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Studies on the efficacy of electrolysed oxidising water to control *Aspergillus carbonarius* and ochratoxin A contamination on grape☆

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ABSTRACT

Ochratoxin A (OTA) occurrence in grapes is caused by black Aspergilli (*Aspergillus carbonarius* followed by *A. niger*) vineyards contamination. It depends on climatic conditions, geographical regions, damage by insects, and grape varieties. Good agricultural practices, pesticides, and fungicides seem adequate to manage the problem during low OTA risk vintages, but the development of new strategies is always encouraged, especially when an extremely favourable condition occurs in the vineyard.

Electrolysed oxidising water (EOW) has become an interesting alternative to chemicals in agriculture, mainly during the post-harvest phase. This study tested the fungicidal efficacy of EOW generated by potassium chloride, *in vitro*, on black Aspergilli conidia, and detached grape berries infected by *A. carbonarius*. Then, during field trials on Primitivo cv vineyard treated with EOW, *A. carbonarius* contamination, and OTA levels were compared with Switch® fungicide treatment (0.8 g/l). Black Aspergilli conidia were killed on plate assay after 2 min of treatment by EOW containing >0.4 g/l of active chlorine.

EOW (0.6 g/l active chlorine) treatment reduced the rate of *A. carbonarius* infections *in vitro* of about 87–92% on detached berries and, more than half in the field trials, although Switch® showed better performance. A significant reduction in the OTA concentration was observed for the EOW and Switch® treatments *in vitro* (92% and 96%, respectively), while in the field trials, although the average decrease in OTA was recorded in the treated grapes, it was not statistically significant.

These results highlighted that EOW could be considered effective, as a substitute for fungicides, to reduce the contamination of *A. carbonarius* and OTA on grapes.

1. Introduction

The vine (*Vitis vinifera*), according to the cultivated hectares and the economic value, is one of the main fruit crops in the world (OIV, 2019). Grape is considered a perishable fruit because the surface of the berries is characterized by the coexistence of various microorganisms, including filamentous fungi, that have different effects on the quality and shelf life of the final product (Barata et al., 2012; Kántor et al., 2017), but the main concern for human health is related to mycotoxins. Ochratoxin A (OTA), a naturally occurring mycotoxin, is a secondary fungal metabolite produced by *Aspergillus* and *Penicillium* species. It has been proven to be nephrotoxic, hepatotoxic among other toxic properties, and is classified as potentially carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC, 1993). It is found in a wide range of products and after cereals, wine and grape juice are the main sources of OTA in the human diet (JECFA, 2008). Based on the links between OTA and the effects on human health, regulatory limits have been established for food of plant origin intended for human consumption by Commission Regulation (EC, 2006). Among the agricultural products associated with OTA contamination, wine as a source of OTA is highlighted by considerable and continuous global increases in wine consumption (De Jesus et al., 2018). OTA in wine is a problem that originates mainly in the vineyard. *Aspergillus* species belonging to the *Nigri* Section are responsible for the accumulation of OTA in grapes and derived products, mainly produced by *Aspergillus carbonarius* (Perrone et al., 2008; Ponsone et al., 2012). These ochratoxigenic fungi are

