Alpha-terthienyl: A plant-derived new generation insecticide

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Alpha-terthienyl, a naturally occurring secondary plant metabolite is found in abundance in the roots of *Tagetes* species (family Asterasceae). It is activated by ultraviolet light and is toxic to a number of insect species. It generates oxygen radical species and has capacity to inhibit several enzymes like both *in vivo* and *in vitro*. Alpha-terthienyl possesses all the desirable properties of a good insecticide/pesticide. It is fast acting, non-toxic, economic and a property of degradation makes it more user friendly and safe. Secondary plant metabolites will play an important role in future insecticide development programme.

MAN has suffered from the activities of mosquito since time immemorial. It is believed that mosquitoes rank as man's most important insect pest. They influenced and still influence his selection of living and working sites. They scourge him with their vicious biting attacks and continuous singing, but more serious are the diseases that they transmit to man and his pets (Table 1).

Synthetic insecticides such as organochlorines, organophosphorous, carbamates, synthetic pyrethroids, etc. are commonly used to control these pests. Consumption of these insecticides/pesticides is very high, specially in India (Figure 1).

The greatest harm from chemical insecticides is that, once introduced into the system, they may remain there forever or for a very long duration. Thus they pose a threat to life and help insects to develop resistance against them. This is the reason there has always been a need for such an insecticide which is more powerful, with lesser side effects and degrading after sometime, reducing the chance to develop resistance against it. Scientists are now accepting the fact that such resistance is inevitable and that it is just matter of time before all pesticides are neutralized by some adaptive mechanism of the pests[1]. The exact impact of these residues is not known, but tests conducted on the affected animals show frightening evidence of genetic defects and cancer[2].

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Plant-derived insecticides

Plants have always been a rich source of chemicals and drugs for man[3]. During the 20th Century, a few of these natural compounds like nicotine, rotenone and pyrethrins have been used commercially as insecticides[4]. However, plants produce thousands of other compounds that are insecticidal and have diverse modes of action like hormonal, neurological, nutritional or enzymatic[5]. Phototoxins are unique among these plant compounds in that their toxicity is greatly increased when irradiated with light.

A number of plant families are known to produce alkaloids, phenolics and oils which have been used for insect control since a long time. They were called as insect killers and were used by Romans and Chinese[6]. Another group of plant products discovered in the last two decades are known as secondary plant metabolites. These were considered as waste products from plants for a long time, because they were of no nutritional significance to insects. These secondary plant metabolites having insecticidal/pesticidal properties are of several types, viz. repellents, antifeedants, phagostimulants and toxins[7].

Secondary plant metabolites

The plant family which is important in producing such secondary plant metabolites is Asteracae, which has been considerably exploited to extract phototoxic com-

Table 1. Diseases of humans and animals transmitted by mosquitoes

Disease	Vector	Host
Malaria (several kinds)	*Anopheles* species	Humans, monkeys, birds
Yellow fever	*Aedes aegypti* and other sp.	Humans, monkeys
Filariasis	*Anopheles* and *Aedes*	Humans, dogs, wild carnivores
Dengue fever	*Aedes* and *Culex*	Humans
Encephalitis	*Aedes* and *Culex*	Humans
Equine encephalitis	*Aedes*	Horses

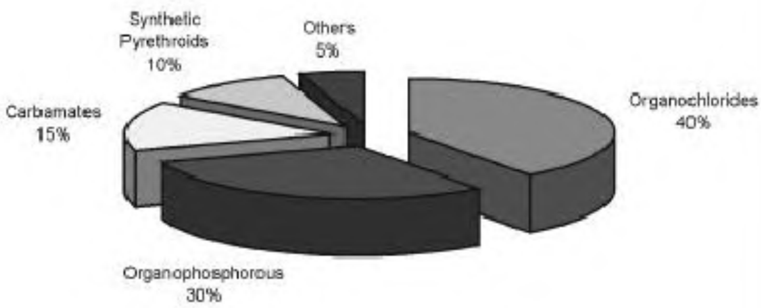

Figure 1. Consumption pattern of different synthetic insecticides in India.

