Efficacy of environmentally friendly disinfectants against the main postharvest pathogen of stone fruits on plastic and wood surfaces

Abstract

Surface facilities disinfection during postharvest handling operation is an important practice to avoid secondary fruit infections at stone fruits packinghouses. The aim of this work was evaluates the effect of six disinfectants environmentally friendly against to *Monilinia fructicola*, *Penicillium expansum*, *Rhizopus* spp. and *Alternaria* spp. on plastic and wood surfaces. Hydrogen peroxide, peracetic acid, sodium hypochlorite, Mico-E-pro®, Proallium FRD-N® and DMC Clean-CNS® were used as disinfectants. Untreated and treated surfaces with water were used as controls. Plastic and wood surfaces were sampled with Rodac plates at 2 and 24 hours after treatments and the number of colonies were counted. In general, all disinfectants reduce the number of viable conidia from all studied surfaces. Hydrogen peroxide used in a concentration of 150 mg L^{-1} was less effective disinfectant in all studied pathogens. The commercial product Mico-E-pro® composed by oregano, onion and orange extract at dose of 10 mg L^{-1} was the most effective disinfectant. *Rhizopus* spp. was the pathogen more resistant to disinfectant followed by *P. expansum*. *M. fructicola* and *Alternaria* spp. Water decreased the number of conidia adhered to surfaces. In addition, the untreated control showed substantial conidia reduction after 24 h of artificially inoculation.

Keywords: antifungal, *Monilinia* spp., *Penicillium* spp., *Rhizopus* spp., *Alternaria* spp.

INTRODUCTION

Infections by postharvest fungi in stone fruits can occur during crop blossom, at harvest or during handling operation. In Mediterranean countries, the main postharvest diseases of stone fruits are brown rot caused by *Monilinia fructicola* and *Monilinia laxa*, and Rhizopus rot caused by *Rhizopus stolonifer*. Other minor pathogens are blue mold caused by *Penicillium expansum* and black rot caused by *Alternaria alternata*. However, fungal infections are reported to have a greater ability to infect a broader range of host throughout the whole postharvest chain (Bautista-Baños, 2014).

Whether infection occurs in orchard or in packinghouse, rot symptoms are mainly developing during storage and transportation (Hong et al., 1997). So, fruits rot in the bin may sporulate and conidia often contaminate the surfaces of bins and packinghouses facilities (Spotts and Cervantes, 1969). These conidia may survive for a long period of time and serve as a source of new inoculation for healthy fruits.

The current way to control postharvest losses is using conventional fungicides at field or in postharvest to reduce conidia infection. In the Ebro Valley (Spain) peaches and nectarine orchards, fungicides are usually applied between three to five times during each growing season (Usall et al., 2010). Tebuconazole, iprodione, cyproconazole and fenbuconazole are the systemic fungicides commonly employed to control postharvest disease such Rhizopus rot and brown rot in peaches (Egüen et al., 2016; Malandrakis et al., 2012; Miessner and Stammler, 2010). In postharvest, the use of fungicides in Spain and other EU countries is limited and only fludioxonil (MAPAMA, 2015) and pirimetanil (MAPAMA, 2017) are allowed to use. The applications of synthetic fungicides are restricted because of consumers concern for human health conditions, the undesirable effects on the environment, and the development of fungicide-resistant strain that have necessitated the search for alternative methods for controlling postharvest decay (Usall et al., 2015; Mari et al., 2014).

Measures adopted to reduce the level of inoculums present on the fruits and bins surface can contribute to disease control. Therefore, effective sanitation practices are needed to minimize the amount of inoculums available in packinghouses facilities (Bancroft et al., 1984; Smilanick et al., 2013). Nowadays, chlorine or hypochlorite is commonly employed aqueous sanitizer used in packinghouses to disinfect fruits when arrived from field and also to clean surfaces of bins or facilities. It is commonly used because is cheap and effective to kill propagules of pathogens but their effectiveness is influenced by water ph and decrease with organic matter (e.g. fruit, soil) which mean a constant monitoring chlorine solution (Feliziani et al., 2016a).

