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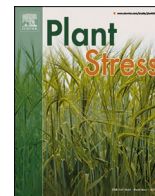
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Review

Applications of rhamnolipid biosurfactants in agriculture

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ABSTRACT

Rhamnolipids are glycolipid biosurfactants naturally produced by *Pseudomonas* and *Burkholderia* bacteria, well-known for their surface-active properties and eco-friendly advantages. Indeed, these compounds efficiently reduce the surface and interface tensions, possess potent biological activities against pathogens, and display low toxicity. Rhamnolipids thus hold significant promise for various industrial applications, including agriculture, which is in need of a transition to greener and more sustainable practices by reducing synthetic inputs. This review describes the knowledge about the antimicrobial properties of rhamnolipids against different phytopathogens, mainly fungal species, as well as their ability to trigger plant defense mechanisms and phytoprotection efficacy in different species. The recent literature investigating rhamnolipids as potential plant biostimulation agents, thanks to their abilities to improve soil health and plant tolerance to abiotic stresses, is also addressed. Finally, the prospect of rhamnolipids as a biopesticide and their overall contribution to sustainable agricultural practices is discussed.

1. Introduction

Global agriculture is currently facing numerous challenges. One of the most urgent is the need for increased productivity and quality to feed an ever-growing population while coping with climate change. Alternative and innovative strategies for achieving sustainable agriculture are being explored. However, diseases and pests pose major challenges, leading to significant yield losses and economic consequences (Savary et al., 2019). Synthetic pesticides and fertilizers are routinely used to protect crops. Their use can cause deleterious effects on human health, the environment and biodiversity (Pathak et al. 2022). In this context, some biosurfactants, produced by microorganisms, have been proposed as interesting safer alternatives to chemically synthesized products. Among biosurfactants, different types of compounds are found such as glycolipids, lipopeptides, phospholipids and fatty acids (Abdel-Mawgoud et al. 2010).

Rhamnolipids (RLs) are glycolipids naturally produced as secondary metabolites by *Pseudomonas* bacteria, mainly by the pathogen *Pseudomonas aeruginosa*, and by some *Burkholderia* species (Abdel-Mawgoud et al. 2010). RLs have notoriously been the most studied microbial biosurfactants (Sekhon Randhawa and Rahman 2014). They are surface-active amphiphilic compounds with low molecular weight

(Rosenberg, Ron 1999). They were first discovered after isolation from *P. aeruginosa* fermentation and described as glycolipidic compounds composed of two l-rhamnoses and two β -hydroxydecanoic acids linked by a glycosidic bond (Bergström et al. 1946; Jarvis and Johnson 1949). Their structure has been elucidated later as one or two hydrophobic alkyl chains linked through a O-glycosidic bond to one (mono-rhamnolipids; mono-RLs) or two (dirhamnolipids; di-RLs) rhamnoses hydrophilic head group linked to each other by an α -1,2-glycosidic linkage (Fig. 1). As biosurfactants, they lower surface and interfacial tensions by reducing the repulsive forces between two phases (Banat et al. 2010; Pacwa-Płociniczak et al. 2011). RLs serve multiple biological and physiological roles for RL-producing bacteria: biofilm development, motility, antimicrobials and nutrition by substrate solubilization and assimilation (Abdel-Mawgoud et al. 2010; Chrzanowski et al. 2012).

RLs produced from fermentation by RL-producing microbial strains are typically a mixture of mono-RLs and di-RLs. The main di-RLs and mono-RLs congeners identified from *P. aeruginosa* fermentation are as follows: α -l-rhamnopyranosyl- α -l-rhamnopyranosyl- β -hydroxydecanoyl- β -hydroxydecanoate (Rha-Rha-C10-C10), α -l-rhamnopyranosyl- α -l-rhamnopyranosyl- β -hydroxydecanoate (Rha-Rha-C10) and α -l-rhamnopyranosyl- β -hydroxydecanoyl- β -hydroxydecanoate (Rha-C10-C10), α -l-

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rhamnopyranosyl- β -hydroxydecanoate (Rha-C10), respectively. The composition of the RL mixture depends on many factors such as bacterial species and strain type, carbon source and culture conditions (Soberón-Chávez et al. 2005). Consequently, there exists a large variety of RL chemical structures with more than 60 homologues with carbon chain length ranging from 8 to 16 (Abdel-Mawgoud et al. 2010).

Surfactants are of crucial use for many industrial processes and products thanks to their surface modifying, emulsifying, dispersing, wetting, thickening and foaming properties (Zhang et al. 2022). Such industries include food industry, cosmetics, detergents and cleaner industries, textiles, pharmaceuticals and agriculture. In those industries, there is an effort to reduce the environmental and human impact of the production and use of synthetic surfactants. Thus, there has been a vast interest in the production and use of RLs. They are mainly produced by fermentation of the opportunistic pathogen *P. aeruginosa*, using water-soluble or insoluble carbon sources, with yields ranging from 1 g l⁻¹ to 200 g l⁻¹ (Li 2017). The main hurdle to the wider adoption of RLs is the production at scale and cost required to replace the current solutions. Recent papers discuss the primary challenges to commercial-scale production of RLs, including optimization of culture conditions, fermentation time, and post-fermentation processing (Blunt et al. 2022; Eslami et al. 2020; Guzmán et al. 2024; Miao et al. 2024). One promising strategy to increase RLs yield is genetic engineering of non-pathogenic recombinant strains. Another solution to reduce costs in a sustainable way consists in the use of renewable and low-cost sources of carbon such as plant-derived oils and various byproducts or sugar-containing wastes from several industries such as refinery, petroleum, fruit, dairy, bakery and agriculture as feedstock for fermentation (Henkel et al. 2012). For instance, the use of lignocellulose, which is one of the most abundant substrates found in many forms of agricultural waste such as sugarcane bagasse, barley pulp, rice-straw and wheat-straw, was demonstrated to be an interesting substrate for RL production (Prabu et al. 2015; Varjani et al. 2021).

RLs exhibit a low Critical Micelle Concentration (CMC), providing an effective surface tension reduction (Zhang et al. 2022). RLs are also known to be stable under extreme conditions of temperature, pH and salinity. The biosurfactant properties of a RL mixture mainly composed of di-RLs produced by the *P. aeruginosa* KT115 strain are unaffected by a wide range of temperatures (20–80 °C), pH (4.0–12.0) and NaCl concentration up to 8 % (Zhou et al. 2019). A cell-free culture broth of *P. aeruginosa* DR1 containing RLs is stable under a similar range of

temperatures, pH and salinity (Sathi Reddy et al. 2016). Moreover, RLs show good biodegradability: they are biodegradable in aerobic conditions and at least partially biodegradable in anaerobic, sulphate reducing and nitrate reducing conditions, contrary to Triton X-100, which is only partially degradable under aerobic conditions (Mohan et al. 2006). Good biodegradability of RLs was also reported in different media such as soil, soil suspensions and wastewater (Hogan et al. 2019; Khaje Bafghi et al. 2012; Pei et al. 2009). Furthermore, RLs show no or low levels of cytotoxicity, mutagenicity and ecotoxicology (Flasz et al. 1998; Das and Mukherjee 2005; Johann et al. 2016).

Besides their surfactant properties, RLs display very interesting biological activities which are valuable assets for applications in agriculture (Banat et al. 2010; Guzmán et al. 2024). Many studies from the last two decades have focused on the use of RLs for agriculture thanks to their favorable environmental and human toxicity profile. RLs can be used for crop disease management as they show direct antimicrobial properties against many phytopathogens. They have affinity for plasma membrane lipids leading to cell permeabilization or lysis (Botcazon et al. 2022; Stanghellini, Miller 1997; Sánchez et al. 2010). This membranotropic mode of action could represent a significant advantage for agricultural applications by lowering the risk of new resistances appearing in targeted microorganisms (Avis 2007).

RLs are also reported to stimulate plant immunity mechanisms (Crouzet et al. 2020). More recently, plant biostimulating properties of RLs have been described, also showing their potential to improve nutrient use efficacy, tolerance to abiotic stress and crop quality traits (Galieva et al. 2023). In parallel, extended research has been conducted on RLs for the bioremediation of polluted soils (Juwarkar et al. 2007; Zhu et al. 2024). Moreover, these compounds can constitute a helpful tool to improve the overall health of agricultural soils by modifying their physical structure and microbial communities (Eras-Muñoz et al. 2022). Finally, the RLs surfactant properties can be a valuable asset for the formulation of agrochemical products (Liu et al. 2016).

Thus, bacterial RLs are increasingly being considered to reduce the use of synthetic pesticides and fertilizers, which is a necessary step to shift towards a sustainable agriculture. We propose in this review a detailed outlook on RLs and their benefits to multiple aspects of crop production. We focus particularly on recent advances in the use of RLs for plant bioprotection and biostimulation, agricultural soil health and formulation of agriproducts.

