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SIDE EFFECTS OF SILICONE-BASED MONOMOLECULAR FILMS ON NON-TARGET ATMOSPHERIC AIR BREATHING AQUATIC INSECTS

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Abstract Silicone-based monomolecular films are becoming widespread both because of their effectiveness and because it seems they do not harm non-target organisms. These products create a barrier on the water surface, lowering surface tension that prevents mosquito larvae and pupae from breathing and adults from laying eggs. The aim of this study is to investigate whether these films have side effects on certain non-target species of Hemiptera and Coleoptera which breathe atmospheric air. The insects used were collected from canals, ditches, lakes, and ponds, and immediately brought to the laboratory, where they were tested with Aquatain® AMF. The results reveal that these insects are extremely sensitive to silicone films, in fact, *Gerridae*, *Corixidae, Dytiscidae, Notonectidae,* and *Gyrinidae* all showed 100% mortality within 24 hours of applying 1 ml/m² of the product. The use of monomolecular films in wetlands must therefore be carefully evaluated.

Key Words Aquatain®, aquatic insects, mosquito larval control, natural environment, non-biocidal larvicide.

INTRODUCTION

In the 1980's (Nayar and Ali, 2003), a new type of mosquito control method was developed as an upgrade of the already existing surface oils against larval stages. Monomolecular surface films (MMFs) emerged as a safer and more efficient alternative, being less harmful to non-target organisms. This method was also introduced to address issues associated with chemical insecticides such as vector resistance. When applied to water, MMFs form an invisible, one-molecule-thick layer on the surface. The reduced surface tension, kills mosquito larvae and pupae which cannot attach their siphons to the water surface to breathe, while adult mosquitoes that normally land on the surface to lay eggs actually sink and drown (Stark, 2005). Aquatain® AMF is certainly the best-known silicone-based monomolecular film, and is primarily composed of polydimethylsiloxane (PDMS). Since Aquatain® kills mosquitoes physically and not chemically, European regulations do not require its registration as a Biocide. However, precisely because Aquatain® is not subjected to the same level of scrutiny as chemical biocides, concerns arise about insufficient data on its efficacy and on potential side effects on non-target species. As regards its efficacy, several studies were conducted on Aquatain®, and its ability to control mosquitoes was demonstrated (Bukhari et al., 2011; Mbare et al., 2014; Dawood et al., 2020; Kavran et al., 2020; Drago et al., 2017, Baz M.M., 2017; Dieng et al. 2022). As for environmental concerns, Polydimethylsiloxane degrades abiotically in soil into smaller molecules, which are then either biodegraded or volatilized into the air, where they decompose in sunlight. In optimal conditions, the final degradation products are inorganic silica, carbon

dioxide, and water vapor. Additionally, WHO Prequalification Team–Vector Control Group (PQT-VC) reports "The ecotoxicity potential of Aquatain® AMF or PDMS is negligible to non-target aquatic vertebrate and invertebrate species." Nonetheless, the statement about the non-hazardous nature of Aquatain® for aquatic organisms is quite general, and it should be analyzed more specifically, perhaps discriminating between different groups. A few studies report the absence of harmful consequences of MMFs on Guppy fish *Poecilia reticulata* (Ngrenngarmlert 2016), *Gambusia affinis* (Levy et al. 1981, Levy et al. 1982 and Batra et al. 2006), Suckermouth catfish *Hypostomus plecostomus,* and tree frog *Hyla cinerea* (Webber & Cochran 1984). In addition, a few studies on snails and crustaceans (Hester et al. 1991, Takahashi et al. 1984 and Su et al. 2014) also showed no negative effects on these organisms. But what about insects? Once again, available studies are few and provide contradictory results. Aquatic insects can be divided into two different non-taxonomic categories, those which take oxygen from the water and those which breathe atmospheric air. As Stark (2005) noted, "The vast majority of studies indicate that MMFs have little effect on non-target organisms. The only species that may be vulnerable are those that make contact with the air-water interface to breathe or live on the water surface.". Considering Aquatain®'s action mechanism, the group breathing atmospheric air should be more exposed to MMFs side effects, and actually our studies focus on this group. Bukhari et al. (2011) observed the consequences of Aquatain® in rice paddies, and found no reduction in most non-target organisms except for backswimmers (Notonectidae spp). Similarly, Karanja et al. 1994 found no adverse effects from the use of Arosurf® on *Dytiscidae, Hydrophilidae, Corizidae, Notonectidae, Nepidae, Belostomatidae* and *Ranidae*. In 2006, Batra et al. did not detect any harmful effects from the use of MMF Agnique® on *Anisops sardae* (Notonectidae). Although White & Garret (1977) did not register damage from the use of various MMFs to *Dytiscidae*, they realised it was harmful to *Gerridae*. Mulla et. al (1983) found Arosurf® to be safe for the *Hydrofilidae Berosus metalliceps*. On the other hand, Takahashi et al. 81984) found Arosurf® to be harmful to *Corisella* sp. (Corixidae), *Notonecta unifasicata* (Notonectidae) and *Tropisternus lateralis* (Hydrophilidae). In summary, given the scanty information about the consequences from the use of MMFs, most of which comes from studies on products other than Aquatain®, concerns about this product remain. The aim of this study is to clarify this important aspect, to better define the best conditions to use these types of products, and thus prevent negative consequences to the environment.

