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Efficacy of Aquatain AMF against *Aedes aegypti* and *Culex quinquefasciatus*

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Abstract
Mosquito is the most notable vector that is responsible for the transmission for various vector-borne diseases, that causes numerous deaths every year. It is necessary to control these vectors as they post huge threat to mankind. Control of mosquito larva is the basic and most important step to control these vector-borne diseases. In this study, we have applied a novel method to control these mosquito larvae by using a new aquatain AMF solution and assessed the efficacy of AMF solution in controlling the mosquito larva. The components present in the AMF control and AMF treated water samples were identified using GC-MS analysis.

Keywords: Mosquito, vector, AMF solution, GC-MS, Vector-borne diseases

Introduction

Mosquito is the most important vector since they cause the highest number of human deaths every year throughout the world. Mosquitoes belong to the Phylum Arthropoda, Class Insecta, Order Diptera, Suborder Nematocera and Family Culicidae. Nearly 3, 500 mosquito species have been identified in the world (Harbach and Howard, 2007). More than 100 mosquito species were identified as vectors of many diseases (Wegner, 2009; Paulraj *et al.,* 2011; WHO, 1996) [46, 29, 48]. In India, the primary vectors of mosquitoes belong to the genera *Anopheles, Culex* and *Aedes* (Rahuman *et al.,* 2009; Borah *et al*., 2010) [32, 9]. Species like *Aedes aegypti*, *Aedes albopictus*, *Aedes stricticus, Aedes cinereus, Anopheles stephensi, Culex quinquefasciatus,* and *Mansonia* spp. cause nuisance for humans and animals every year.

Insect vectors like mosquitoes mainly transmit diseases like Japanese encephalitis, filariasis, dengue, malaria, dengue hemorrhagic fever, yellow fever, Zika, and chikungunya (Rahuman *et al.,* 2009; Borah *et al.,* 2010) [32, 9]. In 1996, the mosquito was declared as public enemy number one by the World Health Organization. These tiny hematophagous insect vectors have a profound impact on public health, labor outputs, and economic burden as well (WHO, 1992) [47]. Mosquitoes are important etiological agents of infections to human beings and native faunas (Snow *et al.,* 2005). Further, mosquito-borne diseases have become more life-threatening and widely dispersed due to ecological and environmental changes, the development of urbanization, and the invasion of insect pests. Although mosquitoes have been studied more extensively than most other insects because of their role as vectors of disease, our taxonomical and phylogenetic knowledge is far from complete. Newer species are being identified year after year.

Life cycle of mosquito

The life cycle of mosquitos includes two main stages: the aquatic immature stage and the free-living stage. The aquatic stage comprises eggs, larvae, and pupae, and the flying adults are free-living and feed on nectar and plant sap. Females need a blood meal for oviposition. The entire life cycle takes approximately 8–10 days. Mosquito species lay their eggs in the water bodies, and the water habitat differs from species to species (Fig. 1).

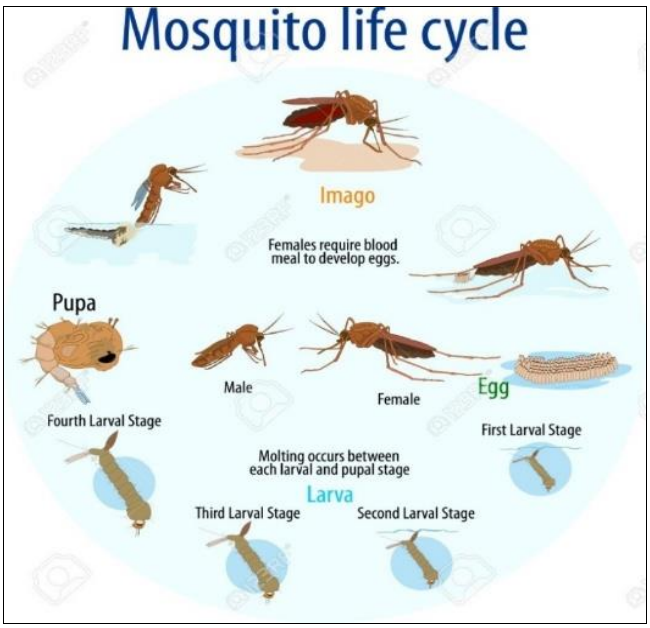

Fig 1: Life cycle of mosquito

Mosquito eggs

Aedes and *Anopheles spp.* lay eggs on fresh water bodies; *Culex* spp. lay eggs in polluted or sewage waters. Eggs are laid either singly, as in the case of *Aedes* and *Anopheles*, or as a bunch, as in the case of *Culex* and *Mansonia*, in which eggs are attached together. Eggs float on the surface of water. Normally *Aedes* mosquito species lay their eggs on the sides of the water storage devices. The oviposition (egg-laying) sites range from small-sized ponds to big water storage reservoirs. Most of the eggs are hatched in 24–48 hrs (Fig. 2).