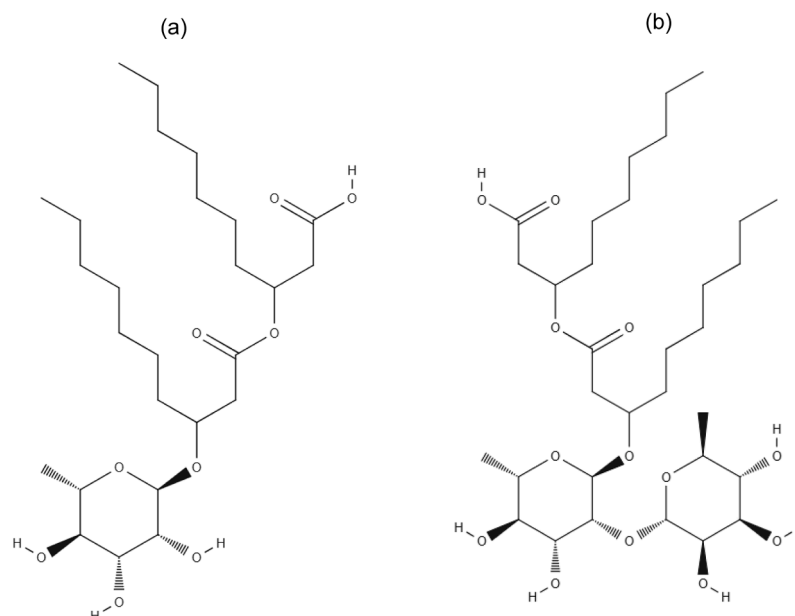


Fig. 1. Structural formulas of the main congeners produced by *Pseudomonas* sp. (a) Mono-rhamnolipid (b) Di-rhamnolipid. ©MolView.

2. Direct effects of RLs against plant pathogens and pests

Biological control, biocontrol of plant diseases, or bioprotection, is the reduction or suppression of plant pathogens by biological substances or organisms, which are generally called biopesticides in the context of crop protection. The United States Environmental Protection Agency defines biopesticides as “certain types of pesticides derived from such natural materials as animals, plants, bacteria, and certain minerals”. In France, biocontrol products are defined by the article L.253–6 of *Code Rural et de la Pêche Maritime* as “agents and products using natural mechanisms in the integrated pest management”. They comprise macroorganisms, microorganisms, chemical mediators such as pheromones and kairomones, and natural substances of plant, animal, or mineral origin. According to International Biocontrol Manufacturers Association (IBMA) France, the French biocontrol market in 2022 accounts for 278 million Euros, which represents an increase of 28.2 % compared to 2019. At the European level, there is no clear definition of biopesticide or biocontrol. Thus, biopesticides are subject to active substance approval criteria set out in the regulation (EC) No. 1107/2009 designed for synthetic pesticides (Villaverde et al. 2014). Currently, the European Union (EU) is discussing a definition for biocontrol to provide an appropriate and unified regulatory framework, in the context of making further progress in the realization of the Farm to Fork and Green Deal policy goals (IBMA 2022). The development and promotion of the use of biocontrol agents is at a critical point in the EU as conventional synthetic pesticides are being banned due to unfavorable toxicological profiles or increasing pest resistance to existing pesticides. On a worldwide scale, the biopesticides market is projected to reach 13.9 billion USD by 2028 from 6.7 billion in 2023 (Markets and Markets 2024).

RLs have extensively been studied for their direct inhibitory effects against a wide range of microorganisms. They exhibit antimicrobial properties on taxonomically diverse animal pathogens, such as fungi, Gram-positive and Gram-negative bacteria and viruses (Vatsa et al. 2010). In the context of biocontrol of phytopathogens, RL biocidal effects have been found to be particularly effective on fungi and oomycetes, whereas no effect was found on phytopathogenic bacteria (Table 1). In these studies, RLs were mainly produced by *P. aeruginosa* fermentation, used as a crude or purified mixtures containing mono-RLs and di-RLs only. Most studied congeners are Rha-C10-C10 and Rha-Rha-C10-C10. Some studies used mixtures enriched in some RL congeners to investigate the link between antimicrobial properties and RL structure. Di-RLs dominate the antifungal activity of a crude RL mix against seven plant pathogens including *Botrytis cinerea*, *Phytophthora* spp. and *Fusarium* spp. (Sha et al. 2012). Similarly, di-RLs are responsible for the antifungal activity of a crude RL mixture against black *Aspergillus* species, while mono-RLs exhibit low inhibitory activity (Rodrigues et al. 2017). Di-RL treatment also results in higher antifungal activity against *Phytophthora*, *Colletotrichum* and *Fusarium* species (Li et al. 2022a). Contrastingly, mono-RLs exhibit better inhibitory activity to phytopathogens such as *Alternaria alternata*, *Pantoea agglomerans* and *Cladosporium* sp (Zhao et al. 2022). No difference between the antifungal activity of purified mono-RLs and di-RLs is detected against *Aspergillus flavus* (Rodrigues et al. 2021). Therefore, it is unclear whether one or two rhamnoses in the RL structure is responsible for greater antifungal abilities, as this may also depend on the pathogens.

As previously described, both crude extracts and purified RLs can exhibit antifungal activity. In some cases, the cell-free culture broth containing mono-RLs and di-RLs showed stronger biological effect than purified congeners, as lower concentrations of mono-RLs and di-RLs were required in the crude broth to reach the half maximal inhibitory concentration (IC50) (Sha et al. 2012). RL-containing crude fermentation products are thus an interesting prospect to control fungal phytopathogens in an eco-friendly and cost-efficient manner.

Besides RL structure differences, various RL concentrations were chosen to perform *in vitro* inhibition assays. From one study to another, it is rather difficult to compare the tested concentrations and the

Minimal Inhibitory Concentrations (MIC), defined as the lowest concentration of antimicrobial agent needed to inhibit visible growth *in vitro* (Andrews 2001). Different experimental designs (inhibition assay methods, culture media, time of measurements) also need to be taken into account for result variability. For instance, inhibitory activity of RLs with Rha-C10-C10 and Rha-Rha-C10-C10 as main congeners against *B. cinerea* growth ranges from 18 to 200 $\mu\text{g mL}^{-1}$, resulting in different MIC values (Abalos et al. 2001; Haba et al. 2003; Monnier et al. 2018; Varnier et al. 2009).

However, general observations can be set. Across all studies reported in Table 1, the concentrations of RLs tested ranges from 1 $\mu\text{g mL}^{-1}$ to 3000 $\mu\text{g mL}^{-1}$. If MIC values obtained are variable, they rarely exceed 200 $\mu\text{g mL}^{-1}$, which demonstrates the potent fungicide activity of RLs. RL inhibitory activity applies to a wide range of fungal and fungal-like families of phytopathogens, including: Aspergillaceae, Ceratobasidiaceae, Davidiellaceae, Glomerellaceae, Leptosphaeriaceae, Mycosphaerellaceae, Magnaportheaceae, Nectriaceae, Pleosporaceae, Sclerotiniaceae for fungi and Peronosporaceae, Pythiaceae for oomycetes. The most studied microorganisms include *Alternaria* sp. (Benincasa et al. 2004; Lahkar et al. 2015; Yan et al. 2014; 2015), *Botrytis* sp. (Abalos et al. 2001; Haba et al. 2003; Monnier et al. 2018; Sanchez et al. 2012; Varnier et al. 2009), *Fusarium* sp. (Abalos et al. 2001; Borah et al. 2015; Borah et al. 2016; Deepika et al. 2015; Goswami et al. 2014; Haba et al. 2003; Li et al. 2022a; Nalini and Parthasarathi 2014; Sathi Reddy et al. 2016), *Phytophthora* sp. (De Jonghe et al. 2005; Kim et al. 2000; Li et al. 2022a; Miao et al. 2015; Stanghellini and Miller 1997; Yoo et al. 2005), and *Pythium* sp. (Perneel et al. 2008; Yoo et al. 2005).

Direct inhibitory effects of RLs against fungi and oomycetes have been identified as mycelial growth inhibition, spore germination delay and/or inhibition, zoospore lysis and motility inhibition. RLs application leads to mycelium alteration and hyphal fusions and clusters formation in *A. alternata* (Yan et al. 2015), *Fusarium verticillioides* (Borah et al. 2016), *B. cinerea* and *Sclerotinia sclerotiorum* (Botcazon et al. 2022). In some cases, deleterious effects of RLs are also observed on spores, resulting in germination delay or inhibition. Such observations were made for the aforementioned pathogens *A. alternata*, *F. verticillioides* and *B. cinerea*. Other pathogens affected in their spore germination by RLs include *Cercospora kikuchii*, *Cladosporium cucumerinum*, *Colletotrichum orbiculare*, *Magnaporthe grisea* (Kim et al. 2000), *Alternaria solani* (Lahkar et al. 2015) and *Fusarium oxysporum* (Li et al. 2022a).

RLs have been proposed to directly interact and disrupt plasma membranes of zoospore plant pathogens, resulting in zoospore lysis and motility inhibition (Stanghellini and Miller 1997). Exposure to RLs damages zoospores from several pathogens such as *Phytophthora capsici* (Kim et al. 2000; Stanghellini and Miller 1997; Yoo et al. 2005), *Phytophthora cryptogea* (De Jonghe et al. 2005), *Phytophthora nicotianae*, *Phytophthora infestans* (Yoo et al. 2005), *Phytophthora sojae* (Miao et al. 2015), *Plasmopara lactucae-radicis* (Stanghellini, Miller 1997) and *Pythium aphanidermatum* (Stanghellini and Miller 1997; Yoo et al. 2005). The lipid composition of the fungal plasma membrane could be a key element in the fungal sensitivity to RLs. The nature of phospholipids and the ergosterol content could explain the higher sensitivity of *S. sclerotiorum* than *B. cinerea* to RLs (Botcazon et al. 2022; 2024).

There is no evidence indicating that RLs can directly inhibit bacteria responsible for plant diseases. RLs present no direct antibacterial activity against phytopathogenic bacterium *Pseudomonas syringae* DC3000 pv tomato (Pst-DC3000) (Sanchez et al. 2012). As for pests, di-RLs present insecticidal activity against green peach aphids (*Myzus persicae*). The aphid mortality reached 100 % at 100 $\mu\text{g mL}^{-1}$ di-RL and was linked to disruption of insect cuticular membrane integrity (Kim et al. 2011).

