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Chlorine dioxide as an alternative disinfectant for disinfection of oyster mushroom growing media

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ABSTRACT

The aim of this study was to investigate the use of ClO_2 on disinfection of two different growing substrates commonly used in oyster mushroom cultivation. Cottonseed hull (CSH) and wheat straw (WS) were immersed for periods of 10, 20, 30 and 40 min in different ClO_2 concentrations (2, 4 and 8 mL/L). The control substrates were immersed in just water at the same times. Efficiency of treatments was evaluated for yield, biological efficiency (BE) and average mushroom weight. Yield and BEs ranged between 93.8–348.5 g/kg and 29.3–108.9% in CSH and 55.3–314.0 g/kg and 14.6–89.7% in WS, respectively. The highest yield and BE were obtained at 4 mL/L concentration of ClO_2 and 30 min immersion time in WS and at 4 mL/L concentration of ClO_2 and 20 min immersion time in CSH substrate. The results revealed that ClO_2 would be a viable and promising technique for disinfection of substrates for oyster mushroom cultivation.

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KEYWORDS
Cotton seed hull; immersion time; *Trichoderma* spp; wheat straw

Introduction

Pleurotus ostreatus (oyster mushroom) is the second most widely cultivated mushroom throughout the world (Royse, Baars, & Tan, 2017). They are able to colonise and degrade a large variety of lignocellulosic materials because of their mechanism of enzymatic lignocellulosic degradation. So, several wastes which are produced in agricultural, forest and food-processing industries are used for the preparation of oyster mushroom growing substrates.

It is well known that substrate is one of the most important contamination sources for disease agents such as *Trichoderma* spp., that prevent the growth of mushroom mycelium. While these agents compete with mushroom mycelium, moreover they secrete toxic substances and negatively affect mycelial growth (Vijay & Sohi, 1987). As a result, mushroom yield significantly is reduced. Moreover, green mould disease (*Trichoderma harzianum*) can cause in substantial losses in the production of cultivated mushrooms including champignon (*Agaricus bisporus*), shiitake (*Lentinula edodes*) and oyster mushroom (*Pleurotus ostreatus*) (Kredics et al., 2010). The disease was able to colonise compost and resulted in crop losses of 30–100% (Seaby, 1998). Therefore, before the mushroom production, effective disinfection of the materials to be used in growing media preparation is of great importance in terms of the success of oyster mushroom cultivation.

Various treatments are used for the preparation of substrate for mushroom cultivation to eliminate competitive fungi. There are some different procedures such as steam pasteurisation, hot-water immersion and chemical sterilisation for substrate disinfection (Atila, 2016; Jaramillo & Albertó, 2013). Although steam sterilisation is the ideal method for disinfection of growing media, the cost of establishing and implementing is high. The scalding method needs less laborious and energy expenditure, but it has some limiting factors such as limited pasteurising capacity and slow rate of pasteurisation. Chemical disinfection is usually done with formaldehyde and pesticides. Disinfection with formaldehyde is easy and cheap, but it can be a problem due to human and environmental health. Because of these problems, there is a great interest in alternative methods of substrate disinfection for mushroom cultivation.

Chlorine dioxide (ClO_2) is a yellow to reddish-yellow manufactured gas, when added to water, chlorine dioxide forms chlorite ion, which is also very reactive. It is a strong oxidising agent and has broad biocidal effectiveness. It has bactericidal, fungicidal and viricidal properties. ClO_2 gas also has strong penetrability and high dissolubility, which makes it promising for reducing the numbers of microorganisms on food or food contacting surfaces (Du, Han, & Linton, 2002). Its antimicrobial effects are primarily due to oxidative attack on the cell surface membrane proteins, including enzymes involved in transport (Jeng & Woodworth, 1990). Ingols and Ridenour (1948) suggested that the bactericidal effectiveness of ClO_2 is due to its adsorption on the cell

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wall with subsequent penetration into the cell where it reacts with enzymes containing sulfhydryl groups. Benarde, Snow, Olivieri, and Davidson (1967) demonstrated that ClO_2 abruptly inhibited protein synthesis. Subsequently, Olivieri (1968) reported a dose-response in the inhibition of protein synthesis in bacteria that had been treated with ClO_2. Food and Drug Administration (FDA) (1998) has allowed the use of aqueous ClO_2 in washing fruits and vegetables. Moreover, ClO_2 has been used as a drinking water treatment agent since 1944 and is a widely used bactericide (Aieta & Berg, 1986).

