorational larvicide formulations: Teknar (B.t.i.), Arosurf MSF (Monomolecular Surface Film), Arosurf MSF combined with Teknar, and SAN-809-I ([s]-methoprene combined with B.t.i.), against *An. albimanus*. All formulations reduced the mean number of larvae per sample area to 0 within 48 h after treatment, and gave significant (*P* < 0.05) control when compared with similar untreated areas for at least 10 days after treatment.

Yunus *et al.* (1990) investigated on three Bactimos formulations of *B. thuringiensis* H-14 for their toxicity against late larval instars of *Ae. albopictus* (Skuse) from laboratory and field populations. In the 24 h after application of mortalities were higher and residual effects longer in field populations than in laboratory ones. Briquets were the
most effective formulation (mortality 96-100% after five weeks; 76-92% after eight weeks). In the field, late instars were reduced by 62-87% after 24 h and 69-72% after one week compared to increases in an untreated population of 160% and 176% respectively.

Ceianu (1991) evaluated an Immediate (24 hours exposure) and long-term (until the emergence of the adults) effects of different test concentrations of a primary powder of *B. thuringiensis* subsp. *israelensis* (B.t.i.) against first and second instar larvae. The long-term effect was test concentrations-dependent and was materialized by a prolongation of the preimaginal development and continuous cumulative mortality until the emergence of the adults. The larvicidal effect was most effective against fourth instars with a higher mortality rate, decreasing size and 2-4 times longer larval stage. The long-term effect was more intense as the treatment was applied earlier during the larval development.

Knepper *et al.* (1991) established that the liquid B.t.i. (Vectobac 12AS), when mixed with water at a 1:3 ratio and applied against spring *Aedes* larvae in snowmelt pools by helicopter at a rate of 1.17 liters *B. thuringiensis* (4.68 liters mix) per ha, was 99% effective in a small (mass median diameter on dye cards: 178 microns) droplet size but ineffective (65%) in a large (553 microns) droplet. Results indicate that liquid formulations of *B. thuringiensis* could be aerially applied for spring *Aedes* control at a considerable cost savings and efficiency over aerially applied, granular formulations.

Montero *et al.* (1991) evaluated the effectiveness of the *B sphaericus* strain 2362 in liquid formulation, at a 10 ml/m² test concentrations, in 157 breeding sites of *Cx. quinquefasciatus* mosquitos, 2 breeding sites of *Cx. quinquefasciatus* and *An. albimanus* and 1 breeding site of *Ae. taeniorhynchus*, consisting of 1 river, 2 oxidation ponds, 1 pond, 4 dams, 2 microdams and 150 pits. The results 1800 l of biolarvicide show its effectiveness within 24 hours of treatment, (100tality rates), in a wide range of ecological conditions, its permanence up to 5 months in breeding sites without stream and innocuousness for other hydrobionts.
Chavenko et al. (1992) established the optimal bactoculicide/sphaero-larvicide ratio in combination using against *Ae. aegypti* larvae in laboratory by selection. The field trials of the selected BS/BT sample containing the ingredients in titer: bactoculicide-83, sphaerolarvicide-17 with 1 g/ m², complete mortality of *Culex*, *Aedes* and *Anopheles* larvae was obtained with the residual larvicidal action of 15 days.

Orduz et al. (1992) established a new isolate of *B. thuringiensis* that showed toxicity toward *Cx. quinquefasciatus*, *Cx. pipiens*, *Ae. aegypti*, and *An. stephensi* larvae was isolated. Supernatant fraction of the whole culture was not toxic, and heat-stable exotoxin production was negative.

Schenkel et al. (1992) established four *B. sphaericus* strains, S1, S2, S5, and L2, isolated from Brazilian soils as larvicide which were found to be toxic to larvae of the mosquitoes *Cx. pipiens* and *An. stephensi* at a level similar to that of strain 2362 which is now used operationally. Although the four Brazilian strains were very similar to strain 2362, gas chromatography analysis of the fatty acids revealed that these strains were different from strain 2362 and from each other, except for a possible similarity between strains S1 and S5.

Dua et al. (1993) evaluated a Bactoculicide (*B. thuringiensis*) in field trials for controlling mosquito breeding of *Aedes*, *Culex* and *Anopheles* in industrial scraps such as broken heavy machine parts, iron moulds and discarded drums. A dose of 0.5 g /m² controlled 96-100% mosquito breeding up to five weeks.

Mittal et al. (1993) investigated on Spherix, a powder formulation of *B. sphaericus* strain B-101, serotype H5a 5b, as larvicide against larvae of *An. stephensi* and *Cx. quinquefasciatus* in both the laboratory and field. In laboratory tests the formulation of 0.1 g/ m² produced 100% mortality against both of the larvae at room temperature. The larvicidal activity of Spherix against *An. stephensi* @ 0.5 g/ m² persisted for over 12 weeks under laboratory conditions. Field evaluation of Spherix @ 0.25-2.0 g/ m² produced 95-100% reduction in the larval density of both of the larvae within 48 h in different habitats, and the larvicidal activity persisted for 2-4 weeks in water habitats.
Rasnytsyn et al. (1993) established the efficacy of *B. thuringiensis* and *B. sphaericus* -based insecticides in salty water mosquito breeding sites through laboratory experiments. Species difference of mosquito larvae susceptibility to the insecticides related to salt concentration in water were detected.