Fig 2: Eggs of *Aedes* Mosquitoes

Larval stages of mosquito

When eggs are hatched, the larvae live in the water and come to the surface for breathing. Mosquito larvae go through four molting (instar) stages called wigglers, and the size will increase in each molting stage (Fig. 3).

Fig 3: Larva of mosquito

Larvae hang upside down from the surface of the water. Mosquito larvae have siphon tubes for breathing, except for *Anopheles* larvae, which are devoid of a siphon. *Anopheles* larvae lie parallel to the surface of the water to get oxygen through a breathing opening. Larval stages of mosquitoes display a great variation in morphology among species, and it is used for identification purposes. The larvae feed on microorganisms (bacteria, protozoa, fungi) and organic matter present in the water. During the end of the fourth instar stage, the larvae develop into pupae. Normally mosquito larvae take 4–5 days to develop into pupae.

Pupal Stage of mosquito

The fully developed fourth instar larvae develop into comma-shaped structures called pupae that are enclosed in cocoons. Pupae are also called astumblers. It is an active and non-feeding stage during development. Pupae move faster with a flip of their tails to the top and bottom. They respond to the light changes, and it is also like metamorphosis. Within 2–4 days the development is complete, the pupal skin splits, and adults emerge successfully (Fig. 4).

Fig 4: Pupa of mosquito

Adult stage

Once the adult mosquito emerges from pupae, it rests on the surface of the water for a period to get its body parts dried out and hardened. The reproductive organs of the adult mosquito mature within 1-2 days from the emergence of pupae. Males seek out the female for mating by the sound of their wingbeats. Normally males live up to 3–5 days by feeding on fruit and plant nectar. Once mated, a female mosquito continues to lay eggs after every blood meal. Female mosquitoes can live up to 2 months under the best climatic conditions (Fig. 5).

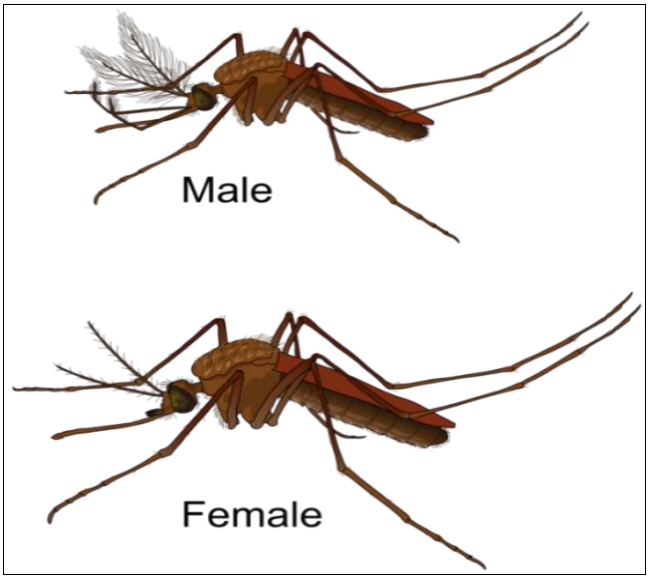

Fig 5: Adult Mosquito

Mosquitoes and Health Hazards

Mosquitoes are well-known vectors involved in the transmission of pathogens that cause various human and vertebrate diseases. Pathogens like protozoans, viruses, and nematodes are transmitted by mosquitoes. More than one million deaths and 2500 million people are infected by mosquito-borne diseases across the globe (WHO, 1996; Carrington and Simmons, 2014) [48, 12]. In India, malaria, chikungunya, dengue, filariasis, and Japanese encephalitis are the major vector-borne diseases causing severe damage to human and public health. (Taubes, 1997).