In literature is easy to find data for using of ClO_2 in managing diseases of mushroom. Szumigaj- Tarnowska, Ulinski, and Slusarski (2012) indicated ClO_2 revealed a high effectiveness in the control of *Pseudomonas tolaasii* causing brown blotch of the white button mushroom *Agaricus bisporus*. A similar result was observed by Royse and Wuest (1980) who reported that chlorinated water reduced bacterial reproduction on the surface of the mushroom. Morever, Oh, Kim, Kim, and Fermor (2000) reported that routine watering with sodium hypochlorite (active chlorine 5.7 mg/L) from mushroom initiation to the end of picking resulted in reduced *Pseudomonas tolaasii* causing bacterial blotch on the cultivated oyster mushroom incidence of 40–86%. However, to the best of our knowledge, there is no study about using ClO_2 on disinfection of mushroom growing substrate. This study investigated the disinfection possibilities of using ClO_2 in two different growing media commonly used in oyster mushroom cultivation. For this purpose, substrate samples were immersed for different periods and ClO_2 concentrations to determine the most effective concentration and disinfection time for the prevention of contamination during oyster mushroom production. In addition, the effects of different ClO_2 concentrations and immersion times on the yield parameters of oyster mushroom were determined.

Materials and method

Materials

HK-35 strain of Oyster mushroom (*Pleurotus ostreatus*) was selected to conduct the present study. The mushroom spawn was obtained from Sylvan Inc (Izmit, Turkey). Wheat straw (WS) and cottonseed hull (CSH) were obtained from local markets (Kirsehir, Turkey). ClO_2 used in this study was supplied from Chemya Co. Inc, Istanbul, Turkey. The commercial liquid ClO_2solution with a concentration of 1,000 mg/L was diluted in the different concentrations.

Experimental design

The study was conducted at the laboratory of the department of Horticulture, Ahi Evran University, Kırsehir, Turkey. Different concentrations of ClO_2 (0, 2, 4, 8 mL/L) and immersion times (10, 20, 30 and 40 min) were tested to determine the effectiveness of ClO_2 in the disinfection of WS and CSH substrates commonly used in oyster mushroom cultivation. The experiments were carried out separately for both substrates and organised by a completely randomised plot design, with 10 replications.

Preparation of substrates

Two different growing media, WS and CSH, were used in the study to determine the efficiency of ClO_2 on disinfection of oyster mushroom growing media. WS was broken into pieces of 1–2 cm before disinfection, while CSH was used directly without shredding. Then, the substrates were immersed in prepared ClO_2 solutions for 10, 20, 30 and 40 min. The control substrates were immersed in just water at the same times. Excess water was drained out until the moisture content was brought to 65–70%. Polyethylene bags were filled 1 kg disinfected substrate and inoculated with approximately 30 g (3% w/w) of spawn using surface spawning technique (Atila, 2017). Some holes (8–10) were punched on the sides of the plastic bags to facilitate ventilation on spawn running period.

The bags were incubated at 25 ± 2°C in the dark for the spawn running. After full colonisation temperature and humidity were changed to 17 ± 2°C and 85%, light provided 12 h/day using fluorescent light. Sufficient air changes were maintained to hold CO_2 concentration below 1,000 ppm. Mushrooms were harvested when the in-rolled margins of the basidiomes began to flatten.

Evaluation of the cultivation parameters

The following cultivation parameters were recorded; number of the contaminated bags, spawn running time (d), time to first primordia initiation (d), time to first harvest (d) yield (g/kg), biological efficiency (BE%) and average mushroom weight (g).

Spawn running time defined as the number of days from inoculation to complete colonisation of the substrate by the mycelium. Time to first primordia initiation defined as the number of days from inoculation to appear tiny fruit bodies of mushroom with a size of greater than (0.01 cm). Total yield was calculated as the sum of three flushes and expressed as grams of fresh mushrooms harvested per gram of wet growing medium (w/w). The biological efficiency (BE%) is defined as the percentage ratio of dry substrate to the fresh weight of harvested mushroom using the formula;

BE (%) = (fresh weight of harvested mushroom per bag/dry weight of substrate per bag) x100 (Royse, 1985). The total fresh weight of fruitbodies divided by their number to determine the average mushroom weight (AMW).