Wilmot et al. (1993) evaluated Vectobac and Bactimos corn cob granules against *Aedes* species mosquito larvae in woodland pools. Both provided greater than 90% control at application rates as low as 100 mg/ m² (0.89 lb/ acre) and greater than 98% control at label-specified field application rates (2.5 or 5 lb/ acre).

Baruah and Das (1994) reported two formulations of *B. thuringiensis* and four strains of *B. sphaericus* (B42, B64, B87 and B33) against larvae of *Anopheles, Culex* and *Aedes* in different breeding habitats. In laboratory condition, *B. thuringiensis var israelensis* (formulation Teknar) was found to be more toxic against *Ae. albopictus, Cx. quinquefasciatus* and *Cx. gelidus* than that of *B. thuringiensis* (Deltox: VCRC B-17).LC₉₀ of *B. sphaericus* strains B42, B64, B87 and B33 against CX quinquefasciatus were 0.055, 0.115, 0.046 and 0.257 ppm respectively. In the field trials, out of four strains of *B. sphaericus* evaluated, strain B87 was found to be the most effective (87-96% mortality rates @ 0.1 kg/ ha dosage) against *Cx. quinquefasciatus, Cx. vishnui* and *An. vagus*.

Knepper et al. (1994) evaluated liquid *B. thuringiensis* H-14 (Acrobe) against *Aedes* larvae. It was applied from fixed-wing aircraft at a rate of 4.68 l of water-insecticide mixture of 1.17 l/ hectare to woodland pools in Michigan. A post-treatment larval survey indicated an 88.5% reduction in *Aedes* species larvae. A volume median diameter of 208 microns was determined.

Ansari et al. (1995) evaluated the Spherimos and Vectolex formulations of *B. sphaericus* against *Anopheles* and *Culex* larvae in both laboratory and field condition. In laboratory condition 0.008 ml/ m² test concentrations showed a higher larval mortality in *Cx. quinquefasciatus* than that of *An. stephensi*. But at higher test concentrations also was unable to kill *An. culicifacies* the similar level of larvae. Single application of a lower dose of Spherimos (2 ml/ m²) caused maximum (100%) reduction of *Cx. quinquefasciatus* larvae with maximal duration (2-3 weeks) in pools.
and wells than that of irrigation channel. Vectolex @ 10 ml/ m² provided 99-100% reduction of Cx. quinquefasciatus larvae up to 9 weeks in wells and 1 week in channels.

Ohba et al. (1995) evaluated eight toxic strains of B. thuringiensis, formed spherical parasporal inclusions against two mosquito species, An. stephensi and Cx. pipiens molestus. But it was not toxic for another dipteran tested: Telmatoscopus albipunctatus, or two lepidopterans, Bombyx mori and Hyphantria cunea. These strains were assigned to a previously undescribed flagellar (H) antigenic group. On the basis of the representative strain, 92-KU-137-4, a serogroup with H antigen 44, B. thuringiensis serovar higo was established as new. The anopheline toxicity (LC₅₀ = 6.3 µg ml⁻¹) was 10 times greater than the activity on the Culex mosquito.

Seleena et al. (1995) isolated a new serovar H28a28c B. thuringiensis strain from soil samples designated as serovar jegathesan and evaluated against Anopheles, Culex and Aedes larvae. Bioassays indicated that Cx. quinquefasciatus larvae are the most susceptible to this new isolate, whereas toxicity to An. maculatus and Ae. aegypti larvae was 10 times lower. The potency of this new serotype is also comparable to most of the Malaysian B. thuringiensis H-14 isolates.

Grovesh and Meisch (1996) evaluated B. sphaericus against larvae of Ae. aegypti, Ae. albopictus, Ae. vexans, An. punctipennis, Cx. quinquefasciatus, Cx. restuans, and Psorophora columbiae in laboratory condition and in the rice field against Ps. columbiae only. 24 hour LC₅₀ and LC₉₀ values were recorded higher than that of 48 hours of exposures. But in the field condition none of the posttreatment mortalities of Ps. columbiae after 24 hours were significantly different from control mortality (P ≤ 0.05). Where as at 48 h posttreatment for both dosages (0.45 and 0.9 g/ ha) were recorded 87 and 90%, respectively.

Smith et al. (1996) evaluated the cloned 135-kDa dually active CryIC delta-endotoxin from B. thuringiensis subsp. aizawai HD-229 and an acrystalliferous B. thuringiensis strain against Ae. aegypti, An. gambiae, and Cx. quinquefasciatus larvae. The
recombinant CryIC delta-endotoxin expressed in *Escherichia coli* was also toxic to *Ae. aegypti* larvae.

