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There must be something in the water: Root-released organic compounds in aquaponics and their potential effects on system performance

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1	There must be something in the water: Root-released organic compounds in aquaponics
2	and their potential effects on system performance
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17	Optimising production in aquaponics requires in-depth knowledge of the plant, fish and microbial
18	compartments. This opinion paper is focused on root-released organic compounds and their implication
19	in aquaponic systems. While there has been no direct research on this to date, literature from distinct
20	fields of study - phytochemicals in aquaculture science and root exudates in plant science - points at the
21	novel topic of the effects of root exudates on cultured fish and the aquaculture microbiome. It shows the
22	need for further research to better understand sources and mechanisms of such molecules and possible
23	ways to harness beneficial effects that can guide new research directions as the role of aquaponics grows
24	in recirculating aquaculture.
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26	Keywords: Aquaponics, integrated aquaculture, bioactive compounds, recirculating aquaculture,
27	hydroponics, root exudates

Introduction

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As the aquaculture sector is facing the challenge of increasing production while reducing environmental impacts, a strategy to achieve more sustainable production systems is to move away from intensive monocultures towards polyculture systems that apply biodiversity in an agro-ecological approach.¹ Aquaponics, an integrated production system combining aquaculture with hydroponic plant culture, aims to move intensive aquaculture towards sustainability by recycling nutrients and optimizing the use of space and water.² In coupled aquaponics, nutrient-rich process water is circulated from an aquaculture subsystem to a hydroponic subsystem, where nutrients are taken up by plants. It is then returned to the aquaculture subsystem, minimizing water use and waste discharge. The use of pesticides can be eliminated and production sites placed closer to markets.³ It is well known that plants do not only takeup molecules via their roots, they also release organic compounds into the rhizosphere, the medium surrounding the roots.⁴ Bacteria settling in this area can also be influenced by root exudates. Similarly, rhizobacteria, which are closely associated with plant roots, can directly influence their environment through metabolites and/or other substances. Thus, there are many different allelopathic interactions in the rhizosphere. Furthermore, the influence of plant compounds on fish and their potential applications in aquaculture as alternatives to chemotherapy and to alleviate stress have been widely investigated and reviewed.^{5–7} Many plant compounds that have been found to modulate fish growth, stress, or immunity can also be released over the roots. This raises the question of their potential diverse roles in a coupled aquaponics system. While to our knowledge there has been no direct research on this to date, the outcomes of some recent studies suggest there is indeed an effect on fish behavior and health while further influences on system components seem likely. We aim to synthesize the evidence that rootreleased organic compounds could influence the functions of an aquaponic system and argue that the subject merits further research to harness potential benefits for systems that integrate natural biological control.

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Part 1: Diversity and effects of plant root exudates

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A wide array of plants can be produced in aquaponics including leafy greens, aromatic herbs, vegetables and medicinal and ornamental plants.⁸ Among these, especially aromatics and medicinal plants have received considerable research attention as feed-additives in aquaculture to improve growth, disease resistance and immunity (Figure 1).⁵ Plants release chemicals called rhizodeposits or root exudates into the rhizosphere in the form of high and low- molecular weight compounds. Those exudates influence a multitude of biotic and abiotic processes.^{4,9} Root exudates include nutrients, chemoattractants or allelochemicals that interact with fungi, bacteria and other plants, to create favorable conditions and alleviate stresses on the host plant.⁹ Plants thus shape the composition of the root micro- and macrobiome, inhibit the growth of pathogens and attract symbiotic i.e. nitrogen-fixing bacteria9 and beneficial mutualistic arbuscular mycorrhizal fungi. 10 Colonization by these mycorrhizal fungi even genetically regulates nutrient transport in the root, improving nitrogen and phosphorus nutrition and subsequent plant growth. 11 Plants also interact with other plants via allelochemicals such as flavonoids, quinones, quinolines and hydroxamic acids, principally released to negatively impact metabolic processes in other plants. 12,13 In a few cases their effects are beneficial to the neighbor plant species through their repellant roles towards herbivores.⁹ It just seems plausible that root exudates would play a role inside the hydroponic unit of an aquaponics system as they shape the microbial communities of the rhizosphere. Especially in systems where different species of crops are produced, exuded chemicals can influence biomass production and nutrient uptake negatively or positively. Similar to findings in soil, various bacteria have been identified in aquaponics, which were associated with stimulating plant growth. 14 These plant growth promoting bacteria include the phyla Proteobacteria, Actinobacteria and Firmicutes as well as the genera Acidovorax, Sphingobium, Flavobacterium and Pseudomonas, genera which have been found in the rhizosphere of lettuce¹⁵; however, their role on roots and root exudates is still unknown. It has been speculated that plant roots create a beneficial environment for symbiotic microorganisms while deterring harmful bacteria by secretion of different compounds. 14 Organic compounds released by roots such as aldehydes, benzenoids, terpenoids and phenylpropanoids, flavonoids and coumarins have potent antimicrobial activity against pathogenic bacteria (16 and references therein).