The objective of the present study was to evaluate the effect of six disinfectants environmentally friendly against to *Monilinia fructicola*, *Rhizopus* spp., *Penicillium expansum* and *Alternaria* spp. on plastic and wood surfaces.

MATERIALS AND METHODS

Disinfectant products

Hydrogen peroxide, sodium hypochlorite, peracetic acid, DMC Clean-CNS®, Mico-E-pro® and Proallium FRD-N® were used as disinfectants. Untreated and treated bins with drinking water were used as controls. The hydrogen peroxide 33% (w/v) stabilized (Panreac Química, S.A.U., Barcelona, Spain) was used in a concentration of 150 mg L^{-1}, sodium hypochlorite 10% (w/v) (Panreac Química, S.A.U., Barcelona, Spain) of 200 mg L^{-1} and Proxitane® 5:23 (Solvay Chemicals, Barcelona, Spain) was used as a PAA-based product at 300 mg L^{-1}. Proxitane® 5:23 is a stabilized mixtures of 5% peracetic acid, 23% hydrogen peroxide and 10% acetic acid. Commercial products such as DMC

Clean-CNS® (DOMCA, S.A., Granada, Spain) composed by ascorbic acid, citric acid and sodium lactate and citric flavors was used at 0.8 mg L^{-1}, Proallium FRD-N ® (DOMCA, S.A., Granada, Spain) composed by organic acids (citric acid, ascorbic acid, lactic acid) and hydro-alcoholic solution flavors of *Allium* spp. at 10000 mg L^{-1} and Mico-E-pro® (DOMCA, S.A., Granada, Spain) composed by oregano, onion and orange extract at 10 mg L^{-1} were tested as recommended by the manufacturer.

Pathogen culture and preparation of spores suspension

Fungal strains of *Monilinia fructicola*, *Penicillium expansum*, *Rhizopus* spp. and *Alternaria* spp. were isolated from decayed stone fruits in Lleida and identify by the Postharvest Pathology Group, IRTA (Catalonia). The strains were maintained on 50% glycerol at -20 ºC in darkness.

The four strains were sub-cultured twice onto potato dextrose agar (PDA) medium (Biokar Diagnostics, 39g L^{-1}) and incubated in the dark at 25 ºC for approximately 1 week. Conidia from PDA dishes were scarped with a sterile loop and transferred to a test tube with 20 ml sterile distilled water added with one droplet of 80% tween. Conidial concentration for each strain was measured with a hemocytometer and the suspension diluted to the desired concentration.

Evaluation of disinfectants

Prior experiment, plastic surfaces were disinfected immersing plastic slice in water containing 20% of commercial bleach during 10 min and wood slices were sterilized in the autoclave. After those processes, a sampling was done in order to know that surfaces are cleaned.

Those pieces of plastic and wood surfaces were submerged during 30 seconds in 2 liters of water with 10^4conidia ml^{-1} of the desired pathogen and were left to dry. The pieces of the surfaces previously infected by the corresponding pathogen were treated submerging in 2 liters of disinfectant product or drinking water during 30 second at the concentration described above. Surfaces of wood and plastic were sampled with 5.5 cm diameter Rodac plates (Replicate Organism Direct Agar Contact) containing PDA medium by contact between the culture medium and the surface, with slight pressure applied to keep spores adhering to the medium. Sampling was done in two moments, at 2 hours (after dry surfaces) and 24 hours after treatment. Then, Rodac plates were incubated at 20 ºC during 3 days. Three replicates were used for each treatment, pathogen, and surface material and sampling time. The number of colony forming units (cfu) per Rodac plates was counted. The experiment was performed twice.

Statistical analysis

Statistical analysis was performed using Statistix 10 (Analytical software, 2013). First analysis of variance (ANOVA) was carried out to found differences between experiments but not statistical differences were found to both experiments for each pathogen, therefore results were analyzed together. To test an appropriated ANOVA, homogeneity of variance was tested by Bartlett's test and normality was tested by Shapiro-Wilk's test. Factorial analysis of variance was performed with the number of colony-forming units (cfu) per Rodac of each pathogen as dependent factor and with treatment, material and time as independent factors. Means were compared using the Tukey's test at the level $P<0.05$. Two interactions between dependent factors were performed.