Dengue

In India, *Ae. aegypti* is the important primary vector, and *Ae. albopictus* is the secondary vector for dengue and dengue hemorrhagic fever (Dutta and Mahanta, 2006;

Georghiou and Lagunes Tejeda, 1991; WHO, 1992) [47]. SPECIES of *Aedes* mosquitoes are diurnal. They feed on human and animal blood by biting and sucking. *Ae. aegypti* and *Ae. albopictus* live in the dark places of the domestic areas, such as the space underneath the cot, curtain, cupboard, etc. These mosquitoes mainly breed in the stagnant water accumulated in materials like coconut shells, tires, the bark of the banana tree, flower pots, and storage containers having a small quantity of water. The eggs of this mosquito can live for more than one to two years.

Chikungunya

Aedes is the primary vector for chikungunya in India. In 2005, there was a serious outbreak of chikungunya virus infection in the Indian Ocean islands, and 1.5 million people were infected by this viral fever (Taubitz *et al.,* 2007). Humans are the major source and reservoir for the chikungunya viral infection. Chikungunya viral fever is mainly transmitted through the mosquitoes biting an infected person and then transmitting the virus when they bite another normal person.

Malaria

Malaria is yet another very important, dreadful disease in India. It is also regarded as an important killer disease, which infects 300–500 million people all over the world. Children become easy prey to malaria. In India, 1000 deaths and 2–3 million cases are recorded every year (Dev *et al.,* 2003; Lal *et al.,* 2010; Garcia, 2010). A total of 460 *Anopheles* species were identified, among which 100 species are reported as malaria vectors. 30–40 *Anopheles* species were reported as vectors that commonly transmit *Plasmodium parasites; Plasmodium ovale, Plasmodium malariae, Plasmodium falciparum, Plasmodium knowlesi, and Plasmodium vivax* are prominent among them (Bronner *et al.,* 2009; Oaks *et al.,* 1991) [11]. In India, malaria is transmitted by six major vectors: *An. culicifacies, An. stephensi, An. sandaicus, An. dirus, An. minimus, and An. fluviatilis* (Das *et al.,* 2012) [16]. *An. subpictus* is a potential vector of *Bancroftian filariasis* and carries *Wuchereria bancrofti* larvae to the human (Amerasinghe and Amerasinghe, 1999; Hoedojo *et al.,* 1980) [2]. In European countries, malaria is transmitted by *An. plumbeus* (Schaffner *et al.,* 2012) [40]. In American countries, malaria is transmitted by *An. darlingi* (Manguin *et al.,* 1999) [23].

The *Anopheles* mosquitoes are commonly present in the areas of human dwellings and cattle sheds. They mainly feed on the blood of humans, cattle, and birds. They breed in rainwater stored in pools and puddles, borrow pits, riverbed pools, irrigation channels, seepages, rice fields, wells, pond margins, and sluggish streams with sandy margins. It is reported that they bite at midnight (2–3 AM). In African countries, malaria was transmitted by *An. merus, An. bwambae, An. arabiensis, An. quadriannulatus, and An. melas* (Kweka *et al.,* 2008).

Filariasis

Culex quinquefasciatus is the primary vector that transmits *Wuchereria bancrofti,* responsible for causing lymphatic filariasis. More than 100 million people from all over the world are infected by filarial diseases (WHO, 1992; Govindarajan *et al.,* 2008) [47]. These mosquitoes are mainly present in tropical and subtropical areas. The infected people transmit the nocturnally periodic *W. bancrofti,* which causes the lymphatic filariasis to the non-infected persons when they are bitten by mosquitoes (WHO, 1992; Bernhard *et al.,* 2003) [47, 6]. They prefer to breed in contaminated waters such as obstructed drains, septic tanks, cesspools, cesspits, or drains close to human dwelling areas. Sometimes they can also breed in moderately clean water pools (WHO, 1992) [47].

Japanese encephalitis

Cu. tritaeniorhynchus is an important vector of Japanese encephalitis in India and in Southeast Asian countries. In India, Japanese encephalitis was highly widespread in a few districts of Tamil Nadu, Southern India (Reuben and Gajanana, 1997; Suman *et al.*, 2008; Ravi *et al*., 1989; Sarkari *et al*., 1984) [34, 33, 39]. Japanese encephalitis is mainly transmitted by mosquitoes like *the Cx. vishnui* group, like *Cx. tritaeniorhynchus, Cx. vishnui,* and *Cx. pseudovishnui* (Tiwari *et al.,* 2012). To eradicate/control the mosquito species, we need some alternatives. Due to this concept, currently the AMF Solution larvicidal bioassays are being carried out.