Statistical analysis

All statistical analyses were performed via SPSS 16.0 software. For the substrates disinfected by different treatments, a two-way ANOVA was used to test the significance of the disinfection treatment and of the growing medium as well as their interaction on yield parameters. The significant interactions were further examined for the main disinfection treatment effects, i.e. the effect of each treatment separately for each growing media. Differences between the means of individual groups were assessed via Tukey's test at a significance level of 5%.

Results

Effect of treatments on the number of contaminated bags

The different disinfection treatments on WS and CSH substrates led to considerable differences in the number of contaminated bags ($p< 0.01$) (Table 1). The mycelium failed to colonise the control substrates of (WS) immersed only in water for different durations due to contamination with green mould (*Trichoderma* spp.), *Aspergillus* spp. and bacterial growth. Similarly, the mycelium colonised substrates disinfected by treatment with C2T10, C4T10 and C8T10, all the bags with these treated substrates were contaminated with green mould at the later stages of production. Mycelial colonisation failed on nine, six and six bags of WS substrate disinfected at 2 mL/L concentration of ClO_2 with 20, 30 and 40 min immersion time, respectively. Moreover, the C8T20, C8T30 and C4T30 treatments exhibited no contamination (0 bags), which did not differ significantly from C4T40 and C8T40 (1 bag).

Although there was no contamination on the CSH substrates disinfected by the C4T30, C4T40 and C8T20, C8T30, C8T40 treatments, and only one bag with the C4T20 treatment was contaminated Green mould infection was observed during mycelial colonisation or before pinhead stage with the control treatments, CSH substrates disinfected at 2, 4 and 8 mL/L concentrations with 10 min immersion time. The contaminated bags were removed from the production room in order to prevent the contamination of healthy bags.

Effect of treatments on spawn running time, time to first primordia and first harvest

Significant differences were observed among the treatments regarding spawn running time, time to first primordia and time to first harvest in the (WS) and CSH substrates ($p< 0.01$) (Table 1). The substrate

Table 1. Effect of disinfection treatments on the number of contaminated bags, spawn running time, days to primordia initiation and days to first harvest of mushroom grown on wheat straw (WS) and cottonseed hull (CSH) substrates.