RESULT

A statistically analysis of four pathogens tested in the experiment were analyses together with pathogen, treatment, material and time as factors (Table 1) to know the level of signification of each individual studied factor and their double interaction.

Table 1. Analysis of variance of *Monilinia fructicola*, *Penicillium expansum*, *Rhizopus* spp. and *Alternaria* spp. (Pathogen) in relation to disinfectant treatments (Treatment), material surfaces (Material) and sample time (Time) two-way interactions on the percentage of colony-forming units (cfu) per Rodac plates. Note: % SS (percentage of sum of square); * Significant (P < 0.05); NS (not significant).

Factor	df	% SS	P>F
Pathogen	3	0,48	0.0002*
Treatment	7	57,73	<.0001*
Material	1	0,01	0.6418NS
Time	1	14,53	<.0001*
Pathogen x Treatment	21	2,28	<.0001*
Pathogen x Material	3	0,25	0.0163*
Pathogen x Time	3	0,52	<.0001*
Treatment x Material	7	1,34	<.0001*
Treatment x Time	7	22,87	<.0001*

Differences between pathogen were found and statistical test show that *M. fructicola* was the pathogen more sensible and *Penicillium* spp. and *Rhizopus* spp. the most resistant. The disinfectant more effective was Mico E-pro and the less effective was hydrogen peroxide to all studied pathogens. No statistical differences between plastic and wood surfaces were found.

Effect of disinfectants against *Monilinia fructicola*

Monilinia fructicola conidia were controlled with all treatments tested both plastic and wood surfaces in the sampling at 2 hours (Figure 1 A) "[insert Figure 1.]". The most effective disinfectants on plastic were sodium hypochlorite, Mico E-pro®, and

Proallium® decreasing from 27 cfu sampled on untreated surfaces to less than 4 cfu. On wood surfaces, the most effective disinfectants on wood were peracetic acid, sodium hypochlorite, Mico E-pro®, Proallium® and DMC Clean -CNS® decreasing from 146 cfu on untreated surfaces to 2.5, 3.5, 0.3, 1.3 and 4.5 cfu respectively. Significant differences between viable conidia on plastic and wood surfaces were found on untreated and on surfaces treated with water and hydrogen peroxide.

After 24 hours of treatment, on untreated surfaces, *M. fructicola* conidia decrease from 27 cfu and 146 cfu in the sampling at 2 hours to 9 cfu and 13 cfu in the sampling at 24 hours on plastic and wood surfaces respectively (Figure 1 A and B).

After 24 hours from treatments and on plastic surfaces, the most effective disinfectant were peracetic acid, sodium hypochlorite, Mico E-pro®, Proallium® and DMC Clean-CNS® decreasing conidia population below 2.5 cfu (Figure 1 B). However, on wood surfaces all treatments except water reduce *M. fructicola* conidia. In untreated and DMC Clean-CNS® treatment at 24 hours *M. fructicola* survived better on wood than on plastic surfaces and with hydrogen peroxide better on plastic than on wood.

Effect of disinfectants against *Penicillium expansum*

In the sampling time at 2 hours after treatment, *Penicillium expansum* conidia were reduced with all disinfectants tested for both surfaces to except when wood surfaces were treated with water and hydrogen peroxide (Figure 2 A) "[insert Figure 2.]". Conidia were totally controlled from 96 and 70 cfu on untreated plastic and wood surfaces respectively when surfaces were treated with Mico E-pro®. In water treatment, *P. expansum* survived better in wood than in plastic surfaces.

After 24 hours of treatment, on untreated surfaces, *P. expansum* conidia decrease from 96 cfu and 70 cfu in the sampling at 2 hours to 11 cfu and 31 cfu in the sampling at 24 hours on plastic and wood surfaces respectively (Figure 2 A and B).