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opportunists (saprophytes) that cause symptoms that are not always visible and commonly linked to limited yield losses (Visconti et al., 2008). Although always present in the field, they may develop massively on berries damaged due to abiotic and/or biotic causes, from veraison to harvest, with a high incidence at ripening. This contamination is strongly related to climatic conditions, geographic regions (the southern Mediterranean climate is very favourable), vines cultivars, damage caused by insects (Cozzi et al., 2009), although, great variations may occur from year to year. Good pest and disease control in the vineyard has been shown to significantly reduce OTA contamination in high-risk areas and years (Battilani and Silva, 2010; Mondani et al., 2020; Visconti et al., 2008). Switch® (37.5% Cyprodinil, 25% Fludioxonil), is a broad-spectrum fungicide that is mainly used in the vineyard to control grey mould (*Botrytis cinerea*), and other secondary fungal pathogens, *e.g.*, black mould caused by black Aspergilli, achieving a significant OTA reduction in grapes (Bellí et al., 2007; Tjamos et al., 2004). Although plant protection products are commonly used to reduce the proliferation of fungi and the production of mycotoxins under field conditions, they must comply with strict legislation on their use and maximum residue levels of pesticides have been regulated in many products, including grapes (EC, 2008). Furthermore, due to the growing number of resistant fungal strains and the impact of fungicides on the environment and human health (De Costa and Bezerra, 2009), new strategies are needed to replace or supplement fungicidal treatments in the control of toxigenic fungi in pre-harvest and post-harvest. In this regard, a possible alternative or supplement to traditional chemicals, with a long history in food disinfection procedures (Hricova et al., 2008), is the use of electrochemical activation technology to produce solutions that effectively control microbial growth without the negative side effects caused by conventional practices. Their sterilizing effect is due to a mixture of inorganic oxidants such as HOCl, ClO^-, Cl_2, OH^-, which are effective to inactivate a variety of microorganisms (Al-Haq et al., 2005; Bari et al., 2003; Fabrizio and Cutter, 2004; Huang et al., 2008). Since in various studies (Guentzel et al., 2010; Tomás-Callejas et al., 2011; Vandekinderen et al., 2009), it was reported that the use of electrolysed water does not leave significant residues of chlorine and does not affect the qualitative, sensory, and nutritional parameters of fruits and vegetables, there is growing interest in seeking its use as an effective component of integrated pest control protocols. Previous research indicates the potential of nearly neutral electrolysed oxidising water (EOW), which effectively mitigated post-harvest surface infections of *Botrytis cinerea* and *Monilia fructicola* on grapes and peaches, respectively (Guentzel et al., 2010). Similarly, good control of apple canker (*Nectria galligena*) and fire blight of pear (*Erwinia amylovora*) has been shown in field trials using EOW (Collina et al., 2014). There is currently no information available on the efficacy of EOW to stop the growth of black Aspergilli and its effect on OTA synthesis. Therefore, the purpose of this study was to evaluate the efficacy of EOW in inhibiting conidial germination and growth of ochratoxigenic strains of *A. carbonarius* and *A. niger*. Establish the minimum concentration of EOW for an optimal reduction of black Aspergilli. Evaluate, in the vineyard, through field trials the validity of this new strategy to control toxigenic fungi and the accumulation of OTA in grapes.

2. Materials and methods

2.1. EOW production

The EVA-100© Mod-2 h electrolysed oxidising water unit (Industrie De Nora S.p.A., Milan, Italy) used in this study, is equipped with a 100 l tank, to be filled with tap water and 1.5 Kg of Potassium Chloride.

After the production of EOW, the pH and oxidation-reduction potential (ORP) were measured, in triplicate, using a pH electrode (Liq-Glass, Hamilton, USA) and an ORP electrode (Plast ORP BNC 32200673, XS-Instrument, Italy), respectively.

The measurement of active chlorine was performed with the iodometric method (APHA-AWWA-WEF, 1998), in production, and before each test or treatment, in triplicate. The EOW solution was stored in a closed, opaque container at 4 °C in the dark.

2.2. Inhibition of black Aspergilli growth and conidial germination in vitro

Three ochratoxigenic strains isolated from grapes, *Aspergillus carbonarius* ITEM 7444, *A. carbonarius* ITEM 5010 and *A. niger* ITEM 7090, deposited in the Agro-Food Microbial Culture Collection (www.ispa.cnr.it/Collection/) of the Institute of Sciences of Food Production (ISPA-CNR, Bari, Italy), were first evaluated for resistance to EOW disinfection, then the most resistant strain was used during the *in vitro* assay on grape berries. Working cultures were maintained on Potato Dextrose Agar (PDA) (Oxoid Ltd., Basingstoke, UK) at 25 °C for 5–7 days in the dark.

