FIELD EVALUATION OF AQUATAIN AMF ™ AS A MOSQUITO LARVAL AND PUPAL CONTROL AGENT IN DIFFERENT BREEDING SITES IN NORTHERN ITALY

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INTRODUCTION

Due to the Biocide directive, mosquito control is facing a serious problem of shortage in insecticide products availability. The restriction in the spectrum of insecticides may also increase the risk of resistance in target population.

New products with diversified action mechanisms are therefore required to strengthen the tools box currently available in Europe.

AQUATAIN AMF ™ is a silicone based monomolecular surface film with a physical mode of action interfering with the water surface tension thus blocking the larvae and pupae normal behavior and breathing, which is considered a potential good agent for the management of mosquito larval control.

During the summer 2016 we conducted several field trials in order to evaluate the efficacy and the lasting activity of AQUATAIN AMF ™ in the most important mosquito breeding sites such as rice fields, irrigation ditches and road drains in Northern Italy.

TRIAL1. RICE FIELD COLINIZED BY *AEDES CASPIUS, CULEX PIPIENS MOLESTUS and ANOPHELES MACULIPENNIS* S.L.

The sequence of rice field floodings usually performed in the summer activate the hatching of *Aedes caspius* eggs present on the ground. After a few days from the flooding mainly *Cx. pipiens molestus* but also *Anopheles maculipennis* s.l. may colonizes the water body as well.

Three rice paddies have been tested of which two treated and one untreated as a control. All basins were located in Motta & Bosco's farm (S.Pietro in Casale, Bologna province, Emilia-Romagna region).

AQUATAIN AMF ™ was applied at dose of 1 ml/meter square (calculated on the whole surface of the flooded area) from the perimeter bank – Trial1A- (manual application every 30 meters) or issued at the water point of entry (Trial1B).

Larvae and pupae have been sampled with water net on the perimeter (30 samples/rice field paddy) according to this schedule:

- few hours before treatment;
- at 24 hours post-treatment;
- at 7 days post-treatment;
- at 14-21-28 days post-treatment (these samplings have been conducted only in case the previous sampling has given positive feedback in terms of residual efficacy).

RESULTS

In trial1A a incomplete efficacy was observed at 24 hours post-treatment while high efficacy was observed at day 7 post-treatment. A good residual efficacy was detected on *Cx.pipiens* at 14-21 days post-treatment. In trial 1B the larval mortality resulted limited at 24 hours post-treatment and increased consistently at 7-14 days post-treatment (Table 1A, 1B).

Table1. Trial 1A in rice field: AQUATAIN AMF ™ distributed manually (dose 30 liters/2.87 ha equal to 1.05 ml/m²) every 30 m along the perimeter

Time		N	*Ae.caspius*				*Cx.pipiens*				*An. maculipennis* spp.				All species				Shannon Index	
			Mean	SD	Test* N-K	% Red. (Abbot)**	Mean	SD	N-K test	% Red. (Abbot)**	Mean	SD	Test N-K	% Red. (Abbot)**	Mean	SD	Test N-K	% Red. (Abbot)**	Mean	SD
PRE (june, 24)	**Aquatain**	30	15.00	20.83	A		170.33	135.40	a		0.13	0.43	ns		185.47	148.43	ns		0.23	0.10
	Control	30	57.00	75.87	B		78.60	76.25	b		0.23	0.50			135.83	113.94			0.19	0.09
Post 1 day	**Aquatain**	30	16.30	31.83	A	**20.28**	92.27	136.57	a	**58.00**	0.13	0.57	ns		108.70	149.06	a	**5.54**	0.17	0.06
	Control	30	77.70	101.69	B		101.37	93.80	b		0.00	0.00			179.07	146.51	b		0.20	0.08
Post 7 days	**Aquatain**	30	0.00	0.00	ns	**100.00**	2.63	7.42	a	**99.43**	0.23	0.57	a	**9.65**	2.87	7.40	a	**99.06**	0.45	0.11
	Control	30	0.07	0.25			215.07	150.87	b		7.63	5.51	b		222.77	151.54	b		0.39	0.15
Post 14 days	**Aquatain**	30	0.17	0.46	ns	**-90.00**	16.40	22.29	ns	**86.72**	0.43	0.94	ns	**31.06**	17.00	22.31	ns	**78.68**	0.53	0.10
	Control	30	0.33	0.96			56.97	93.56			1.10	1.56			58.40	94.01			0.41	0.09
Post 21days	Aquatain	30	0.00	0.00	ns		1.00	1.60	ns	**92.27**	0.00	0.00	ns	**100.00**	1.00	1.60	ns	**87.86**	0.48	0.10
	Control	30	0.00	0.00			5.97	6.61			0.07	0.25			6.03	6.63			0.51	0.11
All Groups		270	9.87	33.11			71.03	115.94			1.11	3.03			82.01	126.79			0.37	0.17