On the sampling 24 hours after treatments, the most effective disinfectants were Mico E-pro®, Proallium® and DMC Clean-CNS® both on wood surfaces and on plastic surfaces. All treatments were effective except hydrogen peroxide disinfectant when is compared with untreated surfaces (Figure 2 B). Significant differences were found between plastic and wood surfaces for untreated, water, hydrogen peroxide, Mico E-pro® and Proallium® treatments.

Effect of disinfectants against *Rhizopus* spp.

Rhizopus spp. conidia were reduced with all treatments tested on plastic surfaces at 2 hours (Figure 3 A) "[insert Figure 3.]". *Rhizopus* spp. decreased from 73.5 cfu on untreated surfaces to 6.7 and 11.5 cfu on Mico E-pro® and Proallium® respectively the most effective disinfectants. On wood surfaces, *Rhizopus* spp. conidia were reduced with all treatments tested except when was treated with water since no differences with the untreated surfaces were found.

After 24 hours of treatment, on untreated surfaces, *Rhizopus* spp conidia decrease from 73.5 cfu and 87 cfu in the sampling at 2 hours to 17.7 cfu and 19 cfu in the sampling at 24 hours on plastic and wood surfaces respectively (Figure 3 A and B).

On the sampling 24 hours after treatments, all disinfectants tested on plastic surfaces were effective compared with the untreated surface. However, clean plastic surfaces with water were not an effective treatment for disinfection. In addition, effective disinfectant to wood surfaces were peracetic acid, sodium hypochlorite, Mico E-pro®, Proallium® and DMC Clean-CNS® with 0, 0.7, 1, 1 and 2 cfu recovered respectively

(Figure 3 B). Differences between both surfaces were found to hydrogen peroxide, sodium hypochlorite and Proallium®.

Effect of disinfectant against *Alternaria* spp.

Alternaria spp. conidia were reduced with all disinfectant tested both plastic and wood surfaces after 2 hours (Figure 4 A) "[insert Figure 4.]". The disinfectants more effective were hydrogen peroxide, peracetic acid, sodium hypochlorite, Mico E-pro®, Proallium® and DMC Clean-CNS® decreasing cfu under 1.4 on both surfaces. Water and hydrogen peroxide also were effective compared with untreated surfaces decreasing cfu although conidia recovered were higher than the other disinfectants. Differences between both surfaces were found on untreated and hydrogen peroxide treatment.

After 24 hours of treatment, on untreated surfaces, *Alternaria* spp. conidia decrease from 74.7 cfu and 107 cfu in the sampling at 2 hours to 25 cfu and 45.7 cfu in the sampling at 24 hours on plastic and wood surfaces respectively (Figure 4 A and B).

After 24 hours from treatment, all disinfectants tested were effective on both wood and plastic surfaces when all disinfectants except water reduced *Alternaria* spp. conidia. (Figure 4 B). Differences between cfu recovered on both surfaces were found on untreated surfaces.

DISCUSSION

The antifungal activity of natural products and their effects on postharvest pathogens in *in vitro* and *in vivo* conditions (Palou et al., 2016) and sanitizers of facilities contaminated with human pathogens (Gil and Allende, 2012) have been studied for many years. However, disinfection of packinghouses facilities have been less studied and most information we have is about citrus packinghouses and their main postharvest pathogen (Smilanick et al., 2013). In own knowledge, this is the first report that traditional as hydrogen peroxide, peracetic acid and sodium hypochlorite disinfectants and new environmental friendly commercial disinfectants as Mico-E-pro®, Proallium FRD-N® and DMC Clean-CNS® are tested against stone fruits postharvest pathogen on plastic and wood surfaces.