Different final concentrations of EOW (0.01, 0.05, 0.1, 0.2, 0.4, 0.6, 1.0, 2.0, 4.0 g/l of active chlorine), were screened *in vitro*, in triplicate, to test their ability to inhibit the conidial germination of these three *Aspergillus* sect. *Nigri* strains. Concentrations that showed effective activities were repeated twice.

The conidial germination (%) was evaluated in triplicate in 24 well multiwell plates (Costar, Corning, NY, USA), filled with 1 ml of an aqueous solution containing, 10^3 conidia/ml, 10% PDB, and an adequate EOW volume to reach the desired final concentration of active chlorine, the control samples were prepared replacing EOW with sterile water. After 24–72 h of treatment, each well was observed throughout an inverted microscope searching for germinating conidia.

The fungicidal activity and inhibition of black Aspergilli growth were evaluated using a plate assay. For each isolate, 1 ml of an aqueous solution containing 10^4 conida/ml containing an adequate EOW volume to reach the established concentration of active chlorine, and the control with water replacing EOW was prepared. After 2 and 10 min of treatment, serial dilutions were prepared and viability of the spores was verified by plating, in triplicate, 100 µl of each dilution on the surface of Dichloran Rose Bengal Chloramphenicol Agar (DRBC, Liofilchem S.r.l., Roseto degli Abruzzi, Italy) plates, then incubated at 25 °C for 5–7 days, in the dark.

2.3. In vitro inhibition of A. carbonarius growth and OTA production on grape berries

The efficacy of the EOW treatment (containing 0.2, 0.4, 0.6 g/l of active chlorine) to inhibit, *in vitro*, the growth of *A. carbonarius* ITEM 7444 and the accumulation of OTA on grape berries, was evaluated in two different local varieties of red wine grapes (Primitivo cv and Aglianico cv) and was also compared with the efficacy of the registered fungicide Switch® (Syngenta Italia S.p.A., Milan, Italy), at two different concentrations (0.4 and 0.8 g/l).

The healthy berries were detached from grape bunches freshly harvested (years 2016–2018), the surface was washed, sterilized twice (hypochlorite 0.5%, alcohol 70%), washed with sterile water, and allowed to dry for at least 1 h below the airflow of a biological safety cabinet. The sterilized berries of each variety were immersed in an aqueous suspension of *A. carbonarius* ITEM 7444 conidia (10^4 conidia/ml), 11 berries were placed individually in the wells of each 12 well multi-well plate, allowed to dry and then sprayed with the established concentrations of EOW or Switch® fungicide. In each plate, the twelfth well was filled with sterile water to balance the loss of moisture during incubation. Evaluations were carried out on all berries, after 7 days of incubation at 25 °C in the dark, estimating the symptoms caused by *A. carbonarius* ITEM 7444 using an empirical scale with six infection classes: 0, healthy berry; 1, 1–5% of the contaminated berry surface (cbs); 2, 6–25% cbs; 3, 26–50% cbs; 4, 51–75% cbs; and 5, 76–100% cbs. The use of the empirical scale allowed the calculation of the following parameters: incidence (percentage of infected berries) and severity of the disease, measured as the McKinney Index (McKinney, 1923). All experiments were performed in triplicate for each treatment and

repeated at least twice.

2.4. EOW applications in the vineyard

2.4.1. Experimental design of field trials

The trials were conducted in a conventional field cultivated with the Primitivo vine, a representative vineyard of Faggiano located in Salentum (Apulia), a wine-growing region of southern Italy, during two growing seasons (2017 and 2018). The vines were raised in a covered scaffolding trellis system. The plant density was 0.25 plants m^{-2} and the row spacing was 2.0 m. For the experimental trials, a randomized block design with five plots per treatment was adopted (see Table 1). Each replicate in the vineyard included 10 vines (Fig. 1) and was delimited by a buffer row of unsprayed vines to minimize the drift of spraying. All sprays were applied using a backpack sprayer, distributing a volume equivalent to 1000 l ha^{-1}. Untreated vine plots were used as controls. EOW treatments (Table 1) and the time of application were based on the grapevine growth stage.

