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What is the aquatic toxicity of saponin-rich plant extracts used as biopesticides?☆

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A B S T R A C T

Saponin-rich extracts from *Quillaja saponaria* and *Chenopodium quinoa* have been registered by US EPA as active ingredients in biopesticides, and extract from tea seed powder, *Camellia oleifera* has been proposed for biocidal use. If saponin-rich biopesticides are efficient against pests, they are most likely also bioactive in the aquatic environment against non-target organisms. The aim of this study was to conduct an effect assessment of saponin-rich plant extracts by using species sensitivity distributions based on acute toxicity tests. The maximal concentrations protecting 95% of the aquatic species (HC_5) of saponins extracted from quillaja bark, tea seed coat and quinoa seed coat were 2.91 ± 1.00, 0.22 ± 0.11 and 22.9 ± 5.84 mg/L, respectively. The 100-fold difference in toxicity between the saponin-rich extracts from different plant species, indicate that saponin toxicity depends on the species it origins from, making “read-across” between saponins a dubious exercise. In addition, the predicted environmental concentrations of different saponins are close to or higher than their water quality standard, which means that the extracts might pose a risk to the aquatic environment if not used cautiously.

1. Introduction

Saponins are a class of natural compounds, found abundantly in more than 100 families of plants (Güçlü-Üstündağ and Mazza, 2007). They are characterized by their structure containing a triterpene or steroid aglycone bound to a sugar moiety (Fig. 1). Saponins have antiviral (Güçlü-Üstündağ and Mazza, 2007), antiprotozoal and antibacterial activities (Potter et al., 2010) and are regarded as wormicides (Szakiel et al., 2005), fungicides (Rubio-Moraga et al., 2011) and piscicides (Cannon et al., 2004). These uses indicate that saponins are bioactive and may be used as biopesticide ingredients. The definition of biopesticides by the U.S. Environmental Protection Agency (EPA) is: “Biopesticides include naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants).” (U.S. Environmental Protection Agency, 2017). Saponin-rich plant extracts belong to the first group, and saponins from *Quillaja saponaria* and *Chenopodium quinoa* have already registered by U. S. EPA as biopesticides (U.S. Environmental Protection Agency, 2007, 2005).

Saponins from *Q. saponaria* can be used for control/suppression of pathogenic fungi or plant parasitic nematodes in vegetable or fruit fields using a dose of 1.4–3.7 g liquid extract/m^2 (~8.6% of saponins) (U.S. Environmental Protection Agency, 2007). Saponins from tea seed powder or pellets (*Camellia oleifera*) have been used as soil additives with documented growth enhancing effects with 1.5 g powder/m^2 for pot-grown beet, oat, and barley plants (Andresen and Cedergreen, 2010), or have been used to expel earthworms from golf courses and sport fields applying 30 g pellets/m^2 once a month (Potter et al., 2010). Saponin extracts from *C. quinoa* can be used for control of pathogenic fungi on tubers, legume, and cereal seeds, with 1 g liquid/m^2 (U.S. Environmental Protection Agency, 2005). They also showed potential use against snails, with an application dose of 0.6 g coat/m^2 (10 mg saponin/L in the water of rice fields) (Joshi et al., 2008). The saponin-rich materials are often biowaste products. The tea seed pellets are, for example produced during oil production (Andresen and Cedergreen, 2010), while quinoa coat is generated during cleaning of the quinoa seed for consumption (San Martín et al., 2008). There is a great interest in creating a rational reuse of the biowastes,

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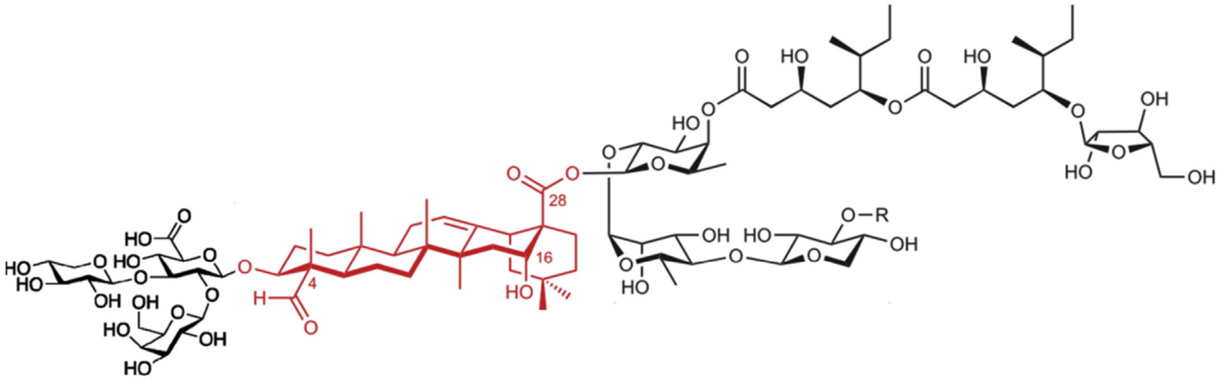

Fig. 1. Structure of quillaja saponin. R may be either -H or substituted by one or more sugar moieties to form different saponins. The lipophilic part (shown in red) located in the middle of the structure is the aglycone. Modified after Fernández-Tejada et al. (2014). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and the saponin-rich bioproducts seem to have a considerable potential. The saponins in the three plant species contain a triterpene aglycone, which means that all saponins have a similar aglycone with different functional groups and sugar chains attached. It is, however, unknown to what extent the naturally produced but different saponins have a similar biological activity. Also, information on their specific toxicity to a wide range of species is lacking. Information on toxic concentration ranges and source-specific saponin toxicity are important, if we are going to use saponin-rich plant extracts in large quantities as biopesticides in an environmentally sustainable way.

The very low octanol/water partition coefficient (k_{ow}) of saponins and hence poor bonding to organic matter make saponins prone to leaching from soils (U.S. Environmental Protection Agency, 2007). Hence, to know their toxicity to aquatic organisms is important. In Europe, there are two regulatory frameworks giving guidance on how to produce water quality standards. One is Environmental Quality Standards (EQS) for chemicals which are used for chemicals in general and used retrospectively described in the Water Framework Directive (WFD) (European Commission, 2011). Another one is Regulatory Acceptable Concentrations (RAC), which is used prospectively for Plant Protection Products and their Residues (EFSA PPR, 2013). Within both frameworks, several options are possible to derive water quality standard for saponins depending on the data available. One of the more comprehensive and preferred methods is to use Species Sensitivity Distributions (SSDs). SSDs describe the variation among a set of species in the sensitivity towards a certain compound (or a mixture of compounds) by applying a statistical distribution to the data (Posthuma et al., 2002). Based on the distribution, a concentration protecting a certain percentage of the species in the community can be determined. Hence, if we want to protect at least 95% of the species, the environmental concentration must be below the concentration that is not hazardous to 5% of the species (HC_5). The SSD based water quality standards underlying the WFD and Plant Protection Products Regulation both use the median HC_5 derived from the SSD curve and an additional assessment factor (AF) for extrapolation to the field, but the methodology used is slightly different as well as the terminology. The WFD makes a distinction in AA-EQS (based on chronic toxicity data) and an MAC-EQS (based on acute toxicity data) and for both AA-EQS and MAC-EQS derivation based on SSDs at least 10 valid toxicity data for at least 8 different taxonomic groups are required. For MAC-EQS derivation the median HC_5 from the SSD constructed with acute toxicity data (50% effect or lethal concentrations, LC_{50} and/or EC_{50} values) and an AF of 10 is selected (European Commission, 2011). In the aquatic effect assessment underlying the PPP Regulation, a distinction is made between an acute (based on acute LC_{50}/EC_{50} data) and a chronic (based on chronic No Observable Effect Concentrations NOEC or EC_{10} values) Regulatory Acceptable Concentration ($RAC_{sw;ac}$ and $RAC_{sw;ch}$, respectively). In deriving the $RAC_{sw;ac}$ and $RAC_{sw;ch}$ by means of the SSD approach at least 8 valid toxicity data for the potentially sensitive taxonomic groups should be provided (European Commission, 2011). For saponins with a more or less biocidal mode-of-action toxicity data of aquatic vertebrates, invertebrates, plants and microorganisms may be used to construct the SSD if the toxicity data comprise at least 6 different taxonomical orders/families (EFSA PPR, 2013). Note that in pesticide risk assessment the functions of microorganisms need to be protected and not necessarily their structural properties. For $RAC_{sw;ac}$ derivation the median HC_5 from an SSD constructed with acute toxicity data (LC_{50} and/or EC_{50} values) and an AF of 3–6 is selected (EFSA PPR, 2013). The size of the selected AF depends on several criteria, amongst others, on the position of the toxicity data in the tail of the SSD, the goodness-of-fit of the SSD curve, the steepness of the SSD curve, the toxicity data used and the 95% confidence limit around the HC_5 estimate. This study will use both the SSD derives MAC-EQS and $RAC_{sw;ac}$ estimates to assess the potential risks of saponin-rich plant extracts to the aquatic environment.

The aim of this study was, therefore, threefold: first, to test the sensitivity of non-target aquatic species to saponin-rich plant extracts to find the more sensitive species among plants, algae, worms, mollusks, arthropods, fish embryo and bacteria. Secondly, to test whether the bioactivity of the saponin-rich extracts of different botanical origin based on triterpene sapogenins was similar, making read across between saponins possible and thirdly, to determine the MAC-EQS and $RAC_{sw,ac}$ for the three different saponin-rich plant extracts and to evaluate them in relation to the Predicted Environmental Concentration (PEC).

2. Materials and methods

2.1. The tested saponin-rich plant extracts

Three kinds of saponin crude extracts from quillaja bark, tea seed coat, and quinoa seed coat were tested in this study, together with one pure quillaja saponin mixture, which was included as a reference and used as a standard for tentative quantification of saponin content of the extracts. The sources of the three saponin crude extracts were: quillaja extract: S7900® from *Q. saponaria* bark (Sigma-Aldrich), tea extract: water extract of tea seed coat powder from *C. oleifera* (NOR-ADD A/S, Denmark), quinoa extract: water