A few studies have investigated the effect of bioinspired RLs synthesized with green chemistry methods on phytopathogens. Synthetic mono-RLs directly inhibit *B. cinerea* growth in tomato (Robineau et al. 2020). Three bioinspired RLs inhibit fungal growth of *Zymoseptoria tritici* with synthetic RLs with a 12-carbon fatty acid tail being the most

Table 1
Direct effects of rhamnolipids (RLs) against pests.

FUNGI							
Phytopathogen or pest family	Phytopathogen or pest species	Type of RL	RL origin	RL doses	RL effects	Reference	
Aspergillaceae	<i>Aspergillus flavus</i> MUM 17.14	9 RL congeners (mainly Rha-C10-C10 and Rha-Rha-C10-C10)	<i>P. aeruginosa</i> #112	45–1500 µg mL ⁻¹	Mycelial growth inhibition	Rodrigues et al. 2021	
	<i>Aspergillus niger</i>	Rha-C10-C10 & Rha-C10-C10 (85 %); Rha-Rha-C10-C12; Rha-C12:1-C10; Rha-C12:2; Rha-C8:2; Rha-C12-C10	<i>P. aeruginosa</i> AT10	16–128 µg mL ⁻¹ (MIC = 16 µg mL ⁻¹)	Fungal growth inhibition	Abalos et al. 2001	
		8 RL congeners (mainly Rha-C10-C10, Rha-Rha-C10-C10, Rha-C10-C10, Rha-Rha-C10-C12:1 as main components	<i>P. aeruginosa</i> LBI	4–250 µg mL ⁻¹ (MIC = 64 µg mL ⁻¹)	Fungal growth inhibition	Benincasa et al. 2004	
	<i>Aspergillus niger</i> MUM 92.13 & <i>Aspergillus carbonarius</i> MUM 05.18	8 RL congeners (mainly Rha-C10-C10 and Rha-Rha-C10-C10)	<i>P. aeruginosa</i> #112	750–1500 µg mL ⁻¹ (mono-RLs), 50–1500 µg mL ⁻¹ (di-RLs)	Mycelial growth inhibition	Rodrigues et al. 2017	
		Rha-C10-C10 & Rha-C10-C10 (85 %); Rha-Rha-C10-C12; Rha-C12:1-C10; Rha-C12:2; Rha-C8:2; Rha-C12-C10	<i>P. aeruginosa</i> AT10	16–128 µg mL ⁻¹ (MIC = 128 µg mL ⁻¹)	Fungal growth inhibition	Abalos et al. 2001	
	<i>Penicillium funiculosum</i>	Rha-Rha-C10-C10, Rha-Rha-C10-C12, Rha-C10-C10, Rha-Rha-C10-C12:1	<i>P. aeruginosa</i> 47T2 NCIB 40,044	16–276 µg mL ⁻¹ (MIC = 16 µg mL ⁻¹)	Fungal growth inhibition	Haba et al. 2003	
		RL mix with Rha-Rha-C10-C10, Rha-C10-C10, Rha-Rha-C10-C12:1 as main components	<i>P. aeruginosa</i> LBI	4–250 µg mL ⁻¹ (MIC = 64 µg mL ⁻¹)	Fungal growth inhibition	Benincasa et al. 2004	
		Rha-C10-C10 & Rha-C10-C10 (85 %); Rha-Rha-C10-C12; Rha-C12:1-C10; Rha-C12:2; Rha-C8:2; Rha-C12-C10	<i>P. aeruginosa</i> AT10	16–128 µg mL ⁻¹ (MIC = 18 µg mL ⁻¹)	Fungal growth inhibition	Abalos et al. 2001	
	Ceratobasidiaceae	<i>Rhizoctonia solani</i>	Main components: Rha-Rha-C10-C10, Rha-Rha-C10-C12, Rha-C10-C10, Rha-Rha-C10-C12:1	<i>P. aeruginosa</i> 47T2 NCIB 40,044	16–276 µg mL ⁻¹ (MIC = 109 µg mL ⁻¹)	Fungal growth inhibition	Haba et al. 2003
	Davidiellaceae	<i>Cladosporium cucumerinum</i>	Rha-Rha-C10-C10	<i>P. aeruginosa</i> B5	1, 5, 10, 25, 50 µg mL ⁻¹ (MIC = 25 µg mL ⁻¹)	Spore germination inhibition	Kim et al. 2000
Glomerellaceae	<i>Colletotrichum destructivum</i>	Rha-Rha-C10-C10, Rha-C10-C10	<i>P. aeruginosa</i> YM4, <i>P. aeruginosa</i> HN, <i>P. aeruginosa</i> PAO1	0, 5, 25, 50, 100, 200 µg mL ⁻¹	Mycelial growth inhibition	Li et al. 2022a	
	<i>Colletotrichum goeosporioides</i>	Rha-C10-C10 & Rha-C10-C10 (85 %); Rha-Rha-C10-C12; Rha-C12:1-C10; Rha-C12:2; Rha-C8:2; Rha-C12-C10	<i>P. aeruginosa</i> AT10	16 to 128 µg mL ⁻¹ (MIC = 65 µg mL ⁻¹)	Fungal growth inhibition	Abalos et al. 2001	
		Rha-C10-C10, Rha-Rha-C10-C8 as major components. Possible other mono- & di-RL: C10-C10, C8-C10, C10-C12, C12-C12 and C14-C10 and C10-C16	<i>Serratia rubidadae</i> SNAU02	0,100, 250, 500 µg mL ⁻¹	Mycelial growth inhibition	Nalini, Parthasarathi 2014	
	<i>Colletotrichum orbiculare</i>	Rha-Rha-C10-C10	<i>P. aeruginosa</i> B5	1, 5, 10, 25, 50 µg mL ⁻¹	Spore germination inhibition	Kim et al. 2000	
	<i>Colletotrichum sublineolum</i>	Rha-Rha-C10-C10, Rha-C10-C10	<i>P. aeruginosa</i> YM4, <i>P. aeruginosa</i> HN, <i>P. aeruginosa</i> PAO1	0, 5, 25, 50, 100, 200 µg mL ⁻¹	Mycelial growth inhibition	Li et al. 2022a	
Leptosphaeriaceae	<i>Glomerella cingulata</i>	Mono-RL	<i>P. aeruginosa</i> spec. DSM 2874	0–4000 µg mL ⁻¹ (MIC = 50–1000 µg mL ⁻¹)	Fungal growth inhibition, conidial germination inhibition	Lang et al. 1989	
Magnaporthaceae	<i>Leptosphaeria maculans</i>	Semipurified RLs (90 %) mix: RL90-A (AGAE Technologies) & RL90-N (NatSurFact)	<i>P. aeruginosa</i>	5–500 µg mL ⁻¹	Mycelial growth inhibition	Monnier et al. 2020	
Mycosphaerellaceae	<i>Magnaporthe grisea</i>	Rha-Rha-C10-C10	<i>P. aeruginosa</i> B5	1, 5, 10, 25, 50 µg mL ⁻¹ (MIC = 50 µg mL ⁻¹)	Spore germination inhibition	Kim et al. 2000	
	<i>Cercospora kikuchii</i>	Rha-Rha-C10-C10	<i>P. aeruginosa</i> B5	1, 5, 10, 25, 50 µg mL ⁻¹ (MIC = 50 µg mL ⁻¹)	Spore germination inhibition	Kim et al. 2000	
	<i>Zymoseptoria tritici</i>	19 RLs, including a natural RL mixture and 18 synthetic bioinspired RLs	<i>P. aeruginosa</i> & green chemistry	39.5, 59.3, 88.9, 133.3, 200, 300, 450, 666.7, 1000, 1500 mM	Fungal growth inhibition (for 3 bioinspired RLs only)	Platel et al. 2021	
Synthetic biosinspired mono-RL (Rh-Est-C12)		Green chemistry	1.9, 3.9, 7.8, 15.6, 31.2, 62.5, 125, 250, 500, 1000 µg mL ⁻¹	Fungal growth inhibition	Platel et al. 2022		

(continued on next page)

Table 1 (continued)

