Evaluation of Aquatain™ monomolecular surface film against mosquito larvae of *Culex pipiens* in a full-grown rice field in Greece

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Summary The impact of the monomolecular surface film Aquatain™ against mosquito larvae was tested in a rice field in Central Greece. Aquatain™ was poured in a 1.2 hectare rice paddy along the one side of the field. Laboratory reared mosquito larvae of *Culex pipiens* were introduced into cages placed in three different transects along the short side of the rice paddy. Larval mortality was counted 3, 6, 15 and 25 days after application. In the line located closer to the site of Aquatain™ application, larval mortality ranged from 100% to 70%, 25 days after application. Mortality ranged from 88% to 25% in the middle transect and from 42%, to 10% in the more distant line. The results indicated that Aquatain™ provides sufficient larval control in wide mosquito larval habitats.

Introduction

Mosquitoes are the most important insects of public health due to the pathogens they transmit. Mosquito larvae require water to breed. Larval habitats vary among extensive surfaces with fresh or brackish water to small containers (used tyres). In agricultural areas, rice fields are often the most important extensive mosquito larval breeding sites for both culicines and anophelines, vectors of serious human diseases (Lacey and Lacey, 1990).

Rice cultivation in Greece covers some 30.000 hectares every year, grown under constant flooding (FAOSTAT, 2012). In areas with rice cultivation, mosquito bites impede human activities, especially at dusk. Moreover, in rice paddies many Culicinae and Anophelinae mosquito species proliferate including the main malaria vector in

Greece, *Anopheles sacharovi* (Voyadjoglou-Samanidou *et al.*, 1989). Mosquito control in rice paddies is currently performed by aerial spraying of larvicides after a specific local authorization and under the supervision of the authorities, as an exception to the prohibition of aerial application of pesticides in EU on the grounds of the threat posed on public health by these breeding sites of mosquitoes. The effectiveness of the applied larvicides is highly impeded by vegetation, especially when rice plants are full-grown.

A method to overcome such problems can be the use of alternative to biocides larvicidal agents which spreads efficiently covering the aquatic surface without accumulating around debris or vegetation. Such a solution can be the use of monomolecular surface films that can be effective on killing mosquito larvae and pupae physically and furthermore non-toxic and safe to the environment (Reiter, 1978; Das *et al.*, 1986; Batra *et al.*, 2006; Bashir *et al.*, 2008). Aquatain™ is a silicone-based monomolecular surface film and its larvicidal and pupicidal properties have been well documented in the laboratory (Bukhari and Knols, 2009; Webb and Russell, 2009). Mosquito control potential of Aquatain™ has also been investigated in

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field trials in backyard habitats in Australia where it successfully managed to keep mosquito larval habitats free of mosquito eggs, larvae and pupae up to 5 weeks post-application (Webb and Russell, 2012). Because of its non-biochemical mode of action no resistance is anticipated. In the present study the effectiveness of Aquatain™ as a mosquito control agent was tested in rice paddies under the common cultivation practices followed in Greece. For this purpose, a field experiment was set up in rice fields close to Anthile, Prefecture of Phthiotida, Greece (38°49'08.36"N- 22°29'51.89"E) in a difficult for mosquito control period when rice plants had completed tillering and flowering, from August to September 2012.

Materials and methods

Cylindrical cages were specially constructed by plastic bottles (30cm high, 8cm diameter) with an opening (3.5x15 cm) at 3.5cm height above the bottom. The opening was covered with gauze of an appropriate mesh capable to prevent mosquito larvae from escaping but allowing the free movement of water in the cages (Figure 1). Nine sampling cages, along three different lines (three cages per line), were selected and marked in a 1.2 ha (120x100m) experimental paddy (Figure 2). The first line (line 1) of replicates was set close to the short edge of the paddy where Aquatain™ was applied, the second one in the middle of the paddy (line 2) and the third one at the far distant edge of the release site (line 3). The sampling cages were 25m apart in the same line and 30m apart from the corresponding point in the adjacent line and from the short edge of the paddy (Figure 2). An adjacent rice paddy of the same dimensions which received the same cultivation and plant protection management as the experimental paddy but was not treated with Aquatain™, served as a control paddy. Sampling cages were set in the same pattern in the control paddy. An irrigation ditch provided water constantly to both paddies (experimental and control), where-