Vilarinhos (1996) evaluated primary powders of *B. sphaericus* strain S2 isolated from soil samples against second-instar larvae of *Cx. quinquefasciatus*, *Anopheles albimanus*, *An. quadrimaculatus*, and *Ae. aegypti*. Toxins of strains S2 and 2362 were extracted at pH 12 with NaOH and having 51 and 42 kDa toxins in both S2 and 2362 *B. sphaericus* strains. Though S2 had a lower LC₅₀ values than that of 2362, but, statistically the differences were found to be insignificant.

Gunasekharan *et al.* (1997) evaluated seven types of formulations as granules using the larvicidal factor of *B. sphaericus* and different concentrations of calcium alginate (which was used as matrix to immobilize and entrap the active ingredient -ai) against *Cx. quinquefasciatus* in disused wells. Among the seven types tested, the type 2 which contained 5% calcium alginate as immobilizing agent, exhibited the maximum larvicidal activity with a persistent control in breeding for 8 weeks.

Rosso and Delécluse (1997) evaluated two new crystal protein genes, cry19A and orf2, isolated from *B. thuringiensis* subsp. *jegathesan* against *An. stephensi* and *Cx. pipiens*. Purified inclusions containing either Cry19A alone or Cry19A and ORF2 together were more toxic to *Cx. pipiens* than to *An. stephensi*. However, inclusions containing Cry19A and ORF2 together were more toxic than inclusions of Cry19A alone but less toxic than the wild-type inclusions of *B. thuringiensis* subsp. *jegathesan*.

Yadav *et al.* (1997) evaluated a large-scale operational field trial for the efficacy of *B. sphaericus* (strain B-101, serotype H5a, 5b) in controlling *Cx. quinquefasciatus*, *Cx.tritaenioryynchus* and *Cx. vishnui*. Application of *B. sphaericus* had good potential for use against disease vectors and mosquito breeding in polluted as well as clean waters. Only at 1 g/ m² in storm drains, wastewater pools, abandoned masonry tanks, peripheral paddy fields, ditches, and other small water collections and at 4 g/ m² in domestic septic tanks, significantly reduced larval and pupal counts (P < 0.0001) and significantly reduced the percentage of habitats containing larvae (3rd-4th instars) (P < 0.0001) as compared with routine antilarval measures.
Yap et al. (1997) evaluated adulticidal and larvicidal performances of a water-based pyrethroid microemulsion Pesguard PS 102 (AI d-allethrin and d-phenothrin, both at 5.0% w/w) and Vectobac 12AS, an aqua-suspension B. thuringiensis israelensis (B.t.i.) formulation (AI 1,200 ITU/mg) were assayed against laboratory-cultured mosquitoes Ae. aegypti, Ae. albopictus and Cx. quinquefasciatus using a Leco ULV Fog Generator Model 1600 and a Scorpion 20 ULV AirBlast Sprayer. The mortality rates were varied with distance from spray nozzle and with strong wind conditions. Both of the larvicidal and adulticidal effects are influenced by using a combination of B.t.i. and Pesguard PS 102 was higher in ULV space spray than that of with the Scorpion 20.

Mulla et al. (1998) evaluated two newly developed B. sphaericus larvicidal formulations, VectoLex CG (corn cob granules) and VectoLex WDG (water dispersible granules) against Cx. quinquefasciatus larvae in 4 highly polluted breeding sites. The VectoLex WDG, had higher potency to larval reduction than that of VectoLex CG. Among the factors influencing longevity of control was dosage of a given formulation, precipitation, presence of moderate numbers of larvivorous fish and flooding of the treated sites; the latter had the greatest impact.

Saitoh et al. (1998) screened Japanese isolates of B. thuringiensis for larvicidal activity against An. stephensi. Anopheles-active strains belonged to more than 12 H serotypes, especially H3ade (serovar fukuokaensis) and H44 (serovar higo). The most active Japanese isolates were H20 strain 89-T-34-14 (LCso 4.4 µg/ ml) and H44 serovar higo strain 74-E-45-24 (LCso 7.6 µg/ ml), which were found to be 13-fold and 23-fold less active than the international standard H14 serovar israelensis (LCso 0.33 µg/ ml).

Sharma et al. (1998) studied on the spray impact of Spherix (B. sphaericus B-101, Serotype H5a, 5b) was assessed against larval and adult populations of anopheline and culicine. High mortality (> 95%) was observed against larvae within 48 h of spray @ 1 g/ m². But the nonrecycling capacity caused the declinment of biolarvicidal effect within a week. The two year intensive field trials, however, had no appreciable impact on adult densities of both malaria vector An. culicifacies and bancroftian
filariasis vector *Cx. quinquefasciatus*. Environmental disturbances and manmade problems also affected the efficacy of the biolarvicide.