In general, hydrogen peroxide was the least effective disinfectant to all pathogen at both sampling time (except *Alternaria spp.* at 24 hours where hydrogen peroxide was effective). On the other hand, pathogens on plastic were more controlled with hydrogen peroxide at dose of 150 mg L^{-1} than in wood surfaces. Smilanick et al. (2013) showed that *Penicillium digitatum* was able to germinate at 100% when was exposed to 10 min to aqueous solution of hydrogen peroxide at dose of 500 mg L^{-1}. In addition, when dose increased to 2000 mg L^{-1} *P. digitatum* germination was reduced at 70%. Sisquella et al. (2013) tested on peaches artificially wounded and inoculated with *Monilinia fructicola* hydrogen peroxide with 1250 and 2500 mg L^{-1} without decay control. Hydrogen peroxide is an odorless, clear liquid and produces no residues since it is decomposed to water and oxygen therefore it is considered as GRAS (Generally Recognized As Safe) compound (Feliziani et al., 2016b; Moriello and Hondzo, 2014). The low effectiveness of hydrogen peroxide could be due to the low dose of 150 mg L^{-1} tested in our experiment. Despite is the dose used to disinfect packinghouses of our area, it is so low and should be increased to be more effective and control postharvest pathogens on surfaces. However, hydrogen peroxide is corrosive to skin and workers should take special precaution.

Peracetic acid (PAA) is produced from the reaction of acetic acid and hydrogen peroxide (Kitis, 2004). PAA reduces more than 90% conidia viability of *M. fructicola*, *P. expansum*, *Rhizopus* spp. and *Alternaria* spp. on plastic and wood surfaces. Sisquella et al. (2013) reported a reduction of 80% incidence of peach artificially infected with *M.*

fructicola when fruit were immersed for 1 min in 300 mg^{-1}L of peracetic solution. In addition, Mari et al (2004) observed reduction in the incidence on stone fruit wounded and inoculated with *Rhizopus stolonifer* treated for 1 min with 250 mg^{-1}L of PAA. The powerful antimicrobial action and the absence of toxic residuals of the PAA have led to a wide range of its application in food-processing and other industry (Kitis, 2004). Our results show that PAA is effective for surfaces disinfection and their effectiveness appears to be in a very short period of time because no differences between conidia sampling at 2 and 24 hours after treatment were detected.

Both sodium hypochlorite and other chlorine compounds are the most commonly employed sanitizers in the food industry. In the present study, sodium hypochlorite was an excellent disinfectant on surfaces infected with *M. fructicola*, *P. expansum* and *Alternaria* spp. instead to control *Rhizopus* spp. were no sufficient with 200 mg^{-1}L although conidia were reduced more than 80%. Rodney and Reymond (1994) reported least sensitive of *Botrytis cinerea* and *P. expansum* compared with *Mucor piriformis* and *Cryptosporiopsis perennans* when were treated with chlorine dioxide. In other study with *Penicillium digitatum*, Smilanick et al. (2002) reported to inactivate 95% of the conidia in a solution containing 200 mg^{-1}L free chlorine and at pH 8 was necessarily 19.1 seconds. This study also concludes that temperature has a marked influence on the rate of conidia mortality. Our experiment was carried out with tap water which is fairly basic with a pH around 8 and 15 ºC. Total chlorine is the sum of combined (chlorine that has reacted with other constituents) and free chlorine (chlorine that remains untreated in solution and is available in solution for disinfection) (Feliziani et al., 2016b) and it is influenced by water pH and the amount of organic matter present in the solution. Chlorine solution prepared from commercial bleach containing sodium hypochlorite was evaluated by Spotts and Peters (1980) in conidial germination presents in pears. The same study showed that chlorine used with a concentration of 50 mg L^{-1} significantly reduced conidial germination of *M. piriformis* and *P. expansum* after 30 seconds treatment although fruit decay was not controlled. Chlorine solutions were an effective sanitizing agent for bins but when it is used in high levels can cause respiratory discomfort in workers.