Substrate	Concentarion (mL/L)	Immersion time (min)	Treatment codes	Number of contaminated bags	Spawn running time (days)	Days to primordia initiation (days)	Days to first harvest (days)
WS	**2**	**20**	**C2T10**	10 ^{a}	31.6 ± 0.6 ^{a**}	No data	No data
	4	**30**	**C4T10**	10 ^{a}	30.2 ± 0.8 ^{b}	No data	No data
	8	**40**	**C8T10**	10 ^{a}	27.2 ± 0.8 ^{c}	No data	No data
	2	**20**	**C2T20**	9 ^{a}	27.6 ± 0.5 ^{c}	34.4 ± 0.9 ^{a**}	37.8 ± 0.5 ^{a**}
	4	**30**	**C4T20**	2 ^{c}	25.8 ± 0.4 ^{d}	31.2 ± 0.4 ^{cd}	35.4 ± 0.5 ^{c}
	8	**40**	**C8T20**	0 ^{c}	24.0 ± 0.7 ^{e}	30.8 ± 0.8 ^{d}	34.2 ± 0.4 ^{d}
	2	**20**	**C2T30**	6 ^{c}	26.8 ± 0.8 ^{cd}	32.2 ± 0.8 ^{bc}	37.2 ± 0.5 ^{b}
	4	**30**	**C4T30**	0 ^{c}	20.2 ± 0.8 ^{g}	23.6 ± 0.5 ^{e}	27.4 ± 0.5 ^{e}
	8	**40**	**C8T30**	0 ^{c}	21.6 ± 0.5 ^{f}	24.6 ± 0.5 ^{e}	27.8 ± 0.8 ^{e}
	2	**20**	**C2T40**	6 ^{b}	26.4 ± 0.5 ^{cd}	33.0 ± 0.7 ^{b}	36.8 ± 0.4 ^{b}
	4	**30**	**C4T40**	1 ^{c}	20.8 ± 0.8 ^{fg}	24.4 ± 0.5 ^{e}	27.0 ± 0.7 ^{e}
	8	**40**	**C8T40**	1 ^{c}	20.6 ± 0.5 ^{fg}	24.2 ± 0.4 ^{e}	27.4 ± 0.5 ^{e}
CSH	**0**	**10**	**C0T10**	10 ^{a}	33.2 ± 1.9 ^{a}	No data	No data
	2	**20**	**C2T10**	10 ^{a}	30.0 ± 0.7 ^{b}	No data	No data
	4	**30**	**C4T10**	10 ^{a}	27.4 ± 0.5 ^{c}	No data	No data
	8	**40**	**C8T10**	10 ^{a}	27.8 ± 0.4 ^{c}	No data	No data
	0	**10**	**C0T20**	10 ^{a}	33.4 ± 1.1 ^{a}	No data	No data
	2	**20**	**C2T20**	7 ^{b}	27.2 ± 0.8 ^{c}	35.6 ± 0.5 ^{a}	40.2 ± 0.8 ^{a}
	4	**30**	**C4T20**	1 ^{d}	24.2 ± 0.4 ^{d}	28.6 ± 0.5 ^{c}	32.6 ± 0.9 ^{b}
	8	**40**	**C8T20**	0 ^{d}	22.4 ± 0.9 ^{d}	25.8 ± 1.1 ^{e}	29.0 ± 0.7 ^{d}
	0	**10**	**C0T30**	10 ^{a}	33.6 ± 0.5 ^{a}	No data	No data
	2	**20**	**C2T30**	4 ^{c}	26.8 ± 0.8 ^{c}	35.6 ± 0.5 ^{a}	41.4 ± 0.5 ^{a}
	4	**30**	**C4T30**	0 ^{d}	23.6 ± 0.5 ^{d}	27.4 ± 0.5 ^{cd}	30.6 ± 0.5 ^{c}
	8	**40**	**C8T30**	0 ^{d}	23.6 ± 1.5 ^{d}	26.4 ± 1.1 ^{de}	30.2 ± 0.4 ^{cd}
	0	**10**	**C0T40**	10 ^{a}	34.4 ± 0.9 ^{a}	No data	No data
	2	**20**	**C2T40**	3 ^{c}	27.2 ± 0.8 ^{c}	34.2 ± 0.4 ^{b}	40.6 ± 0.9 ^{a}
	4	**30**	**C4T40**	0 ^{d}	23.2 ± 0.8 ^{d}	27.0 ± 0.7 ^{de}	30.2 ± 0.4 ^{cd}
	8	**40**	**C8T40**	0 ^{d}	23.4 ± 0.5 ^{d}	26.2 ± 0.8 ^{de}	29.4 ± 1.1 ^{cd}

Asterisks indicate significance at *P <0.05, **P <0.01, ^{ns} not significant; values within the same column followed by the same letter are not significantly different. by Tukey's test.

Table 2. Effect of disinfection treatments on yield, biological efficiency and average mushroom weight of mushroom grown on wheat straw (WS) and cottonseed hull (CSH) substrates.