FUNGI							
Phytopathogen or pest family	Phytopathogen or pest species	Type of RL	RL origin	RL doses	RL effects	Reference	
Nectriaceae	<i>Cylindrocarpon destructans</i>	Rha-Rha-C10-C10	<i>P. aeruginosa</i> B5	1, 5, 10, 25, 50 $\mu\text{g mL}^{-1}$ (MIC = 50 $\mu\text{g mL}^{-1}$)	Spore germination inhibition	Kim et al. 2000	
	<i>Fusarium graminearum</i>	Crude RLs, di-RLs, mono-RLs	<i>P. aeruginosa</i> ZJU211	100, 200, 400 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition	Sha et al. 2012	
	<i>Fusarium oxysporum</i> f. sp. <i>Pisi</i>	Rha-C9:2, Rha-C10, Rha-C12:3, Rha-C8-C8, Rha-C10-C10:1, Rha-C10:1-C10, Rha-C10-C8, Rha-C8-C10, Rha-Rha-C10-C12, Rha-Rha-C12-C10 Rha-C10-C10	<i>P. aeruginosa</i> SS14	5, 10, 25, 50, 100 $\mu\text{g mL}^{-1}$	Fungal growth inhibition	Borah et al. 2015	
	<i>Fusarium sacchari</i>	Rha-(C8:1), Rha-(C10C10:1), or Rha-(C10:1C10) & Rha-Rha (C10C12:1) or Rha-Rha-(C12:1C10)	<i>P. aeruginosa</i> DS9	500, 1000, 1500, 2000 $\mu\text{g mL}^{-1}$ (culture medium or supernatant)	Mycelial growth inhibition	Goswami et al. 2014	
	<i>Fusarium solani</i>	Rha-C10-C10 & Rha-C10-C10 (85 %); Rha-Rha-C10-C12; Rha-C12:1-C10; Rha-C12:2; Rha-C8:2; Rha-C12-C10	<i>P. aeruginosa</i> AT10	16–128 $\mu\text{g mL}^{-1}$ (MIC = 65 $\mu\text{g mL}^{-1}$)	Fungal growth inhibition	Abalos et al. 2001	
	<i>Fusarium solani</i>	Main components: Rha-Rha-C10-C10, Rha-Rha-C10-C12, Rha-C10-C10, Rha-Rha-C10-C12:1	<i>P. aeruginosa</i> 47T2 NCIB 40,044	16–276 $\mu\text{g mL}^{-1}$ (MIC = 75 $\mu\text{g mL}^{-1}$)	Fungal growth inhibition	Haba et al. 2003	
	<i>Fusarium solani</i>	RL mix with Rha-Rha-C10-C10, Rha-C10-C10, Rha-Rha-C10-C12:1 as main components	<i>P. aeruginosa</i> LBI	4–250 $\mu\text{g mL}^{-1}$ (MIC = 4 $\mu\text{g mL}^{-1}$)	Fungal growth inhibition	Benincasa et al. 2004	
	<i>Alternaria alternata</i>	Mono & di-RL mix	Not described	500 $\mu\text{g mL}^{-1}$	Spore germination and mycelial growth inhibition	Yan et al. 2014	
	Pleosporaceae	<i>Alternaria alternata</i>	Mono & di-RL mix	<i>P. aeruginosa</i> ZJU-211	0, 125, 200, 250, 400, 500, 600, 800, 1000, 2000 $\mu\text{g mL}^{-1}$	Spore germination and mycelial growth inhibition	Yan et al. 2015
		<i>Alternaria solani</i>	Mono & di-RL mix	<i>P. aeruginosa</i> JS29	25–3000 $\mu\text{g mL}^{-1}$	Mycelial growth and spore germination inhibition	Lahkar et al. 2015
<i>Alternaria solani</i>		Rha-Rha-C10-C10 & Rha-C10-C10 (85 %); Rha-Rha-C10-C12; Rha-C12:1-C10; Rha-C12:2; Rha-C8:2; Rha-C12-C10	<i>P. aeruginosa</i> AT10	16–128 $\mu\text{g mL}^{-1}$ (MIC = 18 $\mu\text{g mL}^{-1}$)	Fungal growth inhibition	Abalos et al. 2001	
<i>Alternaria solani</i>		Rha-Rha-C10-C10, Rha-Rha-C10-C12, Rha-C10-C10, Rha-Rha-C10-C12:1	<i>P. aeruginosa</i> 47T2 NCIB 40,044	16–276 $\mu\text{g mL}^{-1}$ (MIC = 170 $\mu\text{g mL}^{-1}$)	Fungal growth inhibition	Haba et al. 2003	
<i>Alternaria solani</i>		Rha-C10-C10 (40 %), Rha-Rha-C10-C10 (60 %)	<i>P. aeruginosa</i> (Jeneil Biosurfactant Company)	100, 1000 $\mu\text{g mL}^{-1}$	Spore germination and mycelial growth inhibition	Varnier et al. 2009	
Sclerotiniaceae	<i>Botrytis cinerea</i>	Rha-C10-C10 (40 %), Rha-Rha-C10-C10 (60 %)	<i>P. aeruginosa</i> (Jeneil Biosurfactant Company)	1000 $\mu\text{g mL}^{-1}$	Spore germination inhibition	Sanchez et al. 2012	
	<i>Sclerotinia sclerotiorum</i>	Crude RLs, di-RLs, mono-RLs	<i>P. aeruginosa</i> ZJU211	100, 200, 400 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition	Sha et al. 2012	
		Rha-C10-C10 (40 %), Rha-Rha-C10-C10 (60 %)	<i>P. aeruginosa</i>	60 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition, spore germination delay	Monnier et al. 2018	
		Synthetic Mono-RLs	Green chemistry	100, 200, 300 μM	Conidia germination and mycelium growth inhibition	Robineau et al. 2020	
		Semipurified RLs (90 % mix: RL90-A (AGAE Technologies)	<i>P. aeruginosa</i>	25, 50 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition, fusions and cluster formations of hyphae, mycelium permeabilization and cell death	Botcazon et al. 2022	
<i>Sclerotinia sclerotiorum</i>	Semipurified RLs (90 % mix: RL90-A (AGAE Technologies)	<i>P. aeruginosa</i>	25, 50 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition, fusions and cluster formations of hyphae, mycelium permeabilization and cell death	Botcazon et al. 2022		
OOMYCETES							
Peronosporaceae	<i>Phytophthora cactorum</i>	Mono-RL and di-RL	<i>P. aeruginosa</i> IGB 83	50, 100, 200 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition	Yoo et al. 2005	
	<i>Phytophthora capsici</i>	Purified mono-RL and di-RL	<i>P. aeruginosa</i>	5–30 $\mu\text{g mL}^{-1}$	Zoospore lysis and motility inhibition	Stanghellini, Miller 1997	

(continued on next page)

Table 1 (continued)

FUNGI						
Phytopathogen or pest family	Phytopathogen or pest species	Type of RL	RL origin	RL doses	RL effects	Reference
		Rha-Rha-C10-C10	<i>P. aeruginosa</i> B5	1, 5, 10, 25, 50 $\mu\text{g mL}^{-1}$	Zoospore germination inhibition and lysis, hyphal growth inhibition	Kim et al. 2000
		Mono-RL and di-RL	<i>P. aeruginosa</i> IGB 83	50, 100, 200 $\mu\text{g mL}^{-1}$	Zoospore lysis and motility inhibition, mycelial growth inhibition	Yoo et al. 2005
		Crude RLs, di-RLs, mono-RLs	<i>P. aeruginosa</i> ZJU211	100, 200, 400 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition	Sha et al. 2012
		Rha-Rha-C10-C10, Rha-C10-C10	<i>P. aeruginosa</i> YM4, <i>P. aeruginosa</i> HN, <i>P. aeruginosa</i> PAO1	0, 5, 25, 50, 100, 200 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition	Li et al. 2022a
	<i>Phytophthora cryptogea</i>	25 % RLs in oil (Plantsupport)	<i>P. aeruginosa</i>	12.5, 25 $\mu\text{g mL}^{-1}$	Zoospore lysis, reduction of sporangia formation	De Jonghe et al. 2005
		Mono-RL and di-RL	<i>P. aeruginosa</i> IGB 83	50, 100, 200 $\mu\text{g mL}^{-1}$	Zoospore lysis and motility inhibition, mycelial growth inhibition	Yoo et al. 2005
	<i>Phytophthora infestans</i>	Crude RLs, di-RLs, mono-RLs	<i>P. aeruginosa</i> ZJU211	100, 200, 400 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition	Sha et al. 2012
	<i>Plasmopara lactucae-radicis</i>	Purified mono-RL and di-RL	<i>P. aeruginosa</i>	5–30 $\mu\text{g mL}^{-1}$	Zoospore lysis and motility inhibition	Stanghellini, Miller 1997
	<i>Phytophthora nicotianae</i>	Mono-RL and di-RL	<i>P. aeruginosa</i> IGB 83	50, 100, 200 $\mu\text{g mL}^{-1}$	Zoospore lysis and motility inhibition, mycelial growth inhibition	Yoo et al. 2005
	<i>Phytophthora parasitica</i> var. <i>nicotianae</i>	Rha-Rha-C10-C10, Rha-C10-C10	<i>P. aeruginosa</i> YM4, <i>P. aeruginosa</i> HN, <i>P. aeruginosa</i> PAO1	0, 5, 25, 50, 100, 200 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition	Li et al. 2022a
	<i>Phytophthora sojae</i>	Mono and di-RLs mixes	<i>P. aeruginosa</i>	20, 40, 60, 80, 100, 120, 160, 250 $\mu\text{g mL}^{-1}$	Zoospore motility inhibition	Miao et al. 2015
		Purified mono-RL and di-RL	<i>P. aeruginosa</i>	5–30 $\mu\text{g mL}^{-1}$	Zoospore lysis and motility inhibition	Stanghellini, Miller 1997
	<i>Pythium aphanidermatum</i>	Mono-RL and di-RL	<i>P. aeruginosa</i> IGB 83	50, 100, 200 $\mu\text{g mL}^{-1}$	Zoospore lysis and motility inhibition, mycelial growth inhibition	Yoo et al. 2005
Pythiaceae	<i>Pythium myriotylum</i>	15 % RLs in water (Jeneil Biosurfactants Company), 25 % RLs in oil (Plantsupport)	<i>P. aeruginosa</i>	25–2500 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition	Perneel et al. 2008
	<i>Pythium splendens</i>	15 % RLs in water (Jeneil Biosurfactants Company), 25 % RLs in oil (Plantsupport)	<i>P. aeruginosa</i>	25–2500 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition	Perneel et al. 2008
	<i>Pythium ultimum</i>	Mono-RL and di-RL	<i>P. aeruginosa</i> IGB 83	50, 100, 200 $\mu\text{g mL}^{-1}$	Mycelial growth inhibition	Yoo et al. 2005
INSECTS						
Aphididae	<i>Myzus persicae</i>	Di-RL	<i>Pseudomonas</i> sp. EP-3 (EP-3)	10–4000 $\mu\text{g mL}^{-1}$	Aphid mortality by affecting cuticle membranes	Kim et al. 2011

effective and natural RLs showing no antifungal effect (Platel et al. 2020, 2022). Similarly, to natural RLs, nanoemulsions of synthetic RLs show no direct antibacterial activity against Pst-DC3000 *in vitro* (Mottola et al. 2023). In the aforementioned works, authors have highlighted the role of the acyl chain type and length in the biological activity of synthetic RLs, as the most active tested RLs are characterized by a C12 acyl chain.