Two treatment periods have been scheduled for EOW and Switch®: the first at the pre-bunch closure (14–20 June) and the second at late veraison (8–10 August). However, depending on local weather events, if conditions were too favourable for fungal contamination, the option for the third treatment with EOW at pre-harvest (20 August) was established.

2.4.2. Sampling of grapes

During each field trial, about 20 Kg of grape were sampled at harvest from each replicate of the different treatments. The grapes were pressed during the day with a manual crusher and after 2 h of maceration, grape must samples (1 l for each replicate) were collected and cooled at 4 °C, then processed within 24 h to determine the amount of contamination by black Aspergilli and the OTA level.

2.4.3. Isolation and counting of the Aspergillus section Nigri

The populations of black Aspergilli and *A. carbonarius* (colony forming units (CFU) per ml) were determined for the grape must obtained from each replicate. The grape must was used to make 10-fold serial dilutions in water. From each dilution, 100 µL was spread in triplicate on the surface of the DRBC agar plates. All plates were incubated in the dark for 5–7 days at 25 °C. Black *Aspergillus* colonies were identified based on morphology (Pitt and Hocking, 1997; Samson et al., 2007). The number of colonies of *Aspergillus* section *Nigri* and *A. carbonarius* was counted and confirmed at 40 X directly on the plate. The population of black Aspergilli and *A. carbonarius* was reported as CFU per ml of grape must, and the values were log-transformed (Hirano et al., 1982) for statistical analysis.

2.5. Chemical analysis of OTA

The method of Solfrizzo et al., 2008, for the determination of OTA in grapes, dried vine fruits, and winery by-products, has been slightly modified and used herein for the determination of OTA in grapes. In particular, the sample extracts were analyzed directly by HPLC without immunoaffinity cleanup. Briefly, 5 g of slurried berries (containing 4 ml of water) was extracted with 26 ml of acetonitrile/methanol/water (90:90:80, *v*/v/v) by shaking for 60 min and filtered through a Whatman No. 4 filter paper. One milliliter of extract was diluted with 1 ml of HPLC mobile phase (acetonitrile/water/acetic acid; 99:99:2, v/v/v), and 100 µl, corresponding to 0.008 g of solid sample was injected into the HPLC-FLD apparatus by a full loop injection system.

The Official Method AOAC 2001.01 (Visconti et al., 2001) has been slightly modified and used for the determination of OTA in grape must samples. Briefly, 5 ml of centrifuged grape must were diluted with 5 ml of an aqueous solution containing PEG (1%) and $NaHCO_3$ (5%), mixed, filtered through a Whatman No. 4 filter paper, and 5 ml of diluted sample cleaned-up by OchraTest immunoaffinity columns (Vicam, Watertown, MA). The column was washed with 5 ml of an aqueous solution containing NaCl (2.5%) and $NaHCO_3$ (0.5%), followed by 5 ml of distilled water at a flow rate of 1–2 drops/s. The eluates were discarded, and the OTA was recovered in a vial by passing 1 ml of methanol +1 ml of water through the column. Fifty microliters, corresponding to 0.0625 ml of grape must were injected into the HPLC-FLD.

The HPLC-FLD apparatus for the determination of OTA in berries and grape must was an Agilent 1260 Infinity (AgilentTechnology, Inc., Wilmington, DE, USA) consisting of a binary pump (G1312B), an autosampler (G1367E) with a 100 µl loop, a fluorescence detector (G1321B) fixed at 333 nm (λ_{ex}) and 460 nm (λ_{em}) set at 30 °C and a software for Microsoft Windows 7 (OpenLAB, CSB, ChemStation Edition). The column used was a Zorbax® C18 column, 150 mm × 4.6 mm, 5 µm particle size, 0.5 µm pore size guard filter (Agilent). Chromatographic separation was performed under isocratic conditions using a mixture of acetonitrile/water/acetic acid (99:99:2, *v*/v/v) at a flow rate of 1.0 ml/min.

Table 1
Active ingredients used in the trials performed during 2017 and 2018 on Primitivo grape variety.