Substrates	Concentarion (mL/L)	Immersion time (min)	Tratment codes	Yield			Total Yield (g/kg)	BE (%)	Average mushroom weight (g)
				Flush 1 (g)	Flush 2 (g)	Flush 3 (g)			
WS	**2**	**20**	**C2T20**	55.3 ^{h}	0.0 ^{f}	0.0 ^{d}	55.3 ± 17.0 ^{e}	14.6 ± 4.5 ^{f}	20.4 ± 0.7 ^{cd}
	4	**30**	**C4T20**	116.3 ^{g}	38.6 ^{cd}	47.2 ^{a}	202.1 ± 12.7 ^{c}	53.2 ± 3.3 ^{d}	26.1 ± 2.0 ^{a}
	8	**40**	**C8T20**	182.6 ^{d}	41.6 ^{c}	20.2 ^{c}	245.4 ± 8.5 ^{b}	64.6 ± 2.2 ^{c}	23.9 ± 2.1 ^{abc}
	2	**20**	**C2T30**	128.9 ^{e}	28.8 ^{e}	0.00 ^{d}	157.7 ± 19.1 ^{d}	45.0 ± 5.5 ^{e}	21.8 ± 2.7 ^{bcd}
	4	**30**	**C4T30**	241.6 ^{a}	49.6 ^{b}	22.7 ^{c}	314.0 ± 7.3 ^{a}	89.7 ± 2.1 ^{a}	24.4 ± 2.8 ^{ab}
	8	**40**	**C8T30**	226.3 ^{b}	35.7 ^{d}	30.7 ^{b}	292.8 ± 14.3 ^{a}	83.7 ± 4.1 ^{ab}	25.2 ± 1.8 ^{ab}
	2	**20**	**C2T40**	158.7 ^{e}	25.6 ^{e}	0.00 ^{d}	184.3 ± 18.1 ^{c}	52.7 ± 5.2 ^{d}	20.2 ± 2.3 ^{d}
	4	**30**	**C4T40**	226.3 ^{b}	35.7 ^{d}	30.7 ^{b}	292.7 ± 9.4 ^{a}	83.6 ± 2.7 ^{ab}	26.5 ± 1.8 ^{a}
	8	**40**	**C8T40**	198.4 ^{c}	59.9 ^{a}	32.1 ^{b}	290.4 ± 14.6 ^{a}	83.0 ± 4.2 ^{b}	24.7 ± 2.5 ^{ab}
CSH	**2**	**20**	**C2T20**	93.8 ^{f}	0.0 ^{e}	0.0 ^{g}	93.8 ± 14.0 ^{e}	29.3 ± 4.4 ^{e}	21.2 ± 2.3 ^{c}
	4	**30**	**C4T20**	210.3 ^{a}	79.3 ^{b}	58.9 ^{a}	348.5 ± 14.4 ^{a}	116.2 ± 4.3 ^{a}	24.9 ± 2.2 ^{ab}
	8	**40**	**C8T20**	188.6 ^{c}	75.5 ^{b}	58.3 ^{a}	322.4 ± 11.7 ^{ab}	100.8 ± 3.7 ^{b}	26.9 ± 1.1 ^{a}
	2	**20**	**C2T30**	145.3 ^{e}	49.6 ^{d}	21.4 ^{f}	216.3 ± 5.6 ^{d}	69.8 ± 1.8 ^{d}	24.6 ± 2.1 ^{ab}
	4	**30**	**C4T30**	195.3 ^{bc}	90.5 ^{a}	41.8 ^{d}	345.0 ± 25.9 ^{a}	115.0 ± 7.7 ^{a}	27.2 ± 1.3 ^{a}
	8	**40**	**C8T30**	204.1 ^{ab}	61.6 ^{c}	49.2 ^{bc}	314.9 ± 9.2 ^{b}	101.6 ± 3.0 ^{b}	27.8 ± 1.4 ^{a}
	2	**20**	**C2T40**	168.3 ^{d}	55.3 ^{cd}	33.0 ^{e}	256.5 ± 27.6 ^{c}	85.5 ± 8.2 ^{c}	23.6 ± 2.0 ^{bc}
	4	**30**	**C4T40**	201.9 ^{ab}	90.9 ^{a}	52.2 ^{b}	327.6 ± 8.9 ^{ab}	105.7 ± 2.6 ^{b}	27.2 ± 2.0 ^{a}
	8	**40**	**C8T40**	168.9 ^{d}	97.5 ^{a}	45.7 ^{cd}	312.1 ± 6.8 ^{b}	104.0 ± 3.2 ^{b}	27.6 ± 2.4 ^{a}

Asterisks indicate significance at *P <0.05, **P <0.01, ^{ns} not significant; values within the same column followed by the same letter are not significantly different. by Tukey's test.

pre-treatment had varying effects on the spawn running time, ranging from 20.2 d (C4T30) to 31.6 d (C2T10) for WS substrate. The time taken to colonise the substrate disinfected by C2T10 was significantly longer than that taken to colonise the substrates disinfected at the same concentration by immersion for 20, 30 and 40 min. The time to the appearance of primordia for the treatments varied from 23.6 d (C4T30) to 34.4 d (C2T20) on the WS substrate. The first flush started 27.0 d after the inoculation for the C4T40 treatment, followed by the C4T30, C8T30 and C8T40 treatments, while it started between 36.8 and 37.2 d after the inoculation on substrates disinfected using 2 mL/L concentration of ClO_2.