Objectives

- To evaluate the efficacy and residual activity of AMF in controlling Aedes *aegypti* and *Culex quinquefasciatus* larvae and oviposition deterrent.
- Proven the AMF as a mosquito control agent.
- Effect on Living Beings in water bodies.

Materials and Methods

Collection of Mosquitoes

Mosquito specimens were collected from different taluks of Chennai, Tamil Nadu, India, from February to April 2025.

The collected mosquitoes from various parts of Chennai were maintained at the Entomology Research Institute, Loyola College, Chennai, India. The hatched larvae were kept in enamel trays containing faucet water. The larvae were kept unfastened from pathogens, repellents, or pesticides and were fed on dog biscuits and brewer's yeast (3:2). Then pupae were collected and transferred from the trays to a cup containing tap water and were placed in screened cages (23×23×32 cm in dimension) for adult emergence. A sucrose (10%) solution was provided in a jar with a cotton wick for adult mosquitoes. Adult mosquitoes will be deprived of sucrose for 12 hours, after which they will be provided with a membrane blood feeding. After 3 days, the ovitrap was kept inside the cages and maintained at 28±1°C temperature at 65-70% relative humidity.

Larvicidal bioassay

The third instar larvae obtained from the F2 generation adults were subjected to the Larvicidal bioassay. The AMF solution was used in 1ml/m^2. The third-instar larvae were introduced into the container using an ink pillar and brush (Fig.6).

Fig 6: Experimental Setup.

Two sets of controls were used, one with larvae and the other without larvae. The experimental setup was maintained for observation. The dead larvae and live larvae were observed according to the time intervals for further usage (Vetal *et al.*, 2019).

Effect on Living Beings in Bodies

Guppies, with 2 pairs in each, were used in 5 replicates. These replications are also the experiment by AMF solution and Control maintain for 40 days.

Result

AMF Solution against *Culex quinquefasciatus*

The study shows the larvicidal activity of Aquatain AMF against *Culex quinquefasciatus* larvae using an experimental setup developed in the entomological study. All ten replicates had ten larvae exposed to Aquatain AMF, and mortality was recorded after the lapse of intervals from 30 minutes to 48 hours. In sharp contrast to the treated groups, the control—without any exposure to Aquatain AMF—had minimal mortality, only scattered deaths at 300 minutes and 24 hours, which further entrenched the integrity of the bioassay.

Increasing time saw progressively higher mortality among the exposed larvae. At 30 minutes, merely 8% had died; however, mortality rose to 64% by 180 minutes and reached a total of 100% by the end of the 48-hour observation time. Interestingly, much of this mortality was localized between 180 minutes and 24 hours, highlighting the latent but powerful mode of action of Aquatain AMF. This kind of trend implies that the effectiveness of the formulation increases with exposure duration (Table. 1).

Table. 1 AMF Solution against *Culex quinquefasciatus* (Mortality)

Replication	Total No. Larva	30 min	60 min	120 min	180 min	240 min	300 min	24 hrs	48 hrs
Control	10	0	0	0	0	0	1	1	0
1	10	0	2	1	3	1	1	2	0
2	10	1	1	1	2	0	2	1	2
3	10	1	0	2	0	1	2	4	0
4	10	0	2	1	0	1	1	2	3
5	10	1	1	1	1	2	2	2	0
6	10	0	0	2	2	1	2	1	2
7	10	1	0	1	5	2	0	1	0
8	10	2	0	1	3	1	0	3	0
9	10	1	3	0	3	3	0	0	0
10	10	1	1	0	3	2	2	1	0
Total	100	08	10	10	22	14	12	17	07

AMF Solution against *Aedes aegypti*

The experiment gives the time mortality profile of Aquatain AMF-treated *Aedes aegypti* larvae and its larvicidal activity. Ten replicates of ten larvae were considered for ten incremental observation periods up to 48 hours. The control had minimal mortality, with one larva being dead at 300 minutes and another at 48 hours, hence validating the integrity of the experiment by supporting no significant natural death.