Product	Active ingredient	Application rate (g ha^{-1})	Growing season
EOW 0.4	*Active chlorine (0.40 g/l)*	400	2017
EOW 0.6	Active chlorine (0.60 g/l)	600	2017 and 2018
Switch® (Syngenta Crop Protection)	Cyprodinil 37,5% + Fludioxonil 25% (0.80 g/l)	800	2017 and 2018

2.6. Statistical analysis

Statistical analyses were performed with GraphPad Prism 8 (Graphpad Software LLC, USA), Shapiro-Wilk test and Kolmogorov-Smirnov test were performed to test the hypothesis of the normal distribution of values. The relationship between disease, mould contamination, level of OTA and treatments was studied through Dunn's multiple comparisons test.

3. Results

3.1. EOW production parameters

After 2 h of work, the EOW unit generates 100 l of an aqueous solution containing potassium hypochlorite, with 4.4 ± 0.2 g/l of active chlorine, pH 9.1 ± 0.1, ORP 740 ± 10 mV and 1% residual KCl. During storage in a closed, opaque container at 4 °C in the dark, the concentration of active chlorine shows a decrease of approximately 0.3 g/l every 10 days.

3.2. In vitro inhibition of black Aspergilli growth and conidial germination

Conidial germination appears to be mostly inhibited even at lower concentrations (0.0125 g/l) of EOW, the viability of the conidia of black Aspergilli was effectively inhibited after only 2 min of treatment with EOW containing active chlorine concentrations of 0.2–0.4 g/l, depending on the strains considered (Supp. Fig. S1). *A. carbonarius* ITEM 7444, proved to be the most resistant strain to EOW, while the others were completely inhibited even at 0.2 g/l. Summarizing the EOW fungicidal activity starts from 0.2 g/l but was fully effective at 0.4–0.6 g/l for *A. carbonarius* ITEM 7444. These results were used to set the concentration of EOW to be used in the *in vitro* experiments on red wine grape berries.

Fig. 1. A treated vineyard in 2017 (A) and 2018 (B). In green the untreated control area, in orange the treated area, in light blue grapes treated with EOW 0.4 g/l of active chlorine, in dark blue grapes treated with EOW 0.6 g/l of active chlorine, in yellow grapes treated with Switch® 0.8 g/l. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. *A. carbonarius* growth and OTA production on grape bunches in vitro

EOW has been shown to be effective in reducing both the growth and incidence of *A. carbonarius* disease on the surface of the berries of the two grape varieties. In particular, Aglianico grapes were less susceptible to infection with *A. carbonarius* ITEM 7444, compared to Primitivo grapes. All the treatments were highly effective in inhibiting the fungal growth and the disease index on Aglianico berries. Incidence and severity index of the untreated Aglianico berries contaminated with *A. carbonarius* ITEM 7444, decreased respectively from 45% and 26%, both to zero when EOW >0.4 g/l and Switch® 0.8 g/l treatments were performed, thus demonstrating its greater resistance to black Aspergilli contamination.

In the experiments on Primitivo grape, the EOW treatment 0.4 g/l reduced the infection by *A. carbonarius* ITEM 7444 by about 55–64%, compared to the inoculated control, while EOW 0.6 g/l showed a reduction of approximately 87–92%. The Switch® treatment at 0.8 g/l (recommended dose for grapes) was slightly more effective (95% reduction) than the Switch® treatment at 0.4 g/l (93% reduction) (Fig. 2A).

Chemical analyses on *in vitro* treated grape berries revealed that there was a significant ($p < 0.02$) reduction in OTA production compared to the untreated control. In particular, an average reduction of 92% and 96% in OTA levels was observed for EOW 0.6 g/l and Switch® 0.8 g/l, respectively (Fig. 2B).

3.4. Efficacy of EOW treatments in Primitivo vineyard

Field trials during the 2017 growing season showed that the overall level of black Aspergilli contamination was ubiquitous, ranging from 10^5 to 10^6 CFU/ml in grape must samples, while *A. carbonarius* was more frequently isolated from 10^3 to 10^5 CFU/ml.