Ganushkina *et al.* (1999) tested the recombinant strain of *Methylobacillus flagellatum* with the cloned synthesis gene Cry 4B of the toxic *B. thuringiensis* var. *israelensis* protein proved to be effective against larvae of the *An. stephensi*, *An. atroparvus*, *An. pulcherrimus*, *An. superpictus*, and *An. sacharovi* cultured in the laboratory. Their combined use shows a 6-fold increase in the rate of strain action and a 4-fold decrease in the concentration of the agent. The optimum effects are shown following 24-hour combined intubation of *M. flagellatum* and *Tetrahymena pyriformis*.

Srivastava and Tilak (1999) revealed the efficacy of three formulations; wettable powder (W.P.) floating pellet and beads of *B. thuringiensis* var. *israelensis* (Bti), against larval Anophelines and Culicines. The early Anopheline larval instars were found to be greater susceptible to Bti, sensitivity of anophelines to floating pellet, culicines to bead and equal efficacy and faster kill of W.P. to all the mosquitoes tested. A greater persistence of the slow release formulations, floating pellet and beads for 49 and 28 days against anophelines and culicines respectively was observed in contrast to a maximum persistence for 21 days in case of W.P. formulation.

Dominic *et al.* (2000) evaluated the efficacy of crude suspension (AS) and granular (G) formulation of *B. thuringiensis* var. *israelensis* (Vectobac) against the immatures of *Cx. quinquefasciatus*, *Ae. aegypti* and *An. stephensi* in the laboratory and under field conditions. Laboratory tests showed that the crude suspension was relatively more effective against *Cx. quinquefasciatus* than *Ae. aegypti* and *An. stephensi*, the respective LC₅₀ values being 0.046, 0.060 and 0.190 mg/l. In field condition polluted water bodies showed much effectivity of formulations than that of clear one with out having any significant difference in the effectiveness between the two formulations and the two application rates.

Ganushkina *et al.* (2000) investigated on the duration of action of sporocrystalline mass of *B. thuringiensis israelensis* (Bti) of 6 months and *B. sphaericus* (Bsph) of 2
months larvicidal activity against three-age larvae of *A. stephensi* mosquitoes in the laboratory setting. The duration of action depends on its initial concentration with $10^5$ spores/ml as the optimal. Only the decreasing the test concentration of and increasing the number of the larvae reduces the duration of larvicidal action of Bti. The presence of dead larvae in the test vessel increases the duration of action by Bti only by 4 times whereas Bsph acts rather long.

Wirth et al. (2000) evaluated the capacity of various combinations of the cytolytic protein Cyt1A from *B. thuringiensis* subsp. *israelensis* to enhance the toxicity of *B. sphaericus* toward *Ae. aegypti*. A ratio of 10:1 of *B. sphaericus* to Cyt1A was 3, 600-fold more toxic to *Ae. aegypti* than *B. sphaericus* alone. Statistical analysis showed this high activity was due to synergism between the Cyt1A toxin and *B. sphaericus*.

Xu et al. (2000) studied on larvicidal activity of *Anabaena* strains expressing the binary toxin genes of *B. sphaericus* against *Culex* larvae with living cells with the cooperation of the two 51-kDa and 42-kDa toxin proteins. Outdoor tests with the genetically altered *Anabaena* could keep containers with natural water free from *Culex* larvae for over 2 months.

Lone et al. (2001) tested seven field isolates of *B. thuringiensis* from the Lower Silesia, region of Poland, the Osola plain and phylloplane niches and soil samples from the Karkonosze National Park in vitro for insecticidal activity against mosquito larvae *Ae. aegypti*. Both the spore/crystal mixture and pured crystals from *B. thuringiensis* strains KpC1, KpF3 and OpQ3 (belonging to the first physiological group including the subspecies *japonensis*, *yoso*, *jinghongiensis*) proved to be the most active against insects (61-65% of corrected mortality). The lowest toxicity (7-28% mortality) was caused by *B. thuringiensis wratislaviensis* strains (PO12 and 13).

Mittal et al. (2001) investigated on toxicity of selected larvicidal formulations of *Bacillus sphaericus*, *B. thuringiensis* H-14 *israelensis* (Bti) and insect growth regulators against *A. stephensi* and *Ae. aegypti*. Bti formulations were more toxic against *Ae. aegypti* ($LC_{50} = 0.06$ and $0.14$ mg/l) than against *A. stephensi* ($LC_{50} = 0.14$ and $0.81$ mg/l), while *B. sphaericus* formulation was more toxic against *An. stephensi* $LC_{50} = 0.031$
mg/ l than *Ae. aegypti* LC$_{50}$ = 0.294 mg/ l. IGR compounds were found to be toxic against both the mosquito species at very low concentrations (EC$_{50}$ values ranging between 0.0001 and 0.0004 ppm).

Mulla *et al.* (2001) investigated several larvicidal treatments of *B. sphaericus* strain 2362 water dispersible granular (WDG) formulations were made at 50 to 200 mg/ m$^2$ in mosquito developmental sites to determine whether larviciding dense populations would results in a noticeable reduction of adult mosquitoes in small treated areas. In the treated area the decline in immature populations was followed by a substantial decline in adult mosquitoes. There was a lag of 7 to 14 days post-larval treatments before maximum decline in adults was noted, with out a drastic reduction soon after treatments. Adults that emerged prior to treatments survived for 7-14 days or longer. During the last 2 weeks (17 days post last treatment) of the experimental period, female populations reached the pre-treatment level.