For the treatment groups, mortalities of the larvae commenced early, with nine deaths at only 30 minutes following treatment. Mortality increased to 13 and 12 at the 60- and 120-minute levels, respectively, indicating an early onset of toxic effect. The plateau effect appeared to set in between the 240- and 300-minute points, with 16 and 19 deaths, respectively. While fewer new deaths were being reported at a decelerating pace after 24 hours—with a total of just 11 having occurred by then and a further four within 48 hours—the overall trend suggests that Aquatain AMF is a highly time-effective product (Table.2: Fig. 6). The visibility of the AMF film could be shown in the photograph (Fig. 7).

Such a mortality pattern, with the nadir between 180 and 300 minutes, suggests that the active ingredient is extremely rapid-acting, immobilizing larvae before protracted exposure. Variability among replicates, as moderate as it may be, represents naturally occurring biological variability. In the general context of wider evidence generated in this study, this dataset confirms that Aquatain AMF is a potent tool in vector control, particularly pertinent in integrated mosquito management programs against Aedes vectors, which have been the vectors of diseases such as dengue and chikungunya.

Fig 7: AMF film visibility

Table 2. AMF Solution against *Aedes aegypti* (Mortality)

Replication	Total No. Larvae	30mints	60 mints	120mints	180mints	240mints	300mints	24hrs	48hrs
Control	10	0	0	0	0	0	1	0	1
1	10	0	1	2	1	2	2	2	0
2	10	1	2	1	1	1	2	1	1
3	10	1	1	1	2	2	1	2	0
4	10	1	2	1	0	2	2	2	0
5	10	1	2	1	2	2	2	0	0
6	10	2	1	2	2	1	2	0	0
7	10	1	1	1	1	1	3	2	0
8	10	2	1	1	1	2	1	1	1
9	10	0	1	2	2	1	2	0	2
10	10	0	1	0	4	2	2	1	0
		09	13	12	16	16	19	11	04

Effect on Living Beings in Bodies

This data provides the environmental safety and biological effects of Aquatain AMF in aquatic ecosystems—particularly where fish are present. Three conditions were maintained under observation for 40 days: plain control (no fish), control with fish, and experimental group with fish and AMF treatment. In the control group that is simple, symbolized by the consistent "√" for 35 days, there was no reported adverse effect on aquatic life. However, from days 36 to 38, symbols become "0," possibly indicating areas of observation gaps or where the experiment was ended. Generally, this is an indication of consistent water quality without the application of external treatments. In the control + fish group, 10 fish were visible. There was no mortality reported during the first 24 days. There was one death of a fish on day 25 (labeled "1D"), and there was no further mortality reported. This indicates that there was minimal natural mortality or death due to stress, further confirming the baseline health of the water environment.

The experiment and fish trial, in which 10 fish were treated with Aquatain AMF, yield comparable trends. One death occurred on day 30, showing that the larvicide treatment did not lead to acute fish toxicity within the short term. What is notable is that new births were seen on day 36 (Nb 21) and day 40 (NB 7), implying that not only did the fish survive but also retained reproductive viability in treated conditions (Fig. 8 & Table 1).