*Newman–Keuls method: significant difference with different letters; ns= no significant difference

**Abbott's correction for natural mortality

Table2. Trial 1B in rice field. AQUATAIN AMF ™ administered at the water point of entry (tot. 30 liters/3.01 ha, 1.00 ml/m²)

Time		N	*Ae.caspius*				*Cx.pipiens*				*An. maculipennis* spp.				All species				Shannon Index	
			Mean	SD	Test* N-K	% Reduction (Abbot)**	Mean	SD	Test* N-K	% Reduction (Abbot)**	Mean	SD	Test* N-K	% Reduction (Abbot)**	Mean	SD	Test* N-K	% Reduction (Abbot)**	Mean	SD
PRE July, 7	**Aquatain**	30	40.37	37.84	ns		6.10	7.62	ns		0.00	0.00	ns		46.47	39.19	ns		0.27	0.08
	Control	30	30.10	21.57			0.27	0.74			0.00	0.00			30.37	21.65			0.19	0.09
Post 1 day	Aquatain	30	25.30	26.47	A	**51.75**	9.67	12.38	ns	**91.82**	0.00	0.00	ns		34.97	35.27	ns	**48.38**	0.30	0.09
	Control	30	39.10	19.95	B		5.17	7.48			0.00	0.00			44.27	25.38			0.25	0.08
Post 7 days	Aquatain	30	2.57	3.08	ns	**-218.98**	71.17	80.78	a	**98.51**	1.93	2.03	a		75.67	81.83	a	**76.77**	0.36	0.08
	Control	30	0.60	1.16			208.37	109.81	b		3.93	4.39	b		212.90	111.80	b		0.35	0.11
Post 14 days	Aquatain	30	0.03	0.18	ns	**96.45**	18.33	17.58	a	**98.65**	0.83	1.21	ns		19.20	17.55	a	**79.24**	0.55	0.12
	Control	30	0.70	1.68			59.20	37.45	b		0.53	0.82			60.43	38.50	b		0.49	0.10
All Groups		240	17.35	25.58			47.28	82.69			0.90	2.19			65.53	80.03			0.35	0.15

*Newman–Keuls method: significant difference with different letters; ns= no significant difference

**Abbott's correction for natural mortality

TRIAL2. IRRIGATION DITCHES COLONIZED BY *AEDES CASPIUS* AND *CULEX PIPIENS MOLESTUS*

In the case of occasional or periodic flooding due to irrigation purposes, ditches become important breeding sites for *Ae.caspius* followed mainly by *Cx. pipiens molestus*.

Five ditches (length 250 m, width 1m) have been selected in the agricultural area of Comacchio of which three have been treated and two untreated as a control.

Larvae and pupae have been sampled with water net according to this schedule:

- few hours before treatment (June, 29);
- at 15 hours post-treatment;
- at 8 days post-treatment.

In each sampling session samples have been performed by water net every 15 meters along the entire stretch of the ditch.

AQUATAIN AMF ™ have been distributed every 15 meters along the ditch at a dose of 1 ml / square meter (calculated on the whole surface of the ditch).

RESULTS

At 15 hours post-treatment the efficacy resulted close to 100% and remained very high at day 8 post-treatment (Table3).