Darriet and Hougart (2002) studied on a new strain of *Bacillus circulans* isolated from a larva of *Cx. quinquefasciatus* against 3 mosquitoes. Its insecticidal potentiality was found in the spores but not in the chitinases or exotoxins. Compared to *B. sphaericus* strain 2362, this *B. circulans* isolate proved less toxic to *Cx. quinquefasciatus* and *A. gambiae* but was 107 times more toxic to *Ae. aegypti* and as pathogenic as *B. thuringiensis* var. *israelensis* in *Ae. aegypti*.

Zahiri *et al.* (2002) investigated on *B. sphaericus* (B. spi) strain 2362 at reversing previously established Bsph resistance in a laboratory colony of *Cx. quinquefasciatus* Say by selections with Bti alone, Bti and Bsph in rotation, or mixture. The selections with Bti and Bsph in rotation increased susceptibility to Bti in Bsph-resistant colony. It is promising that selection with Bti alone, Bsph and Bti in rotation or mixture have a potential for developing practical strategies to overcome acquired resistance to Bsph in *Cx. quinquefasciatus* populations.

Fillinger *et al.* (2003) evaluated the efficacy of new water-dispersible granular (WDG) formulations of *B. thuringiensis* var. *israelensis* (Bti; VectoBac) and *B. sphaericus* (Bs; VectoLex), Valent BioScience Corp., Illinois, USA) for the control of
larval *A. gambiae* sensu lato Giles mosquitoes in Western Kenya. The *A. gambiae* larvae proved more susceptible to Bs than to Bti and the WDG formulations were slightly superior to the powder formulations. In the open field trials and a minimum dosage of 200 g/ha Bti WDG, was sufficient to fully suppress emergence of mosquitoes when applied at weekly intervals with out having residual effect, irrespective of the concentration applied. The Bs WDG formulation, however, showed significant larval reductions up to 11 days post-treatment at application test concentrations of either 1 or 5 kg/ha.

Lonc *et al.* (2003) tested pure crystals of seven *B. thuringiensis* field isolates from the Lower Silesia region (Poland) against larvae of *Ae. aegypti* L. and *Cx. pipiens* L. (Culicidae, Diptera). The crystals of OpQ3 phylloplane isolate (belonging to the first biochemical type of *B. thuringiensis* subsp. *japonensis*, *yoso*, *jinghongiensis*) killed from 68 ± 7% to 84 ± 7% of the fourth instar larvae of *Ae. aegypti*. The crystals of two other strains (KpF3 and KpC1) of this group caused mortality between 3± 2% and 70± 7%. The effect of *B. thuringiensis* wratislaviensis H-47 crystals was the lowest with larval mortality from 0% to 17 ± 3%. No significant (0%-37 ± 6%) effect of *B. thuringiensis* crystals on the larvae of *Cx. pipiens* was observed, but, the delta-endotoxins of *B. thuringiensis* act very specifically.

Lukshananil *et al.* (2003) tested two recombinant *B. cereus* strains Ae10 and C5 isolated from mosquito larval guts against *Cx. quinquefasciatus* larvae. The production and presence of the 51-kDa toxin protein in both strains was confirmed by Immunoblotting analysis. The strains were transformed with a recombinant plasmid, pBS373, harboring binary toxin genes from *B. sphaericus* 2297.

Sharma *et al.* (2003) studied on the efficacy of a new Bti formulation in the laboratory and small scale field trials against mosquito larvae. Laboratory tests revealed increased efficacy against *Cx. quinquefasciatus* (LC50 = 0.035 mg/l) followed by *Ae. aegypti* (LC50 = 0.0628 mg/l), *An. culicifacies* sp A (LC50 = 0.184 mg/l) and *An. stephensi* (LC50 - 0.2216 mg/l). In the field trials, 100% mortality of mosquito larvae was recorded after treatment with Bti @ 0.5 g/m² surface area on different mosquito
species breeding in different habitats. However, repeated treatments were required due to reappearance of larvae in the breeding habitats within a week. No side effects of Bti was observed during field trial on non-target organisms (NTOs). Environmental disturbances and man made problems affected the spray impact of Bti.

Toma et al. (2003) evaluated the effectiveness and duration of effectiveness of a new formulation of *B. thuringiensis* var. *israelensis* (Bti) - Vectobac DT (ABG-6499) against larval *Ae. albopictus*. A Bti tablet formulation containing 3.4% of active ingredient (3,400 ITU/mg) for control of larval in most favorable season for their development in Italy. Vectobac DT induced 100% larval mortality after 24 h in all experimental breeding sites during the entire study period. Nonetheless, in most cases, the larvicidal activity only lasted about 48 h.