Fig 8. Effects of living being in waterbodies experimental unit

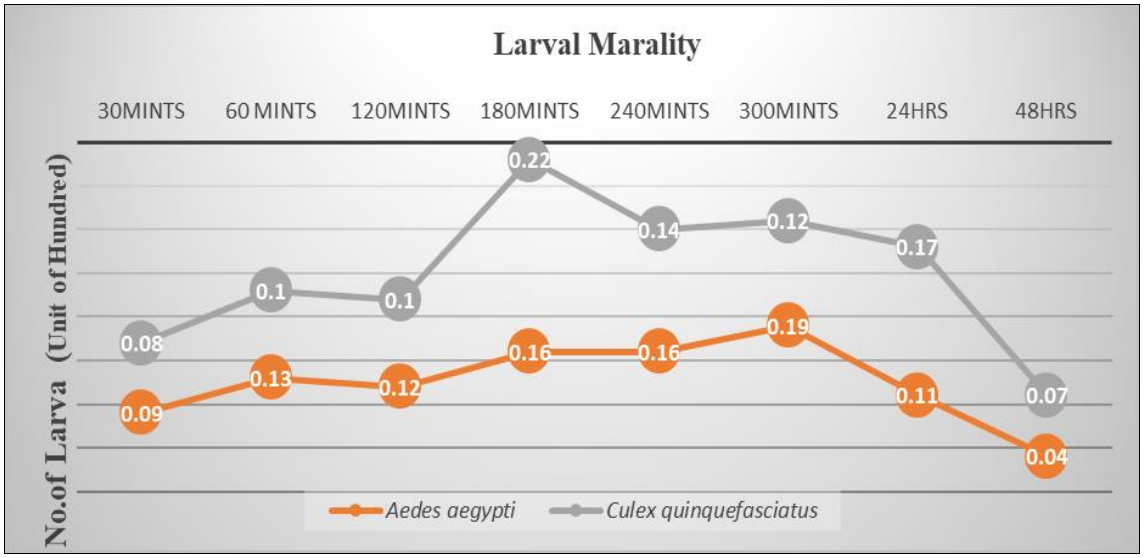

Fig 9: AMF solution's Mortality according to time Interval

In conclusion, the information indicates that Aquitaine AMF has a 100% mortality rate in 48 hours (Fig. 10), is not toxic to fish and remains ecologically safe for a prolonged duration. Oviposition deterrent effect on mosquito species were observed in this experiment. This suggests the potential of the compound in integrated vector management in aquatic ecosystems without harming natural aquatic organisms.

GC-MS Analysis of Residual AMF Solutions:

Both the control and experiment samples underwent analysis using gas chromatography-mass spectrometry (GC-MS) with a "screening method" acquisition method on April 26, 2025. The core inference from comparing these analyses is that the "Experiment" sample exhibits a significantly different chemical composition compared to the "Control" sample.

The control sample was characterized by the presence of compounds such as ethanol, 2-(butylamino)-; L-valine, N-[2-(chloroimino)-3-methyl-1-oxobutyl]-; butanoic acid, 4-(2-methylcyclohexanon-3-yl), (1-phenyl-2-dimethylamino) propyl ester; benzen-1,4-diol, 2,6-difluoro-; multiple entries of 5-fluorotryptophan and 1,2,3-benzenetricarboxylic acid; and various complex organic compounds, including substituted isoquinolines and quinolones. In contrast, the experiment sample contained a distinct set of compounds, with a notable presence of 1-proline, n-heptafluorobutyryl propyl ester; 2,4-di(2,2,6,6-tetramethyl-1,2,5,6-tetrahydro-4-pyridyl) pyrrole; multiple entries of benzyl alcohol, alpha. -(1-(dimethylamine)ethyl)-;S-[4Cyanophenyl-] -N, N-dimethylthiocarbamate; octahydropyrrolo[1,2-a]pyrazine; Ethylenediamine, N,N, N'-trimethyl-N-(4-piperidyl)-; several occurrences of cyclodecylamine; N(1) -[1-[4-Chlorophenyl]-1H-tetrazol-5-yl] - N(2), N(2)-dimethyl-1,2-propanediamine; 2,3-Bisdimethylamino-1,3-butadiene; Pyridostigmine Bromide; and, significantly, Iron pentacarbonyl, which was not detected in the Control sample.

This clear difference in the detected chemical species strongly suggests that the experimental conditions or treatment applied to the "Experiment" sample either introduced new compounds or caused transformations leading to the formation of these distinct substances, differentiating its chemical fingerprint from the "Control" sample (Table 2).

Table 3. Compound present in both experiment and control

Control	Experiment
Ethanol, 2-(butylamino)-	1-Proline, n-heptafluorobutyryl propyl ester
L-Valine, N-[2-(chloroimino)-3-methyl-1-oxobutyl] -	2,4-Di(2,2,6,6-tetramethyl-1,2,5,6-tetrahydro-4-pyridyl) pyrrole
Butanoic acid, 4-(2-methylcyclohexanon-3-yl), (1-phenyl-2 dimethylamino) propyl ester	Benzyl alcohol, α -(1-(dimethylamino)ethyl) - (multiple entries)
Benzen-1,4-diol, 2,6-difluoro-	S-[4-Cyanophenyl-]-N, N-dimethylthiocarbamate
5-Fluorotryptophan (multiple entries)	Octahydropyrrolo[1,2-a] pyrazine (multiple entries)
1,2,3-Benzenetricarboxylic acid (multiple entries)	Ethylenediamine, N, N, N'-trimethyl-N-(4-piperidyl)-
1-(4-Methoxy-6-methyl-5,6,7,8-tetrahydro- [1,3] dioxolo[4,5-g] isoquinolin-5-yl)-5,5,8-trimethyl-nona-3,7-dien-2-one	Cyclodecylamine (multiple entries)
Isoquinoline, 1-[(3,5-dihydroxy) benzyl] -1,2,3,4-tetrahydro -6-hydroxy-	N(1)-[1-[4-Chlorophenyl] -1H-tetrazol-5-yl] -N (2), N (2)-dimethyl-1,2-propanediamine (multiple entries)
1,2-Dihydro-6-methoxy-2-oxoquinoline -4-carboxylic acid	2,3-Bisdimethylamino-1,3-butadiene
4(1H)-Pteridinone	Pyridostigmine Bromide
6-Chloro-3-	Iron pentacarbonyl