The bacterial community in an aquaponics system is often divers with many different families and genera, each potentially with a specific settlement prevalence within the different sections. 15,17 Especially the bacteria located in a biofilter (*Nitrosomonas*, *Nitrobacter* and *Nitrospira*) are a key biotic factor in aquaponics and its nitrifying capacity is essential to the health of animals and plants. To prevent losses of nitrogen in the rhizosphere, plants exude compounds known as biological nitrification inhibitors, examples of which are gallocatechin, caffeic acid and α -linoleic acid. Notably polyphenolics, but also a range of other plant compounds, have demonstrated biological nitrification inhibition (Table 1). Interactions between plants and microbes from the rhizosphere can exert a strong effect on the whole plant, and even on an ecosystem level, 19 with the potential to affect other organisms as well.

Part 2: Effect of plant biomolecules on fish biology and potential pathways in aquaponics

In the past decades, increasing attention has been paid to plants as immunostimulants and alternatives to conventional chemotherapy that reduce stress and improve fish growth and immunity when applied as feed additives or baths.⁵ Many of the positive effects of plant additives are attributed to their polyphenol content (reviewed by ²⁰). As feed additives, they act upon the gastrointestinal tract before entering the circulatory system and being transported to other organs. They were found to stimulate the growth of beneficial gut bacteria (probiotics), thus preventing infection by pathogens. Furthermore, they can reduce oxidative stress on tissues by inhibiting the formation of or scavenging reactive oxygen species, and activating the production of antioxidant enzymes. They can also inhibit the proinflammatory pathways activated by exposure to such chronic stressors as animals often experience in aquaculture settings. By reducing the pathogen load in the water, phenolics and other compounds lower the risk of infection for fish. ^{16,21} Such positive effects have been attributed to a variety of molecules, many of which are not only found in the plant tissue but also in root exudates (Table 2) and they may therefore be released by the plants into an aquaponics system.