Chansang et al. (2004) evaluated the efficacy of a larvicide, *B. thuringiensis* var. *israelensis* (Bti), using singly and jointly, against *Ae. aegypti* in water containers. In a laboratory test, copepods alone produced mortality of 98-100% in 1st instar larvae of *Ae. aegypti* at copepod:larvae ratios ranging from 1:1 to 1:4. In an outdoor field simulated experiment that ran for 16 wk, after a single inoculation, the treatment of copepods and Bti combined yielded the better, more sustainable results than the agents used individually. Bti alone kept the larvae at the zero level for the first 4 wk after a slight increase in numbers and that low levels were sustained up to 12 wk. The number of copepods in the joint treatment was significantly higher than in the copepod alone treatment for the first 8 wk (t = -4.97, df = 14, P<0.01). The density of copepods, however, for the whole 16-wk period was not significantly different in these two treatments (t = -1.51, df = 30, P>0.1).

Gunasekharan et al. (2004) investigated on an improved biolarvicidal formulation of *Bacillus thuringiensis* ssp. *israelensis* (Bti), larvicidal efficacy of Teknar HP-D, against *A. stephensi*, *Cx. quinquefasciatus* and *Ae. aegypti* in the laboratory, and in field against *Cx. quinquefasciatus* breeding in cesspits, unused wells and drains. *Ae. aegypti* showed greatest susceptibility to the Bti toxin in the laboratory. The two
predatory water bugs, Notonecta sp. and Diplonychus indicus that fed on the surviving larvae of *Cx. quinquefasciatus* exposed to the sub-lethal test concentrationss (LC$_{50}$ and LC$_{90}$) of Teknar HP-D were safe with out having any mortality. Teknar HP-D @ 1, 1.5 and 2 l/ha was field tested at three selecting five habitats for each dosage. Another five habitats were kept untreated as controls. The residual activity of the formulation was for 17 days from the day of treatment in unused wells. However, in drains, >80% reduction of pupal recruitment was observed for only 3 days and hence, application of Teknar HP-D at 2 l/ha that caused significantly higher level of reduction twice in a week at 3-day interval is necessary.

Zhakhongirov *et al.* (2004) studied on the effect of granules, emulsion and powder like drug from Bti on *Culex, Aedes* and *Anopheles* larvae. Within the first 24 hours after treatment the numbers of larvae of pp. *Culex* and *Aedes* were drastically reduced (92.3-100% death) at all test test concentrationss (0.01-5.0 g/ m$^2$). and the efficacy of the drugs retained for more than 7 days (100% death). The second experiment have shown that all the test dosage forms of BTI drugs against *An. superpictus* larvae within 5 days after treatment of water reservoirs displayed virtually a 100% efficacy; on day 7$^{th}$ day and onwards, their efficacy slightly decreased, and on day 14, drugs as granules and emulsions showed a noticeable reduction in their efficacy than that of powder-like drugs (64.2-100%).

Lee *et al.* (2005) Studied on the bioefficacy and residual activity of *B. thuringiensis israelensis* H-14 (Bti) (water-dispersible granules of VectoBac ABG 6511 and liquid formulations of VectoBac 12AS) and pyriproxyfen (insect growth regulator, Sumilarv 0.5%) as direct applications for control of larvae of *Ae. aegypti* and *Ae. albopictus*. The higher dosage (570 ITU/liter) for both Bti formulations was only partially effective at the end of 1 wk after being diluted. Water-dispersible Bti granules provided effective initial control activity for both test designs (with and without replenishment of water). After 1 wk, water-dispersible Bti granules provided greater larval mortality and a negative effect on adult emergence after
day 1 against both mosquito species when integrated with pyriproxyfen. Pyriproxyfen (79.5 and 159 mg/liter) on its own showed low larvicidal activity but provided very effective control of adult emergence. Daily replenishment of water increased Bti activity and provided slightly better larval control.

Zahiri and Mulla (2005) have studied on the ovipositional response of Bsph susceptible and resistant Cx. quinquefasciatus to crude suspensions of Bti and Bsph water dispersible granules (WDG) and an inverse relationship between Bacillus product concentrations and oviposition was recorded. The combined effects of reduced oviposition, reducing the number of gravid females and male, female adult mortality on imbibing on Bti and Bsph suspensions.

Fillinger and Linsay (2006) determined the microbial larvicides of B. thuringiensis var. israelensis and B. sphaericus in a 4.5 km² area to reduce the malaria and implemented for 28 months. Overall, larviciding reduced Anopheles larval density by 95% and human exposure to bites from adults by 92%. The estimated cost of providing this protection to the human population in the study area was less than US$ 0.90/person/year.

Gammon et al. (2006) studied on the extensively used mosquitocidal toxins producing B. sphaericus and B. thuringiensis subsp. israelensis. The toxin is produced during sporulation and applied in the field for control of mosquito populations. The resulting transconjugant erythromycin resistance-marked pBtoxis plasmid was transferred to B. sphaericus bacteria were significantly more toxic to Ae. aegypti mosquitoes and were able to overcome resistance to B. sphaericus in a resistant colony of Cx. quinquefasciatus, apparently due to the production of Cry11A but not Cry4A or Cry4B.