Proallium FRD-N® and DMC Clean-CNS® commercial products tested are mainly composed of organic acids (OA) and they are used to control food-borne pathogens but in our experiment products were tested again filamentous pathogens. Both commercial products are classified as GRAS and they are composed by citric acid and ascorbic acid. Differences are present in the lactic acid and *Allium* spp. flavors to Proallium and sodium lactate and citric flavors in DMC Clean-CNS®. OA generally refer to organic compounds that have acidic properties and it is commonly accepted that it is the toxic effect of OA components on the functionality and structure of the cell membrane (Sikkema et al., 1995). Proallium and DMC Clean reduced significantly conidia recovered from plastic and wood surfaces (more than 70% in all cases) and had a similar effectiveness against all studied pathogen. The antimicrobial components of citric acid volatiles (Caccioni et al., 1998; Tzortzakis and Economakis, 2007), ascorbic acid (Liu et al., 2014), lactic acid (Romanazzi et al., 2009) and sodium lactate (Palou et al., 2009) against postharvest pathogen in fruit have been widely studied. The flavor compounds are secondary metabolites having unique properties of volatility, and fat and low-water solubility. Being volatile, not very water soluble, and easily adsorbed, they are very useful in postharvest protection (Tripathi and Dubey, 2004). Proallium gives off a very strong odor due to the flavor compounds from *Allium* spp. species which makes it very annoying to workers and feasible on a commercial scale despite it is an effective disinfectant. On other hand, DMC Clean-CNS® is a powder marketed product and it is recommended to apply with hot water to make more effective the powder dissolution. This consideration could be a disadvantage in a commercial scale because of the difficulty of heating large quantities of hot water. Mico E-pro commercial products is composed by oregano, onion and orange extract and it is definitely the best disinfectant tested achieving an efficacy of 100% at 2 hours to all pathogens except to *Rhizopus* spp. achieving a reduction greater than 90%. After 24 h, *Rhizopus* spp. colonies were almost not recovered. Components and efficacy of oregano (Kocic-Tanackov et al., 2012), onion (Kocić-Tanackov et al., 2012) and orange (Caccioni et al., 1998) extract has been tested as antifungal and their results shown inhibition of fungi growth. Antifungal activity of compounds may be due to the severe damage to the fungal membranes and cell walls, which led to the morphological deformation, collapse and deterioration of the conidia (Neri et al., 2006). Mico E-pro is from natural origin, which means more safety to people and environment. No inconvenience as the smell was detected when working with this product making it, along with its highly effective, fully accessible for use on a commercial scale. Sharma and Tripathi (2006) tested the fungi toxicity *of Citrus sinensis* essential oil with the presence of 10 chemical different constituents and it was reported that when a product is made up for several components it is difficult for the pathogen to develop resistance to such mixture of components with apparently different mechanisms of antifungal activity. Therefore, Proallium, DMC Clean and Mico E-pro have to be considered at low risk for resistance development by postharvest pathogens.

Rhizopus spp. was the pathogen more resistant to disinfectants, followed by *P. expansum* and the most susceptible were *M. fructicola* and *Alternaria* spp. In general, only the fact of dipping surfaces with drinking water decreased number of colonies and all the disinfectants tested were more effective in pathogens on plastic than on wood surfaces in the sample time after 2 hours. Conidia viability of all pathogens was significantly reduced after 24 h in untreated samples both on plastic and wood surfaces and in laboratory conditions, which was not expected. Spotts and Cervantes (1969) reported a reduction of 100% of *P. expansum* conidia and 23% of *Alternaria alternata* conidia after 7 days of exposure of bins to sun in Oregon. In our study the experiment was carried out in late summer and plastic and wood surfaces were left in the laboratory, in a shady place and room temperature (20-25 ºC). We do not have a clear reason why conidia decrease drastically on untreated surfaces from 2 to 24 hours, but we attributed this effect to plastic and wood surfaces not provided a suitable place to adhered, germinate and infect conidia. Our results agree with Bernat et al. (2018) who showed that *M. fructicola* conidia viability decrease drastically after some hours at 20 and 30 ºC and 60% HR on inert surfaces.

Conidia viability could be higher if traces of organic matter were adhered to bins surfaces providing nutrients or simple a suitable environment for conidia survival.

Experiment was carried out on plastic and wood surfaces from a piece of bins but results regards plastic could be applied to other similar plastic surfaces in the packinghouses such as belts in handing lines and walls of cold chambers. Disinfection of bins and facilities is a prerequisite for postharvest control and their applicability depends on many aspects i.e. the length of the products storage, the characteristics of postharvest facilities, the possibilities to integrate the disinfection operation with other technologies and the know-