All the treatments seem not to have significantly influenced the presence of black Aspergilli on grapes, which means that this presence could be ascribed to the basal contamination values, naturally present in the Salentum vineyard.

The 2017 grape growing season, in Salentum, was very hot and dry, with almost no rainfall from June to September in the test area, thus, representing very adverse conditions for *A. carbonarius* growth and infection of the grapes.

No OTA was found in the overall sampling of the vineyards, nor in the different treatments, nor in the untreated controls, which means that

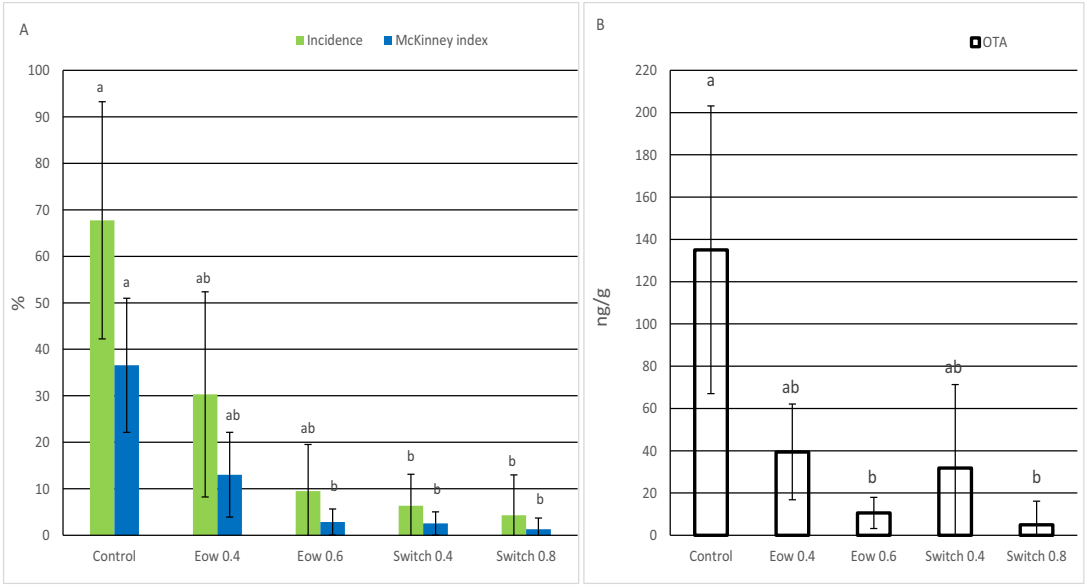

Fig. 2. *Aspergillus carbonarius* incidence rate and McKinney disease index (A); OTA levels (B), detected on Primitivo berries, contaminated *in vitro* with *A. carbonarius* ITEM 7444 and treated with EOW or Switch®. Means of three years survey are shown. Error bars indicate standard deviations. Different letters indicate results significant at the 0.02 level.

even if the grape bunches were contaminated by black Aspergilli and *A. carbonarius*, the climatic condition was not favourable for OTA production.

In the field trial of the 2018 growing season, due to the frequent rains during mid-August and the evident infestation of *Lobesia botrana*, as established, the third EOW treatment, 0.6 g/l active chlorine, was performed at pre-harvest (August 21st). The overall range of contamination in the field of black Aspergilli and *A. carbonarius* in particular was comparable to the previous year, respectively $10^{5–6}$ CFU/ml and $10^{3–5}$ CFU/ml (Fig. 3A).

As expected, OTA was detected in grape must samples from the 2018 trial, but despite the lower average level of OTA level found in treated grapes compared to the untreated control, the observed decrease in OTA contamination was not statistically significant ($p < 0.05$), due to the high variability between replicates (Fig. 3B).