Full colonisation was completed between 22.4 and 34.4 d after inoculation on CSH substrates disinfected by different concentrations of ClO_2. Treatments with 2 mL/L concentrations of ClO_2 promoted slower colonisation than with the other two concentrations. Moreover, the slowest mycelial growth was observed in the treatments with 10 min immersion time; however, there was no difference between the treatments with 20, 30 and 40 min immersion time with all concentrations in terms of spawn running time. The time until the appearance of the primordia varied from 23.2 d to 27.4 d and from 22.4 d to 27.8 d in the substrates disinfected with 4 mL/L ClO_2 and 8 mL/L, respectively. The treatments with 2 mL/L exhibited a later primordia initiation (34.2–35.6 d). Primordia initiation occurred earlier with the 8 mL/L concentration (25.8–26.2 d), no difference was observed between immersion times at this concentration. The first flush started 29.0 d (C8T20)–41.4 d (C2T30) after the inoculation, depending on treatments.

Effect of treatments on yield, BE and average mushroom weight

Significant differences were found among treatments on WS substrate regarding yield, BE and average mushroom weight ($p < 0.01$) (Table 2). The total yield of *P. ostreatus* with the nine treatments varied between 55.3 g/kg and 314.0 g/kg substrate, while the BE varied between 14.6% and 89.7%. For all treatments except for the C2T20, the crops were harvested in three flushes, and the maximum yield was obtained in the first flush, followed by the second and third flushes. The production of the C2T20 treatment was terminated after the first harvest due to contamination. Although the yield increased with increasing immersion time at 2 mL/L concentration of ClO_2 on WS substrate, the results are quite low compared to the applications using a higher concentration. Moreover, according to the marked increase in *Trichoderma* spp. and bacterial growth observed during production cycle on the WS substrate disinfected at 2 mL/L concentration of ClO_2, it can be said that the 2 mL/L concentration of ClO_2 was not effective enough as a disinfection treatment on the WS substrate. Moreover, disinfection with 4 mL/L and 8 mL/L concentrations of ClO_2 was applied successfully on WS at 30 and 40 min immersion time. Average mushroom weight varied between 20.2 g (C2T40) and 26.5 g (C4T40), depending on the treatment.

The total yield (g), BE (%) and average mushroom weight of *P. ostreatus* cultivated on CSH substrate

disinfected by different treatments were affected by the treatments ($p < 0.01$), as shown in Table 2. The maximum total yield of 348.5 g/kg and BE of 116.2% was obtained from substrate disinfected by the C4T20, followed by the C4T40, C4T30 and C8T20 treatments. Although the lowest yield was obtained from the C2T20 treatment, disinfection with 2 mL/L significantly increased the yield performance of the CSH and the effectiveness of the disinfection increased when the immersion time was longer.

According these results, it can be said that the disinfection of wheat straw at 4 mL/L of ClO_2 for 30 min and disinfection of cotton seed hull substrate at immersion in a solution of 4 mL/L of ClO_2 for 20 min would be a viable and promising technique for disinfection of substrates for mushroom cultivation.

The crops for all treatments were harvested in three flushes, and the maximum yield was obtained in the first flush, except for the C2T20 treatment, which was terminated after the first harvest due to contamination. Average weights of fruitbodies grown on CSH substrate disinfected by different treatments ranged from 21.2 g to 27.8 g. The fruitbodies with the highest weight were taken from the C8T30 treatment group, while the lightest fruitbodies were harvested from those with the C2T20 treatment.

The effects of the ClO_2 treatments, the substrates and their interaction on the times to complete spawn-run, primordial initiation, and first flush, and yield, BE and average mushroom weight were all highly significant ($P \leq 0.003$).