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While the vast majority of research has focused on the addition of compounds such as polyphenols to feeds, recent studies suggest comparable positive effects on fish through external application. Most notably, in a study by ²², zebrafish (*Danio rerio*) showed several improved immune parameters as well as increased survival after bacterial challenge when fish were exposed to 1 µg L⁻¹ quercetin in the water for 8 weeks. Many of the effects that were detected - increases of lysozyme, Immunoglobulin M, Myeloperoxidase, activity of superoxide dismutase and glutathione peroxidase as well as decreased malondeldehyde - have also been found in studies when quercetin was administered via feeding.²⁰ Effects on the innate immunity and antioxidant capacity of the intestine suggest that phenol-rich substances are ingested from the water.^{21,22} Other possible pathways include reactivity with the gill mucus or epithelium, interaction with plasma membranes by sufficiently small molecules, and physical irritation or membrane peroxidation with low levels of continuous stress thought to have a "training" effect on defense mechanisms.^{21,23} Additionally, small polyphenols such as resveratrol, quercetin and butein have been found to activate sirtuins, with beneficial effects on cell metabolism.²⁴ Such mechanisms are thought to be partly responsible for the improvements physiological processes and immune responses observed in fish exposed to humic substances, which are largely comprised of tannins and lignin constituents, which in turn have similar properties to plant polyphenols.²³ While it remains to be investigated if direct root exudates would indeed have similar effects on fish, some observations from aquaponic experiments do suggest positive outcomes. Intercropping of lettuce and red chicory, the latter known for its high polyphenol content and presence of antioxidants in the roots, resulted in higher growth of pangasius (*Pangasianodon hypothalmus*) in an aquaponics system.²⁵ The culture of basil (Ocimum basilicum) in coupled aquaponics with African catfish (Clarias gariepinus) led to reduced skin lesions on the fish, suggesting less aggressive behavior. 26 The fish did not come into direct contact with the plants, but were kept in the same process water for several weeks without water changes.

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Part 3: Bottlenecks and future research

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The presence, types and concentrations of root exudates in aquaponic systems and their effect on the physiology or behaviour of fish and other farmed animals remains unexplored, and their role is only postulated based on results of feeding experiments and exposure to similar compounds (Figure 2). It is therefore relevant to investigate the production and composition of exudates in an aquaponics context: Which types of molecules are released? Do they vary between plant species? Do they remain in the water and accumulate? These are a few of the basic questions that remain to be answered. Furthermore, the possible effects on the physiology and behaviour of the farmed animals are to be investigated. How do certain molecules act upon the organism? Are concentrations high or constant enough to influence animals? Can effects be measured? Do the compounds have beneficial or adverse effects? Aquaponic systems vary greatly in their design and management, which may affect the types of phytochemicals released. Allelopathy between different plant species could lead to a diversity or "cocktail" of exudates and possible synergies or antagonisms between them. Furthermore, aquaponics consists of complex production systems of fish, plants and microbes. Which is the role of each biological compartment? Do root exudates influence the biofiltration capacity or other roles of the microbiome? Does the microbial community in turn influence root exudation? Are the compounds released subsequently metabolized? It remains to be seen if it can even be differentiated between the molecules released by plants and those produces by the rhizobiome. In relation to these first points and going forward, the question arises if it is pertinent to manage the plant compartment of an aquaponics system with the objective to promote a "plant biodiversity" that produces vegetables for human consumption, but also achieves a biocontrol of fish welfare and improves their zootechnical performance with the objective to act at several complementary levels such as growth, immune systems and anti-stress. ²⁷ suggested that there may be allelopathic interactions among different organisms within an aquaponic system. Interactions between plants and microbes from the rhizosphere can exert a strong effect on the whole plant, even on an ecosystem level, 19 with potential to influence other organisms. Could a certain composition of plant species achieve such agro-ecological biocontrol as crop auxiliary plants in some applications in terrestrial systems? Could such an approach integrate a control of the microbial compartment and for example limit the emergence or development of pathogens

such as *Aeromonas* or *Vibrio*? If we promote the establishment of plant biodiversity for the biocontrol of fish welfare, such an approach seems easy in an intensively managed system such as a stable and controlled monoculture, but more complex in dynamic or changing systems. Answering these questions requires the involvement of complementary scientific skills in an interdisciplinary research approach to enable advances in knowledge and innovation in aquaponics, at the interface of each of the different research areas concerned.

Acknowledgements

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Tables

Table 1: Examples of vegetable crops, bioactive compounds found to be released by their roots in hydroponics and reported nitrification inhibitory activity, "-" = no information available.