In studies reported in Table 2, phytoprotection tests were performed in parallel of *in vitro* direct pathogen inhibition assays, showing the efficiency of RLs to protect crops mainly from fungal diseases *in planta*.

Plants tested included a variety of economically important crops such as cucumber, pepper, pea, bean, tomato, cherry tomato fruits, maize, grapevine, rapeseed, witloof chicory, and Chinese cabbage. RL treatments provide potent protection against various diseases: *Alternaria* early blight (Lahkar et al. 2015; Yan et al. 2014; 2015), *Botrytis* gray mold (Monnier et al. 2018; Sanchez et al. 2012; Varnier et al. 2009), *Leptosphaeria* blackleg (Monnier et al. 2020), *Fusarium* wilt (Borah et al. 2015; Deepika et al. 2015), *Fusarium* stalk and ear rot (Borah et al. 2016), *Phytophthora* blight (Kim et al. 2000; Yoo et al. 2005), *Pythium* root rot and damping-off (De Jonghe et al. 2005; Perneel et al. 2008). Most of RL treatments were applied preventively on plants with diverse techniques, either in pots with soil or hydroponics, under controlled conditions. Depending on their *in vitro* inhibition assay results, authors

generally chose to test a reduced number of RL concentrations for protection tests on plants, which were identical, double or up to ten times the dose used *in vitro*. RLs were applied by foliar spaying in most assays, as RLs are water soluble (Deepika et al. 2015; Kim et al. 2000; Kim et al. 2011; Lahkar et al. 2015; Monnier et al. 2018; 2020; Platel et al. 2020, 2022; Sanchez et al. 2012). Other types of application were explored such as soil application, seed treatment and nutritive RL-supplemented hydroponic medium. RL solution added to a sand/soil (9:1) substrate before sowing of cucumber seeds helps reducing damping-off disease incidence ratio by one third compared to control (Yoo et al. 2005). Treatment of pea by soaking seeds in RL solutions and treatment of pea seedlings by dipping the roots in RL solutions before contact with soil inoculated with *Fusarium oxysporum* f. sp. *pisii* resulted in the suppression of wilt symptoms. Both methods are efficient as they provide complete disease inhibition from 25 $\mu\text{g mL}^{-1}$ (Borah et al. 2015). In a similar experiment, treatment of maize seeds with RLs at 50 mg l^{-1} leads to the suppression of disease symptoms and colonization by *Fusarium verticillioides* and improvement in biomass and fruit production (Borah et al. 2016).

Preventive treatments with RLs presented the best results for crop protection. On pepper plants, RL treatments performed one day or just before inoculation with *P. capsici* are more effective in controlling

Table 2
Plant protection tests with rhamnolipids (RLs) against pests.

FUNGI						
Phytopathogen or pest species	Type of RL	RL origin	RL doses	Plant species	Type of application	Reference
<i>Alternaria alternata</i>	Mono & di-RL mix combined to <i>Rhodotorula glutinis</i> (yeast)	Not described	500 µg mL ⁻¹	Cherry tomatoes (<i>L. esculentum</i> var. <i>cerasiforme</i>)	Preventive, solution applied on fruit wound	Yan et al. 2014
	Mono & di-RL mix combined to essential oil from <i>Laurus nobilis</i>	<i>P. aeruginosa</i> ZJU-211	0, 500, 1000, 1500, 2000 µg mL ⁻¹	Cherry tomatoes (<i>L. esculentum</i> var. <i>cerasiforme</i>)	Preventive, solution applied on fruit wound	Yan et al. 2015
<i>Alternaria solani</i>	Mono & di-RL mix	<i>P. aeruginosa</i> JS29	300, 600, 750, 1500, 2250, 3000 µg mL ⁻¹	Tomato (<i>Solanum lycopersicum</i>)	Curative, foliar spraying	Lahkar et al. 2015
<i>Botrytis cinerea</i>	Rha-C10-C10 (40 %), Rha-Rha-C10-C10 (60 %)	<i>P. aeruginosa</i> (Jeneil Biosurfactant Company)	5, 10, 25, 50, 100, 1000 µg mL ⁻¹	Grapevine (<i>Vitis vinifera</i> L. cv. Gamay, <i>Vitis vinifera</i> L. cv. Chardonnay 7535)	Preventive, in-vitro plantlets immersion	Varnier et al. 2009
	Rha-C10-C10 (40 %), Rha-Rha-C10-C10 (60 %)	<i>P. aeruginosa</i> (Jeneil Biosurfactant Company)	200, 1000 µg mL ⁻¹	<i>Arabidopsis thaliana</i>	Preventive, foliar spraying	Sanchez et al. 2012
	Rha-C10-C10 (40 %), Rha-Rha-C10-C10 (60 %)	<i>P. aeruginosa</i>	6 µg mL ⁻¹ (ca. 10 µM), 60 µg mL ⁻¹ (ca. 100 µM)	Rapeseed (<i>Brassica napus</i> cultivar Darmor-bzh)	Preventive, foliar spraying	Monnier et al. 2018
<i>Colletotrichum orbiculare</i>	Synthetic Mono-RL	Green chemistry	300 µM	Tomato (<i>Solanum lycopersicum</i>)	Preventive, foliar spraying	Robineau et al. 2020
	Rha-Rha-C10-C10	<i>P. aeruginosa</i> B5	10, 50, 100 & 500 µg mL ⁻¹	Cucumber (<i>Cucumis sativus</i> L cv Baekrokdadaki)	Preventive, foliar spraying	Kim et al. 2000
<i>Fusarium oxysporum</i> f. sp. <i>Pisi</i>	Rha-C9:2, Rha-C10, Rha-C12:3, Rha-C8-C8, Rha-C10-C10:1, Rha-C10:1-C10, Rha-C10-C8, Rha-C8-C10, Rha-Rha-C10-C12, Rha-Rha-C12-C10 Rha-C10-C10	<i>P. aeruginosa</i> SS14	5, 10, 25, 50 & 100 µg mL ⁻¹	Pea (<i>Pisum sativum</i> L)	Preventive, root treatment of seedlings or seed treatment	Borah et al. 2015
	Rha-C10-C10 and Rha-Rha-C10-C10	<i>P. aeruginosa</i> KVD-HM52	100, 200 µg mL ⁻¹	Tomato (<i>Solanum lycopersicum</i>)	Preventive, foliar spraying	Deepika et al. 2015
<i>Fusarium verticillioides</i> FS7	Rha-C8, Rha-C10-C10	<i>P. aeruginosa</i> SS14	10, 25, 50, 100, 200 µg mL ⁻¹	Maize (<i>Zea mays</i> PAC 740)	Preventive, seed treatment	Borah et al. 2016
<i>Leptosphaeria maculans</i>	Semipurified RLs (90 %) mix: RL90-A (AGAE Technologies) & RL90-N (NatSurFact)	<i>P. aeruginosa</i>	100 µg mL ⁻¹ (100 µM)	Rapeseed (<i>Brassica napus</i> cultivars Darmor-bzh, Anastasia, Archimedes, Basalti)	Preventive and curative, foliar spraying	Monnier et al. 2020
<i>Zymoseptoria tritici</i>	Natural RL mixture & 18 synthetic bioinspired RLs	<i>P. aeruginosa</i> & green chemistry	1.5 mM & 50 µM to 3200 µM for Rh-Est-C12	Wheat (<i>Triticum aestivum</i> cultivar Alixan)	Preventive, foliar spraying	Platel et al. 2021
	Synthetic bioinspired mono-RL (Rh-Est-C12)	Green chemistry	500 µg mL ⁻¹	Wheat (<i>Triticum aestivum</i> cultivar Alixan)	Preventive, foliar spraying	Platel et al. 2022
OOMYCETES						
<i>Hyaloperonospora arabidopsidis</i>	Rha-C10-C10 (40 %), Rha-Rha-C10-C10 (60 %)	<i>P. aeruginosa</i> (Jeneil Biosurfactant Company)	200, 1000 µg mL ⁻¹	<i>Arabidopsis thaliana</i>	Preventive, foliar spraying	Sanchez et al. 2012
<i>Phytophthora capsici</i>	Rha-Rha-C10-C10	<i>P. aeruginosa</i> B5	10, 50, 100, 500 µg mL ⁻¹	Pepper (<i>Capsicum annuum</i> L cv Hanbyul)	Preventive and curative, foliar spraying	Kim et al. 2000
	Mono-RLs & di-RLs	<i>P. aeruginosa</i> IGB 83	100, 500, 2000 µg mL ⁻¹	Cucumber (<i>Cucumis sativus</i>)	Preventive, soil application	Yoo et al. 2005
<i>Phytophthora cryptogea</i>	25 % RLs in oil (Plantsupport)	<i>P. aeruginosa</i>	12.5, 25 µg mL ⁻¹	Witloof chicory (<i>Cichorium intybus</i> var. <i>foliosum</i>)	Preventive and curative, hydroponics and soil application	De Jonghe et al. 2005
<i>Pythium splendens</i>	15 % RLs in water (Jeneil Biosurfactants Company), 25 % RLs in oil (PRO1, Plantsupport) with or without phenazines	<i>P. aeruginosa</i>	1 µg mL ⁻¹	Bean (<i>Phaseolus vulgaris</i> L.)	Simultaneous treatment and inoculation, soil application	Perneel et al. 2008
BACTERIA						
<i>Pseudomonas syringae</i> pv tomato	Rha-C10-C10 (40 %), Rha-Rha-C10-C10 (60 %)	<i>P. aeruginosa</i> (Jeneil Biosurfactant Company)	200, 1000 µg mL ⁻¹	<i>Arabidopsis thaliana</i>	Preventive, foliar spraying	Sanchez et al. 2012
<i>Pseudomonas syringae</i> DC3000 pv tomato, <i>Pseudomonas fluorescens</i> PFO	Nanoemulsions of synthetic RLs	Green chemistry	300 µM	<i>Arabidopsis thaliana</i>	Preventive, foliar spraying	Mottola et al. 2023

