Effects of Ozonated Water on Horticultural Products and Future Applications

Ozone, composed of three oxygen atoms, dissociates as easily in air as it does in water for a duration of about twenty minutes. The molecule is naturally formed in small doses in the stratosphere thanks to the action of ultra violet radiation that affects oxygen. The versatility of ozone - in that it is both a strong disinfectant and a strong oxidant that can be applied in the gas and/or liquid phases - plus its fundamental cleanliness and lack of negative environmental impact, means that it is well-suited for many applications in the agriculture and food processing industries. In the gas phase, this versatile chemical can provide an atmosphere for processing, packaging and storage of many agricultural products (fruits, vegetables, meats, poultry, etc.), thereby minimizing the proliferation of spoilage micro-organisms controlling many moulds and odours and ethylene gas. When applied in the aqueous phase, ozone can be used to treat plant influent water, recycled process water, and wastewaters.

Table 1. Microorganism reduction ability for UV radiation, chlorine and ozone (adapted from: Earth Tech Canada, 2005).

Disinfectant	Microorganism Reduction Ability			
	E. Coli	Giardia	Cryptosp-oridium	Viruses
UV radiation	Very effective	Very effective	Very effective	Moderately effective
Chlorine	Very effective	Moderately effective	Not effective	Very effective
Ozone	Very effective	Very effective	Very effective	Very effective

Aqueous solutions of ozone in water are used commercially for spray washing of many different agricultural and food products and for sanitizing processing equipment and for plant wash-downs and cleaning in place. It has been used in hospitals in the US. When combined with ultraviolet radiation and electrolyzed water, ozone provides a new technique for replacing pesticide and insecticide sprays onto growing agricultural crops. Ozone plus UV radiation, electrolyzed water and ultrasound allows close to sterilization of foods during processing and packaging, thus providing significant extensions of shelf-lives of fresh and/or processed foods. In turn, this combination of advanced technologies has created a new concept in restaurants, in which pre-prepared and specially packaged uncooked meals are created in a central processing plant for distribution to restaurants and large institutions where microwave ovens have replaced gourmet chefs and kitchens.

It is highly effective against viruses and bacteria and recognised by the Environmental Protection Agency and Food Safety in Australia and New Zealand. Organics Australia also recognise it for application on organic produce. Unlike other disinfection methods, ozone is considered environmentally friendly because there are no toxic residues administered correctly. In 2001 the U.S. Food and Drug Administration (FDA) formally approved ozone as an Antimicrobial Agent for direct contact with foods. Since that approval, many commercial applications of ozone in treating many foods have developed.

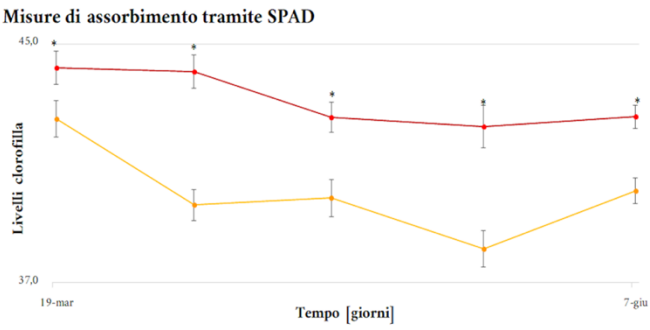
Several studies in addition have revealed that ozone in aqueous solution is a possible candidate as a control agent for micro-organisms, because it is able to exploit antimicrobial character of the oxidant with all the possibilities of water use. This form is therefore called ozonated water which is produced in two phases: the first phase is the generation of gaseous ozone, and the second phase is the injection of ozone into water via porous stones or tools for the production of microbubbles and nanobubbles.

Ozonated water is used nowadays for the control of possible contamination in the most disparate environments: from the treatment of irrigation water in greenhouses to the municipal ones, to the sanitization of vegetable and fruit products after harvest for their conservation and storage. Among all the possible modes of administration, the ozonated water in spray mode is perhaps the one with the most obvious effects on the plant: in the field this mode consists of the nebulization of the suspension directly on the plants, through an atomizer or sprayers.