Zghal et al. (2006) evaluated a newly isolated strain of B. thuringiensis, named BUPM97, was identified as affiliated to the israelensis subspecies against larvae of Cx. pipiens. A 5 kb EcoRI fragment, containing a cry4Ba-type gene, named cry4BLB, was cloned from BUPM97. A two-amino-acid substitution located in domain III of the N-terminal moiety of this protein, which is very important for both toxicity and
specificity of the toxin. cry4BLB transfer to \textit{B. thuringiensis} kurstaki strain synthesizing Cry2A endotoxin known to be weakly toxic to the dipteran insect \textit{Cx. pipiens}. Zhang \textit{et al.} (2006) studied on recombinant strains against \textit{Cx. quinquefasciatus}. Using the shuttle vector pBU4, the mosquitocidal toxin gene mtx1 from \textit{B. sphaericus} strain SSII-1 was introduced into an acrystalliferous strain of \textit{B. thuringiensis} both individually and in combination with the accessory protein gene p20 and the cytolytic protein gene cyt1Aa from \textit{B. thuringiensis} subsp. israelensis. Bioassay results indicated that the recombinants B-pMT4 (Mtx1) and B-pMT9 (Mtx1), both individually containing mtx1, had moderate toxicities to binary toxin susceptible and binary toxin resistant \textit{Cx. quinquefasciatus} larvae during the vegetative growth stage, but that their toxicities declined rapidly during the sporulation phase. Meanwhile, the recombinant B-pMPX2 (Mtx1+Cyt1Aa) expressing Mtx1, P20 alone, and Cyt1Aa in combination had stable toxicities during both the vegetative phase and the sporulation phase. Furthermore, expression of Cyt1Aa appeared to enhance the activity of Mtx1 to target mosquito larvae, suggesting a synergism between Cyt1Aa and Mtx1 toxins.

Beron and Salerno (2007) characterized a novel \textit{B. thuringiensis} isolate native to Argentina (FCC 41) that exhibits a mosquitocidal activity higher than the reference \textit{B. thuringiensis israelensis}. This isolate shows a rounded crystal harboring two major proteins of about 70-80 kDa. Moreover, they have cloned and sequenced the encoding gene of one of the crystal proteins -Cry24Ca consisting of an open reading frame of 2061 pb that encodes a protein of 687 amino acid residues. The deduced amino acid sequence has a predicted relative molecular mass of 78 kDa and is 52% and 45% identical to those of the reported Cry24Aa and Cry24Ba sequences, respectively. The novel Cry protein was designated as, which also exhibited larvicidal activity against \textit{Ae. aegypti} when its encoding gene was expressed in an \textit{Escherichia coli} host strain.
Cetin et al. (2007) reported the efficacy of tank mixtures of commercial *B. thuringiensis israelensis* (Bti) and *B. sphaericus* (Bs) water-dispersable granule (WDG) formulations was evaluated in septic tanks, against *Cx. pipiens* L. (Diptera: Culicidae) larvae. The lowest test concentrations of applications and ratios (VectoLex WDG (Bs) + VectoBac WDG (Bti)) delivering VectoLex WDG provided this level of control for at least 7 days after treatment. A re treatment interval of 2 wk (greater than 80% control after 2 wk) is recommended with the lowest test concentrations and re treatment intervals of 4 or more wk are recommended with the test concentrations equal to or higher than 988 g/ ha, provided more than 90% control for 28 days after treatment.

Park et al. (2007) studied on a highly toxic *B. sphaericus* (WBM 1-1-13) against 4th stage *Cx. quinquefasciatus*. It showed significantly higher toxicity compared with strain 2297 and equivalent; Furthermore, the Winter Beach marsh isolate produced more Bin per unit medium than strain 2362.

Sorensen et al. (2007) studies on the effects of two widespread environmental pollutants, perchlorate and hexavalent chromium, on the efficacy of *B. thuringiensis israelensis* (Bti) and *B. sphaericus* (Bsph) against 4th instar of *Cx. quinquefasciatus* Say (Diptera: Culicidae) in 24 hours laboratory bioassays. 250 mg/ l perchlorate did not affect the control provided by either larvicide but, 1.04 mg/ l hexavalent chromium, an increased the efficacy of both Bti and Bsph by 21 and 80%, respectively.