MATERIAL AND METHODS

In this study, two orders of insects were examined, Coleoptera - and in particular species within the families Dytiscidae and Gyrinidae - and Hemiptera, with species from the families Gerridae, Notonectidae, and Corixidae. Although these insects spend all their lives in water, they breathe atmospheric oxygen, except for Gerridae, which live above water, and are therefore called "waterstriders" or "pondskaters", as they stride across the water exploiting its surface tension. The insects used were collected from various wetlands around the city of Padua from July to September. Samples were collected with a net and the contents were immediately transported to the laboratory at a constant temperature between 27 and 30 °C. The tests were conducted separating the insects in mono-specific plastic containers 28x20x14 cm, filled with 2 Liters of tap water left to dechlorinate in the preceding days. The product was then applied - Aquatain® AMF from a 50 mL domestic package distributed by Bleuline - at the recommended dose of 1 mL/m². Given that the surface area of the test containers measured 28x20 cm, 56 µL were applied. Water salinity, temperature, and pH were measured before proceeding with the tests.

Mortality was monitored at intervals of 1, 2, and 24 hours. At the end of the test, the insects that died during the test were identified using both dichotomous keys and DNA analysis when morphological identification was uncertain. Mortality rates were further explored using survival analysis in R. Kaplan-Meier curves were estimated and a marginal Cox proportional-hazard model with robust standard errors was applied to account for intra-cluster dependence due to container identity (Martinussen and Scheike, 2007). A Cox model, validated through Schoenfeld residual analysis, was developed for each insect family using insect lifetime as the dependent variable, treatment dose as the explanatory variable, and container identity as the cluster factor. Correlation analysis using Spearman's coefficient was made to measure the relationship between water characteristics and mortality rates.

RESULTS

After morphological and DNA identification, the insects used for the test were found to be Gerridae - *Aquarius najas, Aquarius paludum, Gerris thoracicus, gerris lacustris*. Corixidae: *Hesperocorixa sahlbergi, Sigara lateralis, Sigara nigrolineata, Sigara striata, Micronecta sholtzi.* Dytiscidae - *Hydaticus leander, Eretes griseus, Agabus dydimus.* Gyrinidae - *Gyrinus substriatus*. Notonectidae - *Notonecta maculata.* In Gerridae, the recorded mortality rates were 95.56% within the first hour from application, which reached 100% after 24 hours. In the control group, mortality was 8.42% after 24 hours. *Corixidae* mortality was 31.11% after 1 hour, 84.44% at 2 hours, and 100% at 24 hours. In the control group, 3.81% mortality was registered at 24 hours. *Micronecta's* mortality was 8.89% after 1 hour, 11.11% at 2 hours, and 91.11% at 24 hours. This is the only organism which did not reach 100% mortality. In the control group, mortality was 7.78% at 24 hours. Notonecta showed 26.67% mortality in the first hour, followed by 86.67% after 2 hours, reaching 100% after 24 hours. No mortality was observed in the controls. For *Dytiscidae*, mortality was 8.33% in the first hour, 83.33% the second hour, and reached 100% after 24 hours. In the control group, no mortality events were recorded. In the *Gyrinidae* group, mortality in the first hour was 100%. In the control group, no mortality events were observed. No significant correlations between mortality rates and water quality were found.

DISCUSSION

The implementation of the European biocide regulation has, on the one hand, significantly reduced the number of products available on the market, and on the other, driven up the costs of registering new ones. This stricter regulatory framework has forced the industry to seek alternative solutions, shifting toward the development of products that eliminate insects through mechanical, or physical, rather than chemical means. Since these products are not classified as biocides, they are exempt from the rigorous registration process required for chemical products, allowing companies to sidestep the associated costs. However, this also means that the presentation of data about the impact of such products on the environment is unnecessary, which introduces potential risks for their use. The results obtained in this study demonstrate that Aquatain®, when applied at the recommended dose, causes the death of non-target species breathing atmospheric air, all of which are predators. Water tension reduction causes Gerridae, which are devoid of swimming structures, to struggle to reach the surface before sinking to the bottom and drowning rapidly. Gerridae and Gyrinidae were the most affected by the product, showing high mortality rates from the first hour of exposure. Dytiscidae exposed to the treatment are seen attempting to climb up the walls of the containers. They are frequently observed spreading their wings, although this rarely results in flight. It could therefore be hypothesized

that the film interferes not only with the insects' respiratory system but also with their flying ability. Notonectidae, and mosquito larvae exhibited "cleaning" behaviors, often rubbing their abdomen with their last pair of hind legs. The response of Gyrinidae exposed to the product was extremely rapid. They immediately stopped their typical surface "dance", sank quickly to the bottom of the containers and resurfaced only for short periods. Individuals of this family were observed attempting to climb container walls. Corixidae did not exhibit abnormal behaviors, although they showed high mortality after just two hours of exposure. Micronectidae were the only organisms tested that did not reach complete mortality within 24 hours. Some individuals were observed using air bubbles in the trays to breathe, which could explain their survival.

The results of our study reveal the undoubtedly lethal effect Aquatain® has on organisms breathing atmospheric air, and this confirms the findings of Takahashi et al. (1984), and partially of White & Garret (1977). Other studies revealed opposite results, like Bukhari et al. (2011) whose considerable difference can be explained by the very different testing conditions. While our study was carried out in containers, Bukhari et al. did their research in rice pads, which means in the presence of a very dense crop. On one hand, high vegetation density allows the insects to climb out of the treated surface (like Dytiscidae and Gyrinidae showed to do in our experiment) to breathe, allowing Gerridae to hold onto it to prevent drowning, and on the other, stops the spreading of the product, creating areas where the insects can breathe. The behavour of Gyrinadae, which immediately after the treatment of the water leave the surface and swim to the bottom of the containers, seems to replicate the behaviour of insects trying to flee the hazardous spot to emerge in a clean area. Batra et al. in 2006 also found no impact of MMF on Notonectidae populations, and this study was done in water storage tanks, where vegetation is completely absent and conditions can be compared, although sizes are completely different, to our containers. In that case though, the test product was not Aquatain® but Agnique®. Karanja et al. (1994) didn't find any harmful effects on several aquatic insects, but the study was done using Arosurf® MSF in rice fields, and these results are coherent with what Bukhari et al found. White & Garret (1977) tested the efficacy of 4 MMFs, and did not register any lethal effects on Dytiscidae, but the study was focused on mosquitoes, and the observations of non-target were just "casual", none of the four test products was polydimethylsiloxane-based and the dosage was just 0.04 mL/m^2.

CONCLUSIONS

There are very few published data on the impact of MMFs, and fewer still on Aquatain®. If the impact of Aquatain® on plants, fish and invertebrates breathing oxygen from water seems to be almost absent, many concerns regard insects breathing atmospheric air. Both our results and the inadequate information available show that the use of MMFs is extremely dangerous for this group of animals in absence of abundant vegetation, which is essential for their survival. Since these invertebrates are all predators, the misuse of MMFs can increase productivity of the treated breeding sites, once this important limiting factor is eliminated. On the other hand, aquatic Coleoptera and Hemiptera live in permanent or semi-permanent water, conditions which are not normally suitable for mosquitoes to breed, precisely because of the abundant predators. However, there are situations where the control provided by these limiting organisms is not enough, like during the initial stages of flooding, when the predator population has yet to develop, or when large quantities of floodwater mosquito eggs all hatch the same time, which cannot be controlled by predators.

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