Table 2. Secondary plant metabolites shown to have toxic effects on insects

Secondary plant metabolite	Example
Amino acids	Canavanine
Flavonoids	Pror-Cyanidin tannins
Glucosinolates	Sinirgin
Lignas	Excelsin
Tannins	Glaucolide-A
Steroids	Ecdysones

pounds like thiophenes. A number of secondary plant metabolites have been extracted from plants which are repellents, antifeedants, phagostimulants, toxins, xenobiotics and allelochemicals. Alkaloids and phenolics are two important groups of secondary plant metabolites that regulate the tolerance level of plants and acceptance level of insects (Table 2).

In recent years, a lot of work has been done on control of insects by secondary plant metabolites, e.g. control of *Ostrinia nubulalis* by limnoids from Meliaceae and Rutaceae[8], psilotin growth reducer and feeding deterrent from *Psilotum nudum*[9] and phenylheptatryine, an antifeedant against *Euxoa messoria*[10]. Petroleum ether extract of some plants showed larvicidal activity against mosquito larvae of *Anopheles stephensi* and *Aedes aegypti*[11]. The extract of seven indigenous plants, *Ocimum basilicum*, *O. sanctum*, *Lantana camara*, *Vitex negudo*, *Azadiracta indica*, *Clemone voscosa* and Blumea species showed larvicidal activity against 4th instar larva of filaria mosquito, *Culex quinquefasciatus*[12]. Linseed also exhibits larvicidal activity against mosquito larvae of *Culex pipens*[13]; alcoholic extract of *Cassia holosericea* has been shown to have larvicidal action against larvae of *A. aegypti*[14]. One such secondary plant metabolite discovered few decades back is alpha-terthienyl.

Alpha-terthienyl

Alpha-terthienyl (Figure 2) is a member of a new class of phototoxic compounds, having great potential as a pest control agent and is a potent insecticide/larvicide[15–17]. It possesses a quality of rapid decomposition compared to relative persistence of some synthetic insecticides[17].

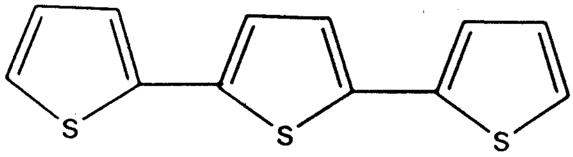

Figure 2. Structure of alpha-terthienyl.

This compound is shown to be phototoxic against a number of organisms, viz. microorganisms of achenes of marigold[18] and nematodes[19], human erythrocytes[20], human skin[21] and some herbivorous and blood-feeding insects like *Manduca sexta*[22–24], *Piaria rapae*[22], *Musca domestica*, *Tribolium casteneum* and *Rhizopertha dominica*[25] and mosquito larvae, *Aedes atropalpus*, *Aedes aegypti*[26] and *A. intrudens*[27].

Alpha-terthienyl, once introduced into the medium containing mosquito larvae, enters into the anal gills which can be visualized by light microscopy or halide leakage, releasing all the electrolytes into the medium leading to death of the larvae[23]. Treatment of alpha-terthienyl on herbivorous insects shows scleretization of pupae[23] and damage to the gut[28].

Many of the biomolecules and enzymes are targets of alpha-terthienyl in the presence of ultraviolet light, viz. DNA *in vitro* and *in vivo*[25], plasma membrane and membrane proteins[20] and enzymes like glucose-6-phosphate dehydrogenase[29], malate dehydrogenase[30], acetylcholinesterase[31] and superoxide dismutase[32–33] in mosquito larvae. The oxygen-dependent phototoxicity of this compound has been reported to involve both Type I and Type II photodynamic reaction.

Mechanism of action of alpha-terthienyl

In presence of ultraviolet light, alpha-terthienyl causes phototoxicity to the mosquito larvae. In an attempt to