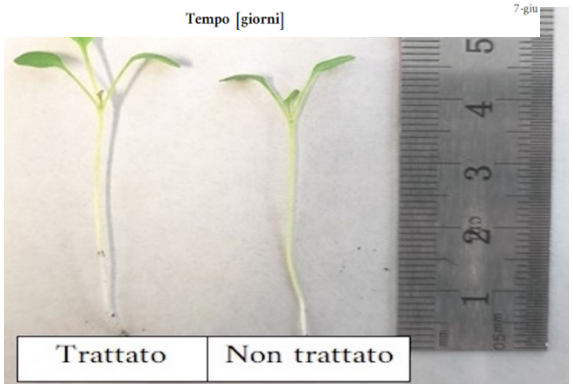
Ozone triggers the plant cells to activate defence processes. As an example following the irrigation of arugula plants (*Diplotaxis tenuifolia*) with ozonized water in the field, an increase in the overall weight of the crop and a better defence of the plant against pathogens, such as fungi and insects, which would otherwise have decimated the production were observed and then proven. (Riccardo Greco)

The research measured the levels of chlorophyll in the plant directly in the field, for the measurement period that went from March to July. This made it possible to observe how ozonated water raised the levels of chlorophyll in arugula leaves. Additional chlorophyll facilitates increased growth and plant resilience.

Measuring chlorophyll levels in the field using the Spad
(Source: Riccardo Greco)



In the laboratory, under controlled environmental conditions, the arugula was irrigated with ozonated water from the seed stage. The seedlings have been observed for two weeks since the first germination, thus being able to notice that those irrigated with ozone tend to germinate earlier and develop more rapidly in the first days of growth. Finally, molecular analyses were conducted that provided promising preliminary results. An analysis of gene expression for the defence of the plant made it possible to prove a slight induction of the insect defence pathway induced by treatment with ozonized water.



Growth differences between arugula seedlings treated and not treated with ozonated water.

The promising results obtained in the greenhouse on various types of horticultural crops are achieved by ozone technology achieving important recognition by the European Community, of this environmentally friendly technology seriously engaged in agricultural production that respects the healthiness of the product with no residual and good for the environment. Ozone is the natural solution.

Some further results:

Location	Crop	Target	Pathogens	Results
Borgo Piave (LT), Lazio	Actinidia chinensis (kiwifruit plants)	Total fungal and bacterial load	Many	- Reduction of airborne fungal load by 58%-70% - Reduction of airborne bacterial load by 97%-98%
Bologna BO), Emilia-Romagna	Basil	Fungal disease: Downy mildew	Peronospora belbahrii	- Disease controlled in 90% leaves (O3MET) compared with only 40% with chemical defence (Mandipropamid and Fosetil) - Reduction of microbial load in the air; propagules are destroyed. Plants are restored in just 10 days
Monte San Biagio (LT), Lazio	Zucchini	Fungal disease: Powdery mildew	Podosphaera xanthii Erysiphe cichoracearum, Fusarium spp, Botritys cinerea	- Blocked the colonization of mildew in apical leaves maintaining same growth as conventional treatment - Clearly enhanced appearance of zucchini flowers
Battipaglia (SA), Campania	Spinach, Rocket salad	Fungal diseases: Spinach Downy Mildew, Lettuce Downy Mildew	Peronosporaspp. Bremia lactucae	Spinach: - 34% increase in green mass in O3MET vschemica Rocket salad: - 90% leaves free of Mildew vs

Location	Crop	Target	Pathogens	Results
				80% in chemical defence - Greater vigour and 23% increase in green mass in O3 vs chemical
Paese (TV), Veneto	Nursery trays	Total fungal and bacterial load	Many	- 99% reduced microbial load in trays (fungi, bacteria and microalgae). No colonies detected after spray treatment
Rosoloini (SR), Sicilia	Zucchini, artichoke	Total fungal and bacterial load	Many	- Reduction of airborne fungal load by 58%-70% - Reduction of airborne bacterial load by 97%-98%
Ispica (RG), Sicilia	Cherry tomato	Total fungal and bacterial load	Many	- Reduction of airborne fungal load by 58%-70% - Reduction of airborne bacterial load by 97%-98%

These results are supported by research on other seedlings including tomatoes and corn. Provisional research has shown ozone has lengthened the shelf life of kiwi fruit in storage We are undertaking further research and have a research paper on its application in an avocado orchard. Research in broccoli showed that the application of ozone had a 15% greater food density.

In conclusion, ozonated water could mark a turning point in organic and/or eco-sustainable crops as it induces plants to defend themselves from the attack of pathogens, increases vegetative development, allows growth even in adverse seasons and much more. However, it is a still new method that still needs a lot of scientific experimentation but in field research shows significant benefit with no chemical residuals, both in the field and in universities, in order to produce new results and understand how it can be used and what other mechanisms it can induce.

Greco R. 2021, Study into the Effects of Aqueous Ozone on arugula plants (*Diplotaxis tenuifolia*) University of Verona Master's

Rice R. G. (2001) - *Recent Developments In Agri-Food Applications Of Ozone* - OZONE SCIENCE AND TECHNOLOGY