Plant	Compound	Concentrations found in hydroponics (µmol L ⁻¹) reported by ^{28,29}	Compounds has nitrification inhibitory activity summarized by ¹⁸
Lettuce	Benzoic acid	0.3 – 11.5	-
	Cinnamic acid	5.8	-
	Ferulic acid	6.4 - 7.8	Yes
	Gallic acid	2.4 - 3.0	Yes
	Lauric acid	2.7 – 8.0	-
	Palmitic acid	0.75 - 12.46	-
	Phenylacetic acid	4.8 – 5.9	-
	Phthalic acid	1.9 – 14.3	-
	Salicylic acid	5.9 – 10.3	-
	Stearic acid	8.3	-
	Tannic acid	0.3 - 0.4	-
Basil	Caffeic acid	-	Yes
Cucumber	Phenylalanine	-	Yes
Tomato	Quercetin	-	Yes

Table 2: Examples of plant active compounds with known influence on fish physiology or water bacteria

load (based on reviews by ^{6,7,30,31}) found in root exudates of plants of agronomic interest.

Active compound	Effect on fish	Root exudate	Reference
Allicin	Antioxidant, antibacterial	garlic 32	
Benzoic acid	Stress reducing	beans (Phaseolus vulgaris, Vicia faba)	33
		cucumber (Cucumis sativus)	34
		lettuce (<i>Lactuca sativa</i>)	29
Cinnamic acid	Stress reducing,	cucumber (C. sativus)	
	antiparasitic	eggplant (S. melongena)	34
	•	lettuce (L. sativa)	28
Epicatechin	Immunity	cucumber (C. sativus)	35
Gallic acid	Immunity	lettuce (L. sativa)	29
Heneicosane	Antibacterial	tomato (Solanum lycopersicum)	36
Oleic acid	Wound healing	lily (Lilium davidii)	37
Palmitic acid	Wound healing	lily (L. davidii)	37
		lettuce (L. sativa)	28
Quercetin	Immunity,	tomato (S. lycopersicum)	38
`	antiparasitic,		
	antibacterial		
Solasodine	Immunity	tomato (S. lycopersicum)	38
Stearic acid	Wound healing	lily (L. davidii)	37
		lettuce (L. sativa)	28
Tomatidine	Immunity	tomato (S. lycopersicum)	38
Vanillin	Antibacterial	lily (Lilium formolongi)	39
		eggplant (Solanum melongena)	34

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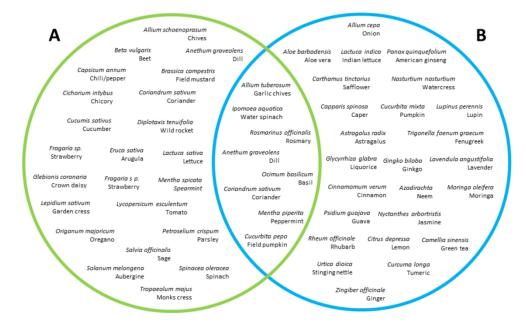


Figure 1: Overlap of (A) plants grown in aquaponics (adopted from Maucieri et al. 2018) and (B) plants investigated as aquaculture feed additives (adopted from Reverter et al. 2014, Elumalai et al. 2020), see original articles for extensive lists.

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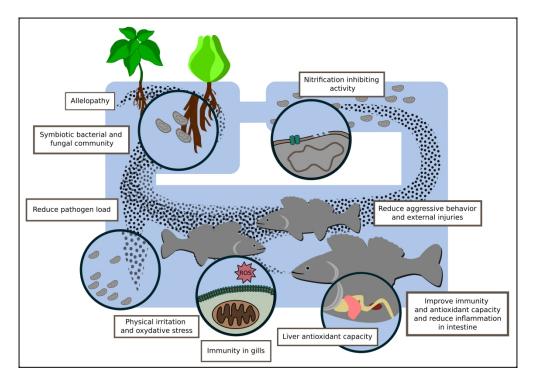


Figure 2: Possible areas of influence of root exudates in an aquaponic system.

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