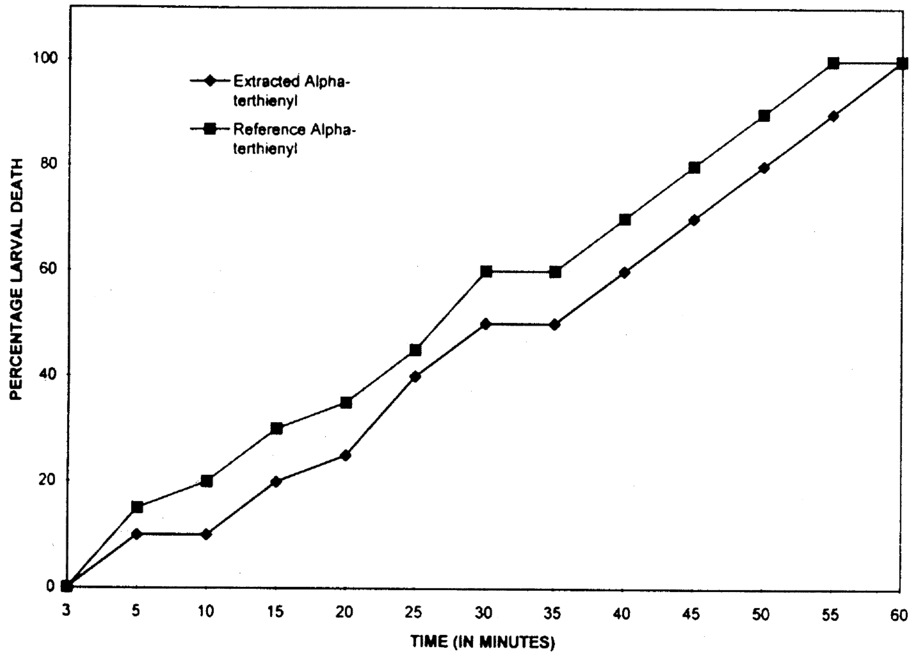

Figure 3. Effect of alpha-terthienyl (crude extract and reference) in presence of ultraviolet light on mosquito larvae of *Aedes aegypti*.

study toxicity of alpha-terthienyl on mosquito larvae in the laboratory, a crude extract of alpha-terthienyl and reference (market preparation) (33 ppb) showed 100% larval death with 55 min of exposure to alpha-terthienyl and ultraviolet light (366 nm)[34] (Figure 3).

Superoxide dismutase and mosquito larvae

Although aerobic organisms are adapted for survival in oxygen in the concentration found in the atmosphere, higher concentration of oxygen has been shown to produce toxic effects[35]. In 1970, Yamamoto and his group[36] reported that rats can be made tolerant to 100% oxygen by prior exposure to 85% oxygen. It was subsequently shown by Crapo and Tierney[37] in 1974 that this increased tolerance is accompanied by an increase in superoxide dismutase activity, an enzyme present in the biological system known to dismutate superoxide anion radical to a less toxic hydrogen peroxide, which is further reduced to water by catalase[38].

Superoxide dismutase is present in all eukaryotic cells such as yeast, plants and animals but absent in prokaryotic cells. This enzyme is present in large quantities in tissues possessing enriched oxygen supply, for example, rete mirable and gas gland epithelium from swim bladder of marine fishes[39]. Anal gills of mosquito larvae are the organs which are comparable to the swim bladder of fishes, i.e. organs rich in oxygen supply.

An increase is observed in the superoxide dismutase activity from 1st instar to 4th instar larval stage[32] (Figure 4). This increase seems to be a protective mechanism against hazardous oxygen derivatives. Superoxide dismutase is present in the entire gill, except in the tracheal network.

Alpha-terthienyl inhibits superoxide dismutase activity completely in all the four instars of mosquito larvae in the presence of ultraviolet light and this is an important step in the phototoxicity of this compound (Figure 4). The inhibition process is still unclear but, this inhibition may lead to accumulation of hazardous oxygen radicals leading to cell damage.

Superoxide anion radical generation

The oxygen-dependent phototoxicity of this compound has been reported by generating alpha-terthienyl radical[40], singlet oxygen[30] and superoxide anion radical[41]. Superoxide anion radical is formed when an electron is added to ground-state oxygen. A number of enzymes such as non-specific peroxidases[42], xanthine oxidase[43], nitropropane dioxygenase[44], galactose oxidase, etc. have been shown to actually reduce oxygen to superoxide anion radical. Even thiophenes can convert ground-state oxygen to superoxide anion radical, as they are unstable and auto-oxidizable[45,46].