4. Discussion

The control of bunch rot is based on good viticultural management practices, although they can often be ineffective when heavy rains occur from late veraison to harvest, resulting in OTA contamination in the final product. Preventing the growth of OTA-producing fungi is the most effective strategy to control the spread of this mycotoxin in grapes and derived products (Zhang et al., 2007). In this regard, the antifungal compounds mepanipyrim, pyrimethanil, fluazinam, iprodione, and cyprodinil/fludioxonil mixture have been reported to reduce both the growth of ochratoxigenic fungi and the levels of OTA in grape bunches (Visconti et al., 2008); being cyprodinil/fludioxonil mixture the most effective treatment in several field trials carried out in the Mediterranean region, namely France, Spain, Greece, and Italy (Bellí et al., 2007; Tjamos et al., 2004). The use of pesticides plays an important role in all agricultural production, but particular concerns have been raised regarding the risks and impacts on human health and the environment. The implementation of the maximum level of pesticide residues in food regulation has increased interest in alternative methods for disease control. This highlight the need to replace or reduce the use of synthetic chemical fungicide treatments with other environmentally and health-friendly methods to control toxigenic fungi at pre- and post-harvest stages. Among the alternative strategies available, biological control has been widely proposed as an alternative to reduce the impact of ochratoxigenic species (Zhang et al., 2016a). Yeasts, for example, have been investigated as potential biocontrol agents of black Aspergilli on grapes (Bleve et al., 2006; Dimakopoulou et al., 2008), but their use seems to be far from being implemented. EO water, an alternative, and cheaper method, recognised as an effective disinfectant, easy to use, and environmentally friendly (Huang et al., 2008), has turned his attention from the food industry to agriculture. It also has the advantage of a generally recognised safe status (GRAS), as well as its rapid on-site production which makes EOW an extremely viable antimicrobial agent. Previous studies have shown that EOW have been used effectively (Rahman et al., 2016) for the reduction of bacteria (Zhang et al., 2016b), yeasts (Zeng et al., 2011) or moulds (Buck et al., 2002; Suzuki et al., 2002) contamination, *in vitro* or in the field, on fruits and vegetables, without deleterious effects on the organoleptic properties of food. The EOW treatments had already been evaluated to: reduce the natural microbiota on radish seeds and sprouts (Zhang et al., 2016c), extend the shelf life of harvested blueberries (Chen et al., 2019), control *Colletotrichum fructicola, B. cinerea,* and *Monilia fructicola* on strawberries (Guentzel et al., 2011; Hirayama et al., 2016), and managing powdery mildew infection in cucumber (Fujiwara et al., 2009).

Post-harvest treatments of grapes with EOW (Cravero et al., 2016; Cravero et al., 2018; Guentzel et al., 2010) showed that properties and characteristics, such as skin hardness were not affected (Laureano et al., 2015) and sensory analysis after the end of fermentation of the grape must did not reveal any defects in the wine (Cravero et al., 2018).

Furthermore, EOW have proven effective in the reduction of pesticide residues (Hao et al., 2011; Qi et al., 2018) and the conversion of harmful toxins into less toxic compounds, such as aflatoxins (Escobedo-González et al., 2016).

Since the EOW used in this study was produced starting from KCl, its application in the vineyard should have no side effects, thus, avoiding problem-related to sodicity that could negatively impact the soil, affecting plant growth and crop yields.

In this study, the EOW fungicidal effectiveness has been mainly evaluated against the spores of *A. niger* and *A. carbonarius*, the main fungal species responsible for OTA contamination in the vineyard, highlighting different results in terms of required EOW concentration and time of exposure. An EOW with a concentration of 0.4–0.6 g/l of active chlorine, after 2 min of contact time, showed the complete fungicidal effect on the conidia of the three black Aspergilli strains studied.

Subsequently, the evaluation of the efficacy of EOW was carried out on berries contaminated with *A. carbonarius* ITEM 7444, the toughest of the tested fungi, for two different grape cultivars. The Primitivo variety was selected because it is widely cultivated in Southern Apulia and is known to be susceptible to contamination by *A. carbonarius* (Battilani et al., 2004), while the Aglianico variety is highly cultivated in the North of Apulia and susceptibility to *A. carbonarius* has not been studied. As