Wirth et al. (2007) reported two mosquitocidal toxins (Mtx: Mtx-1 and Mtx-2) of *B. sphaericus*, against *Cx. quinquefasciatus* mosquitoes, with LC₅₀ of Mtx-1 and Mtx-2 of 0.246 and 4.13 µg/ ml, respectively. The mixture of two Mtx toxins and *B. sphaericus* was 10 times more active against susceptible mosquitoes than *B. sphaericus* alone. The LC₅₀ s were 0.406 to 0.430 µg/ ml when Mtx-1 or Mtx-2 was mixed with *B. sphaericus*, and synergy improved activity and reduced resistance levels. The combination of proteins with a recombinant *Bacillus thuringiensis* produced Cry11Aa, were highly active against Cry11A-resistant larvae and resistance was also reduced.
Hadpad et al. (2008) studied on eco-friendly UV protectants in formulations UV-A, UV-B of an indigenous isolate of *B. sphaericus* Neide, ISPC-8 larvicide under laboratory conditions to get a prolonged efficacy of biopesticides under field conditions, especially in tropical countries. The UV sensitivity of ISPC-8 and standard strain 1593 were when tested against 3rd instar larvae of mosquito, *Cx. quinquefasciatus* Say, the spore viability at 8 hours exposure time was drastically reduced to 2.5% in ISPC-8 and 0.3% in 1593. The spore viability and larvicidal activity patterns were found to be similar to UV-B treatment when spores were exposed to a combination of UV-A and UV-B.

Jones et al. (2008) reported the Cry48Aa/Cry49Aa binary toxin of *Bacillus sphaericus* against *Cx. quinquefasciatus* mosquito larvae to be non-toxic to coleopteran, lepidopteran and other dipteran insects, including closely related *Aedes* and *Anopheles* mosquitoes. Gut extracts from *Culex* and *Aedes* larvae showed differential processing of the Cry48Aa protein, with the location of cleavage sites in *Culex* reflected those same as the activation of Cry4 toxins in mosquitoes. Pre-activation of Cry48Aa/Cry49Aa with *Culex* extracts, however, fails to induce toxicity to *Aedes* larvae. Co-administration of Cry49Aa with Cry4Aa gives higher than predicted toxicity, perhaps suggesting weak synergism against *Culex* larvae between Cry49Aa and other three-domain Cry toxins.

Pravakaran and Hoti (2008) developed a protocol for freeze and spray drying methods of *B. thuringiensis* var. *israelensis* (VCRC B-17) through optimizing parameters such as inlet temperature and atomization type and tested against early 4th instars *Ae. aegypti* larvae. The freeze-dried powders retained the larvicidal activity fairly well (LC50=16.18 ng/ml). Though the spray-dried powder showed a higher larvicidal activity (LC50=17.42 ng/ml) but it moderately lost its larvicidal activity at different inlet temperatures. Between the two types of atomization, centrifugal atomization retained more activity than the nozzle type atomization.

Pravakaran et al. (2008) studied on *B. thuringiensis* var. *israelensis* (Bti) by growing this bacterium in coconut waste product and in comparison with the conventional
medium (NYSM) against early 4th instar larvae of *Ae. aegypti*. The 30 hours old culture having a mosquito larvicidal activity LC_{50} of 14.85 ng/ml. *B. thuringiensis* var. *israelensis* produces parasporal crystal protein spore crystal complex (SCC) during sporulation, which is toxic in the mosquito larvae gut. They have reported tangential flow ultra-filtration yielded the maximum amount of spore crystal complex (SCC) from the fermented broth of *B. thuringiensis* var. *israelensis* (53.3 g/l), maximum number of spores (2.30 x10^{18} CFU/ml) and highest level of larvicidal activity (LC_{50} 28 µl/ml) against *Aedes aegypti* Bora-Bora strain followed by continuous centrifugation and acid precipitation methods.

Rungrod *et al.* (2009) studied on mosquitocidal toxins Mtx1 and Mtx2 produced by some strains of *Bacillus sphaericus* during vegetative phase of growth against *Cx. quinquefasciatus* and *Ae. aegypti*. Mtx1 from *B. sphaericus* 2297 shows higher toxicity against *Cx. quinquefasciatus* larvae than to *Ae. aegypti* larvae whereas Mtx2 from *B. sphaericus* 2297 shows lower toxicity against *Cx. quinquefasciatus* than to *Ae. aegypti* larvae. mtx1 and mtx2 genes were cloned into a single plasmid and expressed in for synergistic effect against *Ae. aegypti* larvae. Cells producing both Mtx1 and Mtx2 toxins exhibited approximately 10 times higher synergistic activity against *Ae. aegypti* larvae compared to cells expressing only a single toxin.

Singh and Prakash (2009) studied on the control of all instars of *An. stephensi* Liston and *Cx. quinquefasciatus* Say using *B. sphaericus*. During the experiments, the mortalities were not found highly effective in dose concentration of LC_{90} 0.01 mg/l. Thereafter, six different concentrations were used in laboratory bioassays (0.05, 0.10, 0.20, 0.30, 0.40, and 0.50 mg/l) for *An. stephensi*. Similarly, in the case of *Cx. quinquefasciatus*, six statistically significant different concentrations were used (0.01, 0.04, 0.05, 0.10, 0.50, and 10.0 mg/l) of *B. sphaericus*. The result of the study distinctly showed that *B. sphaericus* is not effective on selected defined doses against *An. stephensi* and *Cx. quinquefasciatus*. This probably indicates an initiation of resistance. This efficacy study can be useful while detecting early resistance phenomena in environment specific zones.