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Table 2 (continued)

FUNGI						
Phytopathogen or pest species	Type of RL	RL origin	RL doses	Plant species	Type of application	Reference
INSECTS						
<i>Myzus persicae</i>	Di-RL	<i>Pseudomonas</i> sp. EP-3 (EP-3)	Not described	Chinese cabbage (<i>Brassica rapa</i> subspecies <i>pekinensis</i> and <i>chinensis</i>), Pepper	Curative, foliar spraying	Kim et al. 2011
NEMATODES						
	Pure mono-RL and di-RL congeners	Recombinant <i>Pseudomonas putida</i> KT2440	8.3, 50, 200 ppm	<i>Arabidopsis thaliana</i>	Preventive, nutritive RL-supplemented medium	Bredenbruch et al. 2024
<i>Heterodera schachtii</i>	Pure di-RL congeners with hydroxylated prodiginine	Recombinant <i>Pseudomonas putida</i> KT2440	0.7, 1.4, 2.1, 2.8, 3.5 $\mu\text{g mL}^{-1}$	<i>Arabidopsis thaliana</i>	Preventive, nutritive RL-supplemented medium	Kossmann et al. 2023

phytophthora blight disease than those at one day after inoculation (Kim et al. 2000). The addition of RLs nutrient solution in a hydroponic forcing system of witloof chicory effectively controls the spread of *P. cryptogea*, however RLs have limited effects as a curative treatment against brown root rot (De Jonghe et al. 2005).

In some cases, curative treatments with RLs also offered interesting results for disease reduction. Spraying of RLs on tomato plants 1, 8, 15 and 22 days after inoculation with *Alternaria solani* spores provides protection against early blight, with a similar level of efficiency compared to the chemical fungicide at 0.75 g l^{-1} RLs (Lahkar et al. 2015). RL application 7 days after *Leptosphaeria maculans* inoculation on rapeseed seedlings induces a reduction in lesions on cotyledons, though treatments performed before or simultaneously to the inoculation offer better protections (Monnier et al. 2020). On pepper plants infested with green peach aphids, spraying cell-free supernatant containing RLs results in 50 % aphid mortality at 40 $\mu\text{g mL}^{-1}$ and 100 % aphid mortality at 100 $\mu\text{g mL}^{-1}$, which is comparable to 100 % mortality obtained with a commercial insecticide applied at 40 $\mu\text{g mL}^{-1}$ (Kim et al. 2011).

The ability of RLs to control plant diseases in combination with other compounds has also been investigated (Table 2). RLs in combination with the biocontrol yeast *Rhodotorula glutinis* are more effective in suppressing *A. alternata* infection in cherry tomato fruits than when treatments are applied separately. In this case, RLs improved the growth of *R. glutinis* *in vivo* (Yan et al. 2014). On the same pathosystem, RLs combined with the essential oil of *Laurus nobilis*, a known tool to control post-harvest pathogens, exhibited a better infection reduction than the laurel oil used alone (Yan et al. 2015). Authors suppose that, thanks to their membranotropic properties, RLs allow the essential oil to penetrate into the cytoplasm of the fungal cell more easily. Damping-off caused by *P. splendens* is significantly more reduced on bean seeds when RLs are applied together with phenazines compared to treatments with single compounds, indicating a synergistic interaction. Phenazines are another type of antibiotic produced by *Pseudomonas* species. It is proposed that RLs solubilize phenazines and give them better access to the fungal cell (Perneel et al. 2008).

3. Indirect effects by plant defense mechanisms triggering

As they have been described as human and animal immunity stimulators, RLs have also been reported as plant defense mechanism stimulators (Vatsa et al. 2010). The way plants are able to recognize RLs remains unclear. However, they have been described to trigger events involved in the Microbe-Associated Molecular Patterns (MAMP)-triggered immunity. This model describes how plants are able to recognize microbial elicitors known as MAMPs thanks to protein receptors known as Pattern-Recognition Receptors (PRRs) localized on the plant cell surface (Zhang, Zhou 2010). MAMP sensing triggers signaling cascades leading up to early and late plant defense responses, eventually

setting up basal immunity. Nevertheless, to date, no plant receptor has been identified for RLs and the hypothesis of a direct plasma membrane recognition is favored (Crouzet et al. 2020).

RLs trigger early signalization reactions such as Reactive Oxygen Species (ROS) accumulation and hypersensitive response (HR)-like response in grapevine (Varnier et al. 2009) and rapeseed (Monnier et al. 2018). RL-triggered stomatal closure was also shown in rapeseed (Monnier et al. 2018) while calcium (Ca^{2+}) influx and phosphorylation cascades were observed in grapevine (Varnier et al. 2009). Later responses triggered by RLs in grapevine, rapeseed as well as the model plant *Arabidopsis thaliana* include defense gene expression modifications, induction of hormonal pathways (salicylic acid, jasmonic acid and ethylene) and callose depositions (Monnier et al. 2018; 2020; Sanchez et al. 2012; Varnier et al. 2009). In cherry tomatoes, RLs application increases activities of antioxidant enzymes (Yan et al. 2014). A quantitative proteomic analysis highlighted the differential accumulation of defense or stress proteins in response to RL treatments with a clear effect of the type of application (foliar spraying or root absorption) and showed that RLs can activate systemic plant defense mechanisms (Pierre et al. 2023).

Some bioinspired synthetic RLs were also found to promote antioxidant enzyme activity (Platel et al. 2020) and to induce a slight defense gene expression and metabolite accumulation. Most genes and metabolites are down-regulated, with few of them associated with resistance to pathogens. Surprisingly, some differentially accumulated metabolites could be linked to an “abiotic stress-like” effect of RLs on wheat (Platel et al. 2022). In *A. thaliana*, defense genes involved in hormonal signaling pathways are induced by synthetic RL bolafoms (Luzuriaga-Loaiza et al. 2018) and synthetic RL nanoemulsions (Mottola et al. 2023).

Some studies have reported that RLs protect the model plant *A. thaliana* from pathogens not sensitive to RLs, suggesting plant defense stimulation can be sufficient to reduce the disease. RL-triggered immune response leads to a local resistance against the hemibiotrophic bacterium *P. syringae* DC3000 pv tomato, even at the lowest concentration tested (0.2 mg mL^{-1}) (Sanchez et al. 2012). Pure mono-RL and di-RL congeners produced by a recombinant *Pseudomonas putida* strain significantly reduce the plant nematode *Heterodera schachtii* infection on *A. thaliana*, though no direct inhibitory activity of di-RLs was observed against *H. schachtii*. RL treatment on the host plant does not induce hormonal pathways related genes within a 1-hour or a 48-hour treatment, but an increase in hydrogen peroxide (H_2O_2) production in leaves is detected upon RL treatment, suggesting some level of plant defense activity (Bredenbruch et al. 2024). The reduction of nematode infection in *A. thaliana* by di-RL treatment depends on the acyl chains length, with C10-C12 and C10-C12:1 congeners being the most effective at low dose (2 ppm). However, those congeners reduce plant growth at higher doses. Mono-RLs at 8.3 ppm concentration were also found to have deleterious impact on *Arabidopsis* growth, further showcasing a structure-effect

relation (Bredenbruch et al. 2024).

Thus, as potent bioprotection agents, rhamnolipids are able to protect plants by reducing the disease severity caused by a large and diverse range of RL-sensitive and non RL-sensitive plant pathogens. For RL-sensitive phytopathogens, which have been found so far to be exclusively fungi and fungi-like species, there is the possibility of a dual mode of action of RLs, by direct inhibition of fungal growth and plant defense mechanism stimulation (Monnier et al. 2019). On some bacteria and nematodes which proved to be non-sensitive to RLs, RL treatment could provide protection by triggering plant defense responses. It should be noticed that, to date, published research describing field trial efficacy of RLs is lacking.

4. RLs as potential plant biostimulation agents

According to the European Union Fertilizers Regulation (EC) No

2019/1009 implemented on July 16, 2022, plant biostimulants are natural compounds stimulating plant nutrition based on the claims that they improve one or more of the following parameters: nutrient use efficiency, tolerance to abiotic stress, crop quality traits and availability of soil and rhizosphere nutrients (Ricci et al. 2019). In recent years, there has been growing interest in the use of plant biostimulation products to ensure crop yield and quality in a sustainable crop production system. With more and more products being commercially available, the biostimulants worldwide market is projected to reach USD 7.6 billion by 2029 from USD 4.3 billion in 2024 (Markets and Markets 2024). Plant biostimulants comprise substances and microorganisms. Microorganisms have been the focus of many biostimulant products development strategies, as plants live in association with diverse and complex microbial communities, also known as the plant microbiome. However, limitations still exist on the use of living microorganisms in the field, which can eventually impair their efficiency as biostimulants:

Table 3
Biostimulation assays with rhamnolipids (RLs) on plants.