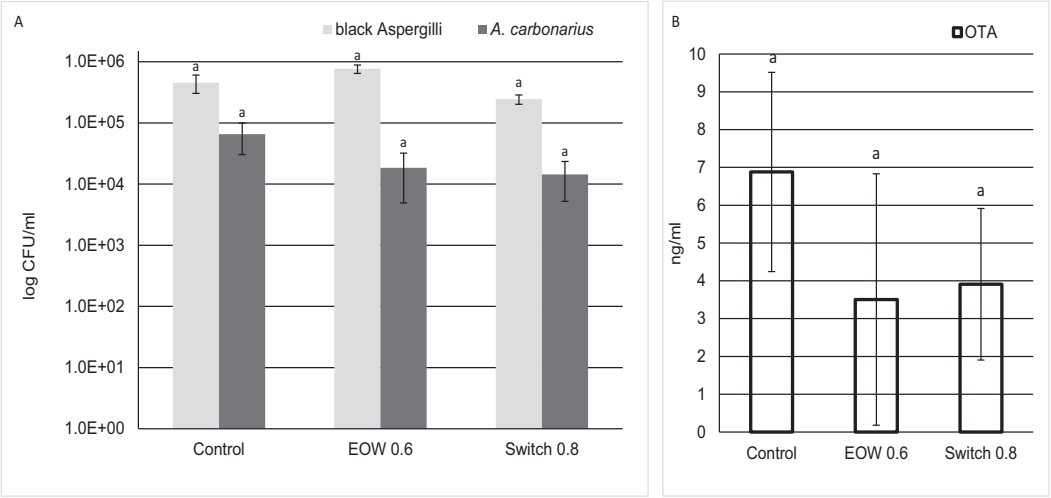

Fig. 3. Microbiological and chemical analysis of Primitivo must samples during 2018 vineyard treatments. In A, Black Aspergilli, and *A. carbonarius* contamination with standard error bars. In B, the OTA level with standard deviation bars. Same letters indicate that OTA levels were not statistically different at the 0.05 level.

Primitivo berries confirmed their weak resistance to contamination by *A. carbonarius* ITEM 7444, compared to Aglianico berries, and since we have noticed that most of the contamination originated from the peduncle area, hypothetically it would suggest that the Aglianico berries have a firmer peduncle junction compared to Primitivo berries.

Field trials were carried out with EOW and Switch®, a fungicide registered against black Aspergilli, in a Primitivo cultivar vineyard. The results obtained during *in vitro* tests, in fact, clearly suggested the possible effectiveness of this treatment in the field (Fig. 2).

During the first year of field trials, treatments with EOW 0.4 g/l and 0.6 g/l of active chlorine and Switch® 0.8 g/l, respectively, do not show a reduction in contamination by black Aspergilli and *A. carbonarius*, compared to the untreated grapes, moreover, no correlation with the level of toxins was possible as OTA was not detected in the samples of grape must.

Field trial treatments performed in 2018, EOW with 0.6 g/l of active chlorine and Switch® 0.8 g/l, showed a 3-fold reduction in mean *A. carbonarius* contamination, compared to the untreated grapes, not statistical (Fig. 3A). Even the 2-fold reduction in mean OTA values observed in both treated grapes was not statistically different from the untreated control in terms of OTA levels (Fig. 3B). The OTA contamination found in the 2018 field trials confirms previous evidence that the production of OTA on grapes in the field is mainly related/due to the contamination rate by *A. carbonarius* (Perrone et al., 2008).

Grape variety, geographic region, agricultural practices, and weather conditions, have a strong impact on the incidence of toxigenic fungi and, consequently, on toxin levels in grapes (Freire et al., 2017). For these reasons, the interpretation of the data of a field trial must take into account the fact that frequent variations of results obtained in different places during the same year or in the same place during different years, can occur.

5. Conclusions

Despite the predictable lack of statistical confidence between *in vitro* tests and field trials, the results of this study suggest that EOW efficacy is comparable to Switch® fungicide to control black Aspergilli infection and OTA contamination in the vineyard when applied with the right timing. It can therefore be suggested that EOW disinfection can alternatively replace conventional fungicides in the vineyard, reducing the problems generated by chemical residues, encouraging its use in organic farming procedures.

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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.

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