Table3. Trial2 in irrigation ditches. Trial in irrigation ditches. AQUATAIN AMF ™ has been distributed along the ditch every 15 meters (tot. 0.25 liters/250/m²)

Time		N	*Cx.pipiens* (L3-4+P)			*Ae.caspius* (L3-4+P)			All species (L3-4+P)			% reduction corrected by Abbott's formula**		
			Mean	SD	Test* N-K	mean	SD	Test* N-K	mean	SD	Test* N-K	*Cx.pipiens*	*Ae.caspius*	All species
PRE (June,29)	**Control**	30	10.40	17.82	NS	16.40	19.63	A	26.80	25.57	a			
	Aquatain	45	16.42	14.81		121.31	120.73	B	137.73	126.11	b			
Post 15h	**Control**	30	14.50	16.38	a	13.50	18.24	NS	28.00	26.16	NS	**99.03%**	**99.51%**	**99.51%**
	Aquatain	45	0.22	0.90	b	0.49	1.75		0.71	2.19				
Post 8 days	**Control**	30	1.80	3.40	NS	0.03	0.18	NS	1.83	3.38	NS	**85.93%**	**100.00%**	**95.75%**
	Aquatain	45	0.40	1.34		0.00	0.00		0.40	1.34				

**Abbott's correction for natural mortality

TRIAL3. CATCH BASINS COLONIZED BY *AEDES ALBOPICTUS* AND *CULEX PIPIENS MOLESTUS*

The trial has been conducted in 70 road catch basins chosen in a urban area of Crevalcore (Bologna province), of which 60 have been treated with AQUATAIN AMF ™, and 10 left untreated as a control.

Three consecutive trials have been performed:

Trial 1: 1 ml/catch basin (June, 8)

Trial 2: 2 ml/catch basin (June, 28)

Trial 3: 5 ml/catch basin (July, 27)

All the treatments have been performed with a lab pipette to assure maximum precision.

The sampling were made with water net immediately before the treatment and then on weekly bases until the average rate of infestation was greater than or equal to 70% calculated in relation to the control.

Each week post-treatment only 10 catch basins randomly selected on the 60 treated catch basins were sampled, thus avoiding the re-sampling of the same catch basin in the following weeks. This precaution is adopted to avoid the risk that the product may be removed during the sampling phase.

Larvae and pupae of each sample were counted by species and age.

During the tests rainfall was recorded (weather station ARPAE –ER).

Fig. 1-2-3 Average density (n. larvae + pupae / net) in catch basins during the three trials

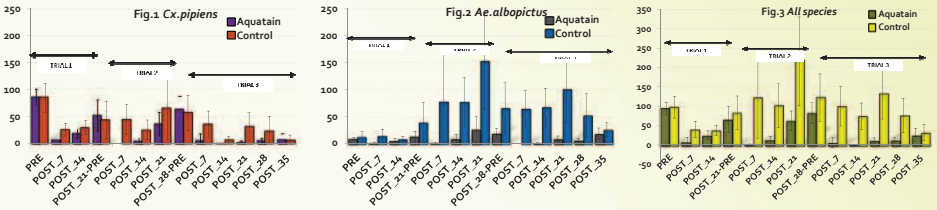
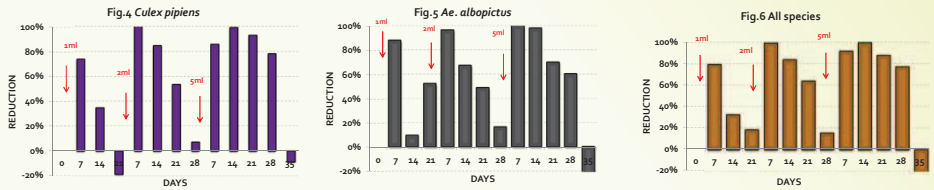


Fig. 4-5-6 Reduction the density of larvae and pupae in the catch basins treated compared to the control



RESULTS

The three trials evidenced that the efficacy of AQUATAIN AMF ™ increases when increasing the dose from 1 ml/catch basin to 5 ml/catch basin.

With the highest tested dose (5 ml/catch basin) larval reduction resulted above 90% during the first three weeks post-treatments and about 80% at week 4 post-treatment (Figg.4,5,6).

During the third trial (dose 5 ml/catch basin) rain was limited with a total of 11.6 mm till the 28th day post-treatment.

Acknowledgment

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