as excess water was exiting to the drainage channel (Figure 2, blue arrows). Plastic cages were tightened on supporting poles and placed in the rice paddy prior to Aquatain™ application. All supporting poles were submerged in such a way that the opening of the cages was half submerged (10cm from the base of the cage, Figure 2). Adjustment of the level of the cages was performed on the assessment days to match water fluctuation. Twenty fourth-instar larvae (L_4) of *Culex pipiens* from a laboratory colony (reared at Benaki Phytopathological Institute, $T=25 \pm 2^\circ\text{C}$ and $75 \pm 5\%\text{RH}$) were placed into each cage just before Aquatain™ application.

Aquatain™ was applied into the experimental field by pouring from five different spots along the short edge (A) of the paddy (Figure 2) at the labelled rate of 1 ml/m² (12L in total). A single application was performed on 27th August 2012. Efficacy of Aquatain™ was assessed by recording larval mortality 3, 6, 15 and 25 days after application. To record larval mortality, the contents of the cages were poured into a white pan in order to count the remained larvae (dead or alive). After each assessment another batch

Figure 1. Plastic cage used in the study.

Figure 2. Map presenting the location of the experimental rice in Phthiotida, Lamia, the irrigation system of the rice fields, and a drawing showing the positions of the cages in the experimental paddy.

of twenty four *Cx. pipiens* larvae were introduced into the cages (each batch of larvae remained for three days in the cages before mortality evaluation) and adjustment of the level of the cages was performed to match water fluctuation.

Statistical analysis

To identify differences in percent larval mortality between lines in the rice paddy, indicating distances from Aquatain™ application site, the data of each assessment were analyzed using Kruskal-Wallis H-test. When significant differences were detected, Mann – Whitney U-test were carried out for pairwise comparisons. All analyses were conducted using the statistical package SPSS 14.0.

Results and Discussion

The results indicated that Aquatain™ managed successfully to cover the entire water surface of the 1.2 ha rice paddy within a time period of 6 days even though it was applied along the one side only (A, short edge, Figure 2).

Larval mortality reached 100% in the proximate line to the application site (line 1) three days after application. Mortality remained at 100% six days after application and ranged from 95% to 73%, 15 and 25 days after application respectively. Mortality in line 1 was significantly higher than mortality in the other lines, in all assessments, indicating that the distance from the release site affected the larval mortality (Table 1). In the middle of the paddy (line 2), larval mortality was quite low (28%) three days after applica-

tion but it increased significantly during the following days (88% and 83%, 6 and 15 days after Aquatain™ application, respectively) and was significantly higher than mortality in line 3. Larval mortality in line 3 increased and reached its highest level (42%) six days after application.

Increase of larval mortality over time in lines 2 and 3 can be attributed to the time needed for the spread of the product. Furthermore, three days after the application of Aquatain™ (first assessment) all cages in the paddy were found above the water surface as the amount of irrigation water could not maintain the water level. Thus, there was adequate water in the traps to keep mosquito larvae alive but Aquatain™ could not enter into the cages except for those in line 1. Adjustment of the supporting poles after the first assessment, allowing the water to cover half of the cage opening, is also associated with time period of the mortality levels increase in lines 2 and 3.

The complete coverage of the experimental paddy occurred at slower mode than that reported in other studies (Bukhari *et al.*, 2011). This could possibly be due to higher density of the rice plants as in our case Aquatain™ was applied relatively late in the season, after tillering had been completed.

Aquatain™ remained highly effective in lines 1 and 2 for at least 15 days (larval mortality rates ranged up to 95% and 83%, respectively). Environmental factors (rainfall, strong winds) as well as cultivation techniques may possibly influence the efficacy of Aquatain™ over extensive and verdant mosquito breeding sites. For this reason Bukhari *et al.* (2011) recommended a reapplication of 10 – 14 days in rice paddies in Kenya in order to effectively control mosquito larvae. However, in container mosquito habitats Aquatain™ provided effective control of mosquito larvae for at least 4 weeks at the labelled dose-rate (1ml/m²) and thus the recommended reapplication frequency is every 4 weeks even though a longer larval control may be achieved with a single application (Webb and Russell, 2012).