Plant species	Type of assay	Type of RL	RL doses	Combination with other compounds	Type of application	Stress	Reference
<i>Abelmoschus esculentus</i> (Okra)	Seed germination assays	Partially purified RLs	0.25, 0.50, 0.75, 1 g L ⁻¹	/	Solution on filter papers	/	Ghazi Faisal 2024
<i>Allium cepa</i> (Onion)	Seed germination assays	Partially purified RLs	0.25, 0.50, 0.75, 1 g L ⁻¹	/	Solution on filter papers	/	Ghazi Faisal 2024
<i>Brassica napus</i> (Rapeseed)	Adsorption assays in hydroponics	25 % RL liquid extract (Jeneil Biosurfactant Co.)	10 μM	Zinc	In nutrient solution	/	Stacey et al. 2008
<i>Camellia sinensis</i> L. (Tea plant)	Pot experiment	Not described	0.4 %	Small peptides (6 g L ⁻¹)	Leaf spraying (every 5 days)	High temperature	Chen et al. 2023
	Pot experiment	Not described	0.4 %	Small peptides (6 g L ⁻¹)	Leaf spraying (every 7 days)	/	Chen et al. 2023
<i>Glycine max</i> (Soybean)	Seed germination and early growth assays	RLs mix	0.25, 0.50, 0.75, 1 g L ⁻¹	/	Solution over seeds on filter papers	/	da Silva et al. 2015
	Imbibition and germination assays Pot experiment	37.6 % mono-RLs, 34.9 % di-RLs RLs mix	0.5 to g L ⁻¹ & 2 g L ⁻¹ 0.1 %, 0.3 % & 0.5 %	/	Seed soaking Drip irrigation	/ Saline soils	Sancheti et al. 2020 Liu et al. 2023
<i>Gossypium</i> (Cotton)	Field experiment	Mono-RLs (7 congeners) & di-RLs (11 congeners) mix RLs mix	0.3 g L ⁻¹	/	Drip irrigation (x10)	Saline soils	Chen et al. 2024
<i>Helianthus annuus</i> (Sunflower)	Seed germination and early growth assays	RLs mix	0.25, 0.50, 0.75, 1 g L ⁻¹	/	Solution over seeds on filter papers	/	da Silva et al. 2015
	Liquid medium and field experiments	RLs biocomplex or cell-free culture liquid	0.01 g L ⁻¹ RL biocomplex or diluted (1:200) cell-free culture liquid	/	Seed soaking	/	Koretska et al. 2020
<i>Lactuca sativa</i> (Lettuce)	Seed germination and early growth assays	RLs mix	0.25, 0.50, 0.75, 1 g L ⁻¹	/	Solution over seeds on filter papers	/	da Silva et al. 2015
	Seed germination assays	Partially purified RLs	0.25, 0.50, 0.75, 1 g L ⁻¹	/	Solution on filter papers	/	Ghazi Faisal 2024
<i>Oryza sativa</i> (Rice)	Field experiment	Not described	0.0156, 0.0312, 0.0624 g L ⁻¹	Transpiration inhibitor (humic acid) at 0.48 g L ⁻¹	Leaf spraying (every 7 days)	Heavy metal (cadmium)	Peng et al. 2023
<i>Solanum lycopersicum</i> (Tomato)	Field experiment	Commercial liquid product with 20 % RLs	3, 7.5, 12 L ha ⁻¹	Choline chloride, organic manure, chemical fertilizers	Not described	Saline soils	Hu et al. 2023
	Pot experiment	25 % RL liquid extract (Jeneil Biosurfactant Co.)	0, 0.75, 2, 4, 6 mg kg ⁻¹	Zinc	Soil (x3)	/	Stacey et al. 2008
<i>Triticum aestivum</i> (Wheat)	Seed germination assays	Crude mixture	0.1, 0.5, 1 g L ⁻¹	/	Solution over seeds on filter papers	/	Galieva et al. 2023
<i>Triticum durum</i> (Durum)	Pot experiment	25 % RL liquid extract (Jeneil Biosurfactant Co.)	0, 0.75, 2, 4, 6 mg kg ⁻¹	Zinc	Soil (x3)	/	Stacey et al. 2008
	Seed germination and early growth assays	RLs mix	0.25, 0.50, 0.75, 1 g L ⁻¹	/	Solution over seeds on filter papers	/	da Silva et al. 2015
<i>Zea mays</i> (Corn/ Maize)	Field experiment	70 % RLs, 30 % other glycolipids	0.75 kg ha ⁻¹ glycolipids	Chemical fertilizers	RL solution sprayed over soil	/	Meng et al. 2024

sensitivity to environmental stresses, interactions with native microbial communities, and relatively short shelf life (Brahmaprakash, Sahu 2012). An alternative and novel approach to microbial biostimulants is the use of microbial metabolites directly in the field, thus bypassing the limitations of living microorganisms. In that regard, some recent works have investigated the potential of RLs as plant biostimulation agents (Table 3).

RLs have been shown to promote germination and early growth *in vitro* for some plant species. RLs extracted from a *P. aeruginosa* fermented broth increase lettuce seed germination rate at the highest doses (0.75 and 1 g l⁻¹) while increase of corn and sunflower germination occurred at a lower dose (0.25 g l⁻¹). RL treatment also leads to improvement of seedling development for sunflower and soybean (da Silva et al. 2015). On wheat, crude RL mixture from *P. aeruginosa* PAO1 fermentation stimulates seed germination by 1.9 and 2 times at 0.5 g l⁻¹ (Galieva et al. 2023). With partially purified RLs from *P. aeruginosa* fermentation, the highest stimulation of okra, lettuce and onion seed germination is observed at 0.25 g l⁻¹. In these conditions, wheat and barley germination rates are reduced by RLs, however the treatment protects the seeds from fungal infections when the incubation time is increased (Ghazi Faisal 2024). Thus, it is necessary to point out that RLs could exhibit phytotoxicity in some conditions, especially when the RL dose is higher than 1 g l⁻¹. With a seed soaking technique, a mix of mono-RLs and di-RLs improves lateral root development and reduces primary root extension of soybean at 0.5 to 1 g l⁻¹ while at 2 g l⁻¹, an improved imbibition rate is observed (Sancheti, Ju 2020). With a similar application technique, diluted cell-free liquid culture from *P. aeruginosa* and 0.01 g l⁻¹ of RL biocomplex improved ion absorption and sunflower plant growth in liquid nutrient medium experiments (Koretska et al. 2020).

In field experiments, RLs improve sunflower yield as well as seed lipids and protein content (Koretska et al. 2020). Adding glycolipids majorly composed of RLs to conventional NPK fertilizers helps reducing the nitrogen fertilizer use without compromising maize yields in the field and increases nitrogen uptake (Meng et al. 2024). RLs have also been previously described to increase trace nutrient uptake by plants. Zinc complexed to RLs has an improved root uptake in rapeseed (*Brassica napus*) grown in hydroponic solution and application of RLs to a calcareous soil increases zinc content and dry matter in durum and wheat (Stacey et al. 2008).

Some recent experiments were also performed to evaluate the interest of RLs to mitigate abiotic stress in controlled conditions and in field (Table 2). In controlled conditions, RLs combined with small peptides applied by spraying on tea plants in high temperature conditions improved photosynthetic parameters, antioxidant enzymes activity, and modified phytohormones and metabolic pathways, resulting in an overall increased survival rate and adaptability to high temperature stress (Chen et al. 2023a). This same treatment caused a decrease in diversity of bacterial and fungal communities and an increase in beneficial microorganisms in the tea plant phyllosphere, thus improving the microbiome community structure in relation to the plant health (Chen et al. 2023b).

In field, a solution of 20 % RL was combined with choline chloride and tested on tomato plants in moderately salinized soil (0.6 % salt content) with different types of fertilization (Hu et al. 2023). Choline chloride is a well-known animal feed additive and has been described to play a positive role on plant growth and tolerance to stress (Riaz et al. 2021; Hussain et al. 2022). The addition of RLs in combination to choline chloride enhances salt tolerance and tomato growth by decreasing superoxide anion and malondialdehyde content, the ratio of Na⁺/K⁺, increase of peroxidase activity, proline content, chlorophyll content and net photosynthetic rate (Hu et al. 2023). In the case of heavy metal stress contamination in paddy fields, foliar spraying of RLs combined with humic acid reduces cadmium content in rice grain and increases plant biomass and yield. Authors suggest enhanced cadmium fixation in leaves resulted in a reduced transfer to the grain in presence of RLs (Peng et al. 2023).

Availability and use of the soil micro and macronutrients are essential for plant growth and crop quality, especially in extreme and abiotic stress affected environments. On the cotton crop, RL treatment by drip irrigation on saline soils decreases soil electrical conductivity, increases soil organic matter and promotes nutrients cycling, resulting in cotton growth. In these works, authors showed that RLs reshapes the composition and function of the fungal and bacterial communities in the rhizosphere, thus supporting the idea of a biostimulating effect of RLs on cotton by improved availability of soil and rhizosphere nutrients (Chen et al. 2024; Liu et al. 2023). Other works also highlight how RLs help soil nitrogen and organic matter cycling by promoting microbial enzyme activity, thus increasing soil fertility (Gong et al. 2017; Ren et al. 2023). In saline-alkali soils, RLs amendment improves the soil aggregates stability and promotes microecological functions such as carbon and nitrogen metabolisms by influencing microbial soil communities (Li et al., 2022b). These results suggest that RLs can constitute an interesting tool to improve the health and quality of agricultural soils by influencing the soil microbiome, resulting in indirect plant growth.