Alpha-terthienyl generates superoxide anion radical both *in vitro* and in the anal gills of mosquito larvae[41]. Bakker *et al.*[30], while studying inhibition of certain enzymes by alpha-terthienyl, claimed involvement of singlet oxygen in the photodynamic action of alpha-terthienyl against the enzyme being studied, since the known singlet oxygen quenchers like bovine serum

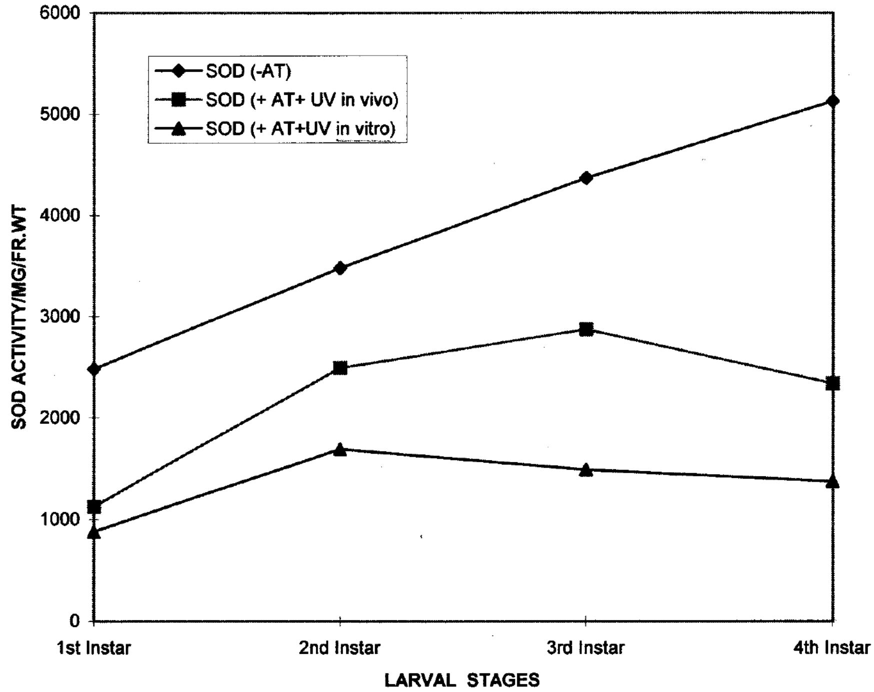

Figure 4. Superoxide dismutase (SOD) activity in mosquito larvae of *Aedes aegypti* without and with alpha-terthienyl (AT) treatment *in vivo* and *in vitro*.

albumin and sodium azide in the medium showed protective effect on the enzyme inhibited by alpha-terthienyl, while superoxide dismutase had no effect, thereby suggesting no role of superoxide anion in the inhibition of the enzyme.

The failure of superoxide dismutase to protect against phototoxicity of alpha-terthienyl can be interpreted, based on the fact that superoxide dismutase activity is inhibited by alpha terthienyl radical *in vitro* and superoxide radical both *in vivo* (in the anal gills of mosquito larvae of 1st, 2nd, 3rd and 4th instar) and *in vitro*, suggesting that alpha-terthienyl acts via oxyradical mechanism.

Membrane damage

Both the facts that alpha-terthienyl inhibits superoxide anion radical and generates free radical species give an idea that this may lead to irreversible cellular damage, which is further confirmed by spin-labelling studies.

The aqueous and lipid compartments of anal gill membrane when labelled with spin labels show cellular damage. Spin-labelling is a very useful tool in the study of membrane structure, viscosity, density and polarity of the local environment[47]. The smallest change in these parameters can be studied accurately. Nivsarkar *et al.*[48] showed that both the lipid and aqueous domains of the anal gill membrane are damaged after exposure of the anal gills to alpha-terthienyl in presence of ultraviolet light, in all the four instars of mosquito larvae.

Singlet oxygen can cause certain damages involving its sensitization by retina in the presence of light that damages the retina, skin eruptions, scarring and thickening caused by porphyrias and lipid peroxidation that damages fatty acid side-chains present in the membrane lipids.

Lipid peroxidation decreases membrane fluidity, increases leakiness of the membrane and inactivates membrane-bound proteins. It has also been demonstrated that superoxide anion radical can cause damages to membrane-bound proteins, membrane lysis, injury to many other organs and lipid peroxidation[42]. This damage is suggested to be due to generation of free radicals and inhibition of superoxide dismutase activity.

Alpha-terthienyl can also be localized in the alimentary canal of mosquito larvae[49], and causes gastric irritation. Thus, it seems that alpha-terthienyl has a multidirectional toxicity in mosquito where it damages the nervous, respiratory and digestive systems of the larvae, giving no chance for survival (Figure 5).

Safety and other activities of alpha-terthienyl

'Insect-visiting pesticide' is an important factor in determining the safety and efficacy of a new pesticide. Several non-target insect species, especially the benefi-

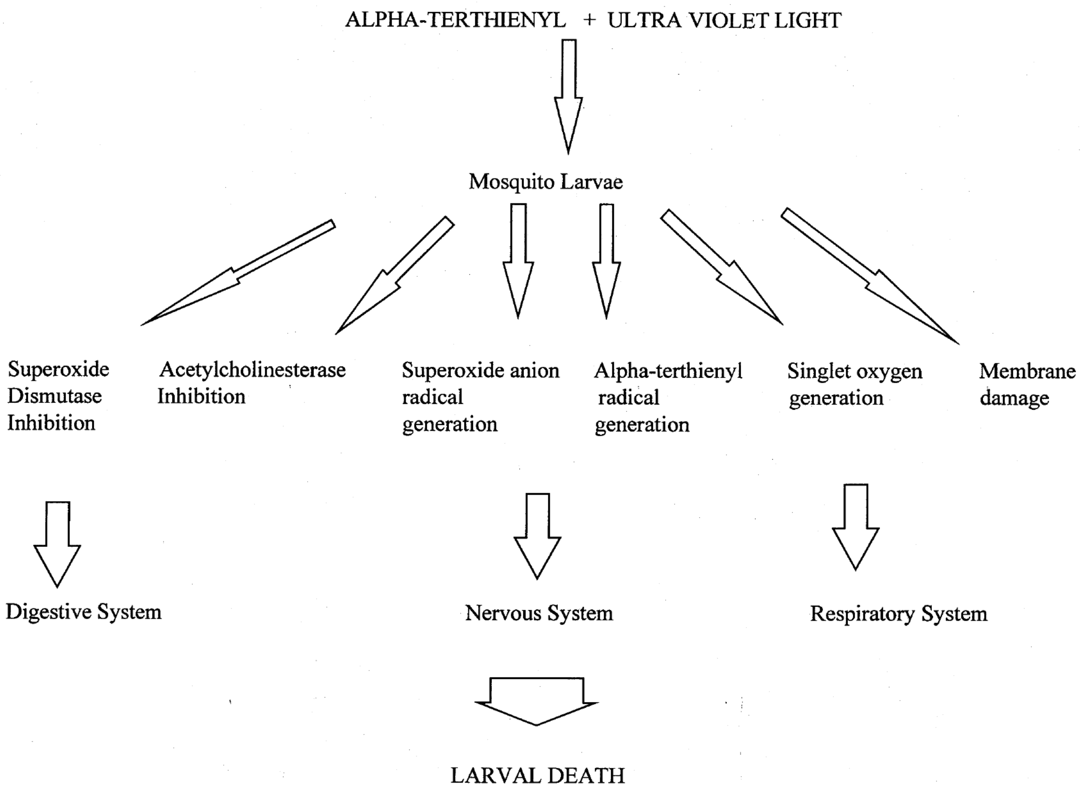

Figure 5. Schematic representation of mechanism of action of alpha-terthienyl in mosquito larvae.

cial insects like bees, silkworms, etc. are targets of synthetic/organic insecticides.

Alpha-terthienyl is of natural origin and has a degradation half-life of ~4 h in sunlight[17]; it is non-residual and environment-friendly. However, toxicity has been reported in certain non-target species such as tadpoles[50] and fishes[51]. However, no toxicity has been reported in other non-target insect species.

Besides being a mosquito-control agent, alpha-terthienyl has been shown to have several other activities in presence of ultraviolet light like antibiotic[52], antifungal[53], anti-HIV[54] activities and a novel protein c kinase inhibitory action[55]. Alpha-terthienyl can be extracted from the roots of *Tagetes* species in petroleum ether and even the crude extract is effective against mosquito larvae[34]. Alpha-terthienyl can be purified further using a silica column (80–120 Å). Alpha-terthienyl is also available commercially with Aldrich, USA.

Conclusion

Alpha-terthienyl is emerging as a potentially sound, potent mosquito control agent, with low cost, easy-to-administer and risk-free properties. In addition to the conceptual facts that this secondary plant metabolite may act as an antifeedant, an inhibitor of various enzymes, generator of active oxygen species and free radicals, it shows tremendous capacity to totally shatter the oxyradical defence mechanism existing in the anal gills, thrusting the larvae to face irreversible free-radical threat.

We suggest that secondary plant metabolites will have their impact on the insecticide development programme of the Third World countries and surely alpha-terthienyl will emerge as a potent larvicide in the next century.

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