In conclusion, our study showed that Aquatain™ is an effective control agent against mosquitoes not only in small or closed water ecosystems (Webb and Russell, 2012), but also in extensive water surfaces such as rice paddies. It causes death of the existing mosquito larvae and pupae of a breeding site (Webb and Russell, 2009) and it can offer sufficient protection from the development of new mosquito generations for at least 3 weeks.

Table 1. Mean larval mortality ($\pm$ SE) and percentage mortality (%) of *L₄* mosquito larvae of *Culex pipiens* in cages over a period of 25 days after application of the product Aquatain™ in a rice paddy in the Prefecture of Phthiotida, Greece.

Days after application of Aquatain™	Experimental paddy			Control paddy*
	line 1	line 2	line 3	lines 1,2,3 (pooled data)
3	20.0 $\pm$ 0.0 (100%) a§	5.67 $\pm$ 5.13 (28%) b	0.0 $\pm$ 0.0 (0%) b	1.0 $\pm$ 1.0 (1.67%) b
6	20.0 $\pm$ 0.0 (100%) a	17.67 $\pm$ 3.21 (88.3%) a	8.33 $\pm$ 2.08 (41.7%) b	1.33 $\pm$ 0.56 (2.22%) c
15	19.0 $\pm$ 1.0 (95%) a	16.67 $\pm$ 0.58 (83.3%) b	5.33 $\pm$ 2.08 (26.7%) c	0.33 $\pm$ 0.57 (0.56%) d
25	14.0 $\pm$ 1.0 (70%) a	5.0 $\pm$ 1.0 (25%) b	2.0 $\pm$ 1.0 (10%) c	1.33 $\pm$ 1.15 (2.21%) c

* Without application of Aquatain™

§ Different letters within rows indicate statistically significant differences.

Authors would like to thank Dr Dimitrios Papachristos for his help in statistical analysis and Dimitris Vlachos for generously providing us the experimental rice paddies.

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Received: 18 April 2013; Accepted: 1 January 2015

Μελέτη της αποτελεσματικότητας του σκευάσματος μονομοριακής μεμβράνης Aquatrain™ εναντίον προνυμφών κουνουπιών *Culex pipiens* σε ορυζώνες

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Περίληψη Η δράση του προϊόντος μονομοριακής μεμβράνης Aquatrain™ εναντίον προνυμφών κουνουπιών εξετάστηκε σε ορυζώνα της κεντρικής Ελλάδας (Ανθήλη, Φθιώτιδα). Το Aquatrain™ εφαρμόστηκε με απλή έγχυση μόνο από τη μία πλευρά ορυζώνα έκτασης 12 στρεμμάτων. Προνύμφες κουνουπιών 4^{ης} ηλικίας (*Culex pipiens* από εργαστηριακή εκτροφή) τοποθετήθηκαν σε ειδικά κατασκευασμένους κλωβούς. Οι κλωβοί εγκαταστάθηκαν σε τρεις παράλληλες γραμμές που βρίσκονταν σε διαφορετικές αποστάσεις από την πλευρά εφαρμογής του Aquatrain™. Η θνησιμότητα των προνυμφών καταγράφηκε 3, 6, 15 και 25 μέρες μετά την εφαρμογή. Στην πλησιέστερη γραμμή στην πλευρά εφαρμογής του Aquatrain™ η θνησιμότητα κυμάνθηκε από 100% έως 70% καθ' όλη τη διάρκεια του πειράμα-

τος. Στη δεύτερη γραμμή στο μέσο του πειραματικού αγρού, η θνησιμότητα κυμάνθηκε από 88% έως 25% ενώ στην πιο απομακρυσμένη γραμμή η θνησιμότητα ήταν 42% έως 10%. Σύμφωνα με τα αποτελέσματα το AquatainTM αποδεικνύεται ικανό να καταπολεμήσει τις υπάρχουσες προνύμφες των κουνουπιών και να εμποδίσει την ανάπτυξη νέων για διάστημα τουλάχιστον τριών εβδομάδων σε μεγάλα υδρόβια συστήματα όπως οι ορυζώνες.

Hellenic Plant Protection Journal **8**: 21-26, 2015