5. Other potential applications of RLs in agriculture and prospects

Currently, a wide a range of synthetic surfactants are used as adjuvants to improve emulsifying, dispersing, spreading, wetting, sticking or penetrating of chemical pesticides (Jibrin et al. 2021). As anionic biosurfactants, RLs represent a natural and effective alternative to synthetic surfactants for the formulation of agricultural products. Thanks to their effective surface tension reduction abilities, they offer an enhanced wetting and penetration of the hydrophobic waxy leaf surface (Bunster et al. 1989; Liu et al. 2016) and show good solubilizing and emulsifying properties, though parameters such as RL homologs ratios and pH have an impact on the activity (Li et al. 2022a). Moreover, RLs are biodegradable as they can effectively be mineralized by soil microorganisms (Lima et al. 2011).

Another promising way of using RLs as alternatives to agrichemicals is to use their biosurfactant properties to formulate and improve the effectiveness of microorganisms-containing biocontrol and biostimulant solutions. In the last few years, some works have investigated the potential of RLs as a formulating agent for Plant Growth-Promoting Rhizobacteria (PGPR) solutions, PGPR being beneficial soil bacteria that colonize plant roots and promote plant growth and health through many mechanisms (de Andrade et al. 2023). The formulation of a PGPR *P. putida* strain amended with RLs provides the best improvement of *Brassica juncea* growth parameters in field conditions (Mishra et al. 2020). Similarly, the formulation of a PGPR *Pseudomonas guariconensis* strain with RL-containing biosurfactant mixture promotes sunflower growth and controls charcoal rot disease caused by *Macrophomina phaseolina* in field conditions (Khare and Arora 2021). As RLs can be used to formulate other solutions, formulation could also be a way to boost RLs performance in agricultural settings. RLs have been already tested in combination with other compounds and showed synergistic effects in some cases (Table 2).

Furthermore, agricultural soils can be affected by organic and inorganic pollution, thus threatening the productivity of crops. Biosurfactants like RLs are known to improve bioavailability and bioaccessibility of hydrophobic compounds such as hydrocarbons by solubilization, emulsification, modification of the cell surface of soil microorganisms or boost soil enzymatic activity, thus helping degrade the hydrocarbons. RLs also help mitigate heavy metal stress by chelating metals. Many papers explore the use of RLs for bioremediation (Aşçi et al. 2008; Camilios Neto et al. 2009; Costa et al. 2010; Juwarkar et al. 2007; Maier et al. 2001; Rahman et al. 2003; Shin et al. 2006; Wei et al. 2020; Zhang et al. 1997) and review papers discuss the potential environmental applications and limitations, sometimes including other biosurfactants (Eras-Muñoz et al. 2022; Liu et al. 2018; Mulligan 2005; Pacwa-Płociniczak et al. 2011; Parus et al. 2023; Singh et al. 2024).

As we have mentioned beforehand, the potent action of RLs on soil and its microbiome can be beneficial in many aspects for crop production. By modifying the microbial communities and their activities, RLs can mitigate abiotic stresses such as alkalinity, nutrient deficiency, pollutants persistence and enhance beneficial plant-microbe interactions. However, these alterations on the structure and diversity of microbial communities raise the question of their long-lasting effects on global ecosystems. Another matter to carefully consider is the determination of effective RL concentrations for their different potential agricultural applications. In some cases, low RL concentrations can be sufficient for intended uses e.g. stimulation of microbial activity or plant defense mechanisms. In other types of application like bioremediation or plant disease control in field, higher RL concentrations are needed. Mono-RLs and di-RLs have been demonstrated to have different efficacies on different phytopathogens *in vitro*. However, this remains to be confirmed in greenhouse and field experiments. The biodegradability of RLs is a considerable advantage compared to synthetic surfactants, however it should not be overlooked to ensure that RLs are not degraded before they can fulfill their task. It is also necessary to proceed with caution when using RLs with a specific aim. For example, if RLs are used for increasing bioavailability of trace essential metals, their property to increase the mobility of toxic metals, such as Pb and Cd, should be taken into account. The phytotoxicity of RLs should also be investigated as RLs can have deleterious effects on some plant species growth (Parus et al. 2023).

6. Conclusion

Rhamnolipids are a promising class of biosurfactants with significant potential for developing sustainable agricultural practices. Their multiple properties, ranging from direct microbial inhibition and indirect plant defense mechanism stimulation to plant biostimulation, soil health and nutrient availability enhancement (Fig. 2), can contribute to increase crop yields while minimizing synthetic chemicals inputs. However, further research is essential to better understand the mechanisms underlying their effectiveness, optimize application methods, and evaluate their long-term impacts on ecosystems. Considering their bio-protection properties, field data are still missing in the academic literature and it can therefore be difficult to draw conclusions on the real RL efficacy to fight specific diseases. Moreover, the scale-up of rhamnolipid production and their cost-effectiveness also call for extended investigation to ensure their practical application for agriculture. As the global demand for sustainable farming practices rises, integrating rhamnolipids into agricultural strategies is a promising way toward achieving sustainable crop production.

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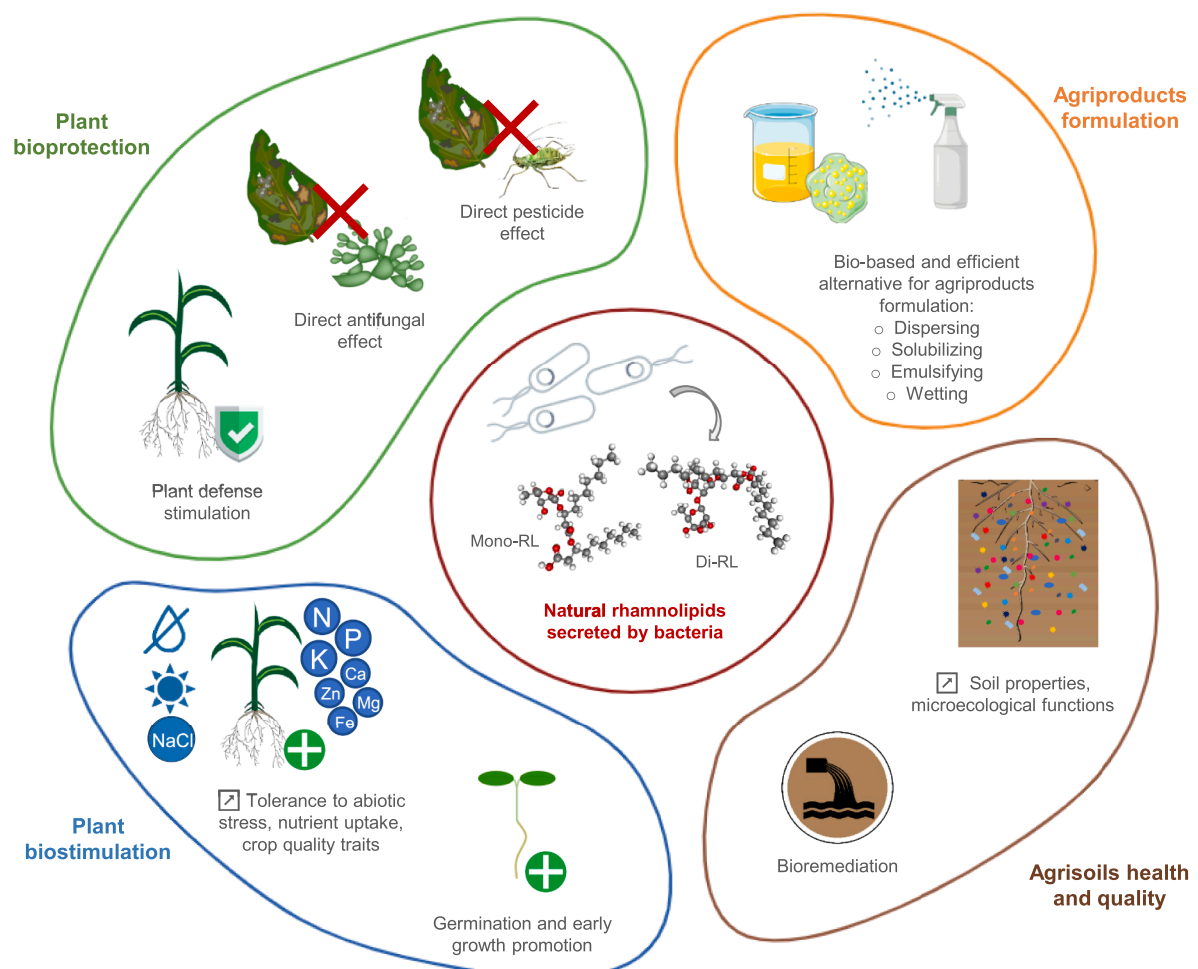


Fig. 2. Potential applications of rhamnolipids for agriculture.

CRedit authorship contribution statement

Elise Pierre: Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Elodie Shaw:** Writing – review & editing. **Brian Corr:** Writing – review & editing. **Karine Pageau:** Funding acquisition, Supervision, Writing – review & editing. **Sonia Rippa:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

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Data availability

No data was used for the research described in the article.

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