Comparative studies over the past decade based on literature

Aspect	Laboratory Study	Field Trials (Based on literature)
Mosquito Species	*Culex quinquefasciatus* and *Aedes aegypti*	*Anopheles*, *Aedes*, and *Culex*
Larval Mortality Rate	*Aedes*: Rapid onset – 90% mortality within 300 mins *Culex*: Slower onset – 64% mortality in 48 hours	Up to 100% mortality in 24 hours for 4th instar larvae of all three genera in some trials
Pupal Mortality	Not explicitly measured	Very high – pupae died within hours; maximum by 24 hours
Onset of Action	*Aedes*: Immediate (30–60 mins) *Culex*: Gradual (most deaths after 180 mins)	Rapid – pupae mortality within a few hours; larvae mortality completes within 1–2 weeks
Residual Activity	Not measured directly; GC-MS showed component breakdown by day 37	Long-lasting – up to 6 weeks residual effect with single application
Oviposition Deterrence	Not assessed	Confirmed – females avoid treated surfaces; some drown trying to lay eggs
Effect on Non-Target Organisms	Safe – no significant mortality in fish; reproduction still occurred	Safe – no toxicity to fish or aquatic plants; approved for use in drinking water
Chemical Profile	GC-MS showed component degradation and transformation after 37 days	Not chemically profiled, but emphasis on non-toxic, physical action mechanism
Mechanism of Action	Suffocation by blocking air access through film; supported by chemical residue changes	Physical suffocation via silicone film; prevents respiration and emergence; resistance-free

Under controlled laboratory conditions, Aquatain AMF showed species-sensitive action, faster on Aedes aegypti than Culex quinquefasciatus, with effective larval kills and no harmful impact on fish. GC-MS analysis showed progressive breakdown of active ingredients, possibly consistent with loss of surface activity with passage of time.

Field trials, on the other hand, showed broader and more consistent efficacy, such as high percentages of death in Anopheles, Aedes, and Culex larvae and pupae. Long duration residual activity (lasting 6 weeks), effective oviposition deterring, and good environmental safety in various eco-zones were also exhibited by the trials.

Thus, while lab tests highlight short-term performance and environmental safety, field tests confirm long-term performance, species-specific suitability, and field feasibility of Aquatain AMF for integrated mosquito control schemes.

Conclusion

Comparative investigations of Aquatain AMF are characterized by its effective larvicidal activity and ecotoxicological safety in diverse biological environments. *Culex quinquefasciatus* and *Aedes aegypti* both exhibit 64% mortality upon exposure, while Aedes larvae respond more

rapidly, with optimal effects observed within the first 300 minutes, indicating species-specific toxicity and a swift toxicological onset. Conversely, the death of *Culex* developed more slowly and reached its peak near the 24th hour, suggesting a more normal type of action. Interestingly, the fish environmental bioassay did not produce many negative effects, but only scattered deaths and some degree of continued reproduction, thus validating the agent's compatibility in aquatic environments. Pyrimidine, 5,5'-(2-methylpropylidene)-bis[2,4,6-tris[(trimethylsilyl)oxy]- its siliconic compound in AMF solution, but it's not present in the experiment after 37 days. It may be due to decomposition or modification so that the film is braked. This synergy of outcomes, speed, and persistence of mosquito control by taxa, with minimal disturbance to the balance of nature, positions Aquatain AMF at the forefront as a viable, selective larvicide for use in integrated vector management programs.

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