Discussion

Green mould competes with the mushrooms for space and nutrients as well as causing chemical alteration of the substrate, which hinders mushroom development (Chang & Miles, 1989). According to the results of the study, substrate disinfection with ClO_2 made it possible to reduce disease agents *Trichoderma* sp. on the mushroom growing substrates. But, the presence of bacterial and fungal growth in the disinfected samples indicated the resistance of these bacteria and fungus to low ClO_2 concentrations and immersion times such as 2 mL/L ClO_2 and 10 min. Han, Floros, Linton, Nielsen, and Nelson (2001) reported that the factors influencing the antimicrobial effectiveness of ClO_2 were its concentration and the contact time. Li, Zhang, Li, Li, and Sun (2017) reported that the spawn run time of *P. ostreatus* was 28.8 d on CSH substrate sterilised by autoclave, while Oseni, Dlamini, Earnshaw, and Masarirambi (2012) confirmed that this varied between 36 and 64 d in different growing media sterilised by autoclave or disinfected by hot-water immersion at 60°C in a steel drum. Although Ali et al. (2007) reported that the steam pasteurisation technique required shorter time to complete mycelial growth than chemical treatment, the data from the present study showed that ClO_2 did not have a prolonged influence on the spawn running time of *P. ostreatus*. The findings of the current study are similar or shorter than those of previous studies.

Substrate pretreatment methods had significant effects of on the average yield, BE and average mushroom weight of oyster mushrooms. Atila (2016) reported that the yield and BE of oyster mushrooms grown on substrates disinfected via different methods were 136.1–271.3 g/kg and 46.9–92.0%, respectively – close to the values of the current study. Moreover, the yields in this study are consistent with the results obtained in previous disinfection experiments with the *Pleurotus* spp. (Dinesh, Babu, & Manasa, 2013; Ficor et al., 2006; Yadav, Mishra, & Singh, 2014).

The absence of disease agents is not the only target point for substrate disinfection. The chemicals used must not damage mycelial growth, yield or quality. No inhibitory effect on mycelial growth was observed at the 2, 4 and 8 mL/L concentrations. Conversely, the decrease of spawn running time and increase of yield in both the 4 and 8 mL/L concentrations of ClO_2 and 30 and 40 min immersion times can be clearly observed, showing that these concentrations provide better disinfection.

Results obtained by ClO_2 as a disinfectant were not consistent among substrates although the application methodology was the same. The highest yield and BE were obtained on CSH and WS substrates disinfected by different treatments. it is highly remarkable that the time of treatment for disinfection could be reduced to only 20 min with the use of the CSH. Oseni et al. (2012) also reported that horse manure compost bags pasteurised for either 2 or 3 h were heavily contaminated by green mould, whereas sugarcane bagasse pasteurised for 3 h exhibited high yield and low contamination. Since ClO_2 concentration and application time were the same in the two substrates, the differences in the yield of WS and CSH could be due to their physical and chemical structure. The advantages of using CSH as a substrate have already been reported elsewhere for the cultivation of some mushroom species such as *P. ostreatus* (He, Bao, Wen, & Lu, 1995), *Hericium americanum* (Atila, Tuzel, Cano, & Fernandez, 2017), *Hericium erinaceus* (Atila & Tuzel, 2016).

The mushroom yield with the other treatments was also significantly different among the flushes. More than 60% of the total fruiting bodies were obtained in the first harvest, while the second and third mushroom harvests were of lower yield. The comparison of the results of these treatments according to the two substrates showed that the yield and BE were higher in addition to the percentage of infection obtained during the production cycle being lowered. With both substrates, the highest percent of mushrooms were harvested at

the beginning of the period, in the first flush. In the case of mushrooms grown on substrate disinfected with the C2T20 treatment, only the first flush represented both of the substrates. The competitive microorganisms inhibited the mushroom yield on the next two flushes because of insufficient disinfectionPhilippoussis, Diamantopoulou, and Israilides (2007) reported that the highest BE and the heaviest fruiting bodies were obtained on the same growing medium. Therefore, it is not surprising that the highest average mushroom weights were obtained from substrate disinfected with 4 and 8 mL/L ClO_2. Onyango, Palapala, Arama, Wagai, and Gichimu (2011) reported that large-sized fruit bodies were considered to be of good quality and rated highly in mushroom production.

According to the results of the present study, it is possible to destroy disease agents by ClO_2 if sufficient time is given for disinfection. This method can be adopted to produce a good oyster mushroom yield in rural areas where autoclave sterilisation or hot-water scalding may not be feasible. But, further research is needed to test the disinfection performance of the ClO_2 for different substrates supplemented with different nitrogen sources. After that, results obtained from the study could be more effectively transferred to the mushroom industry.

Disclosure statement

No potential conflict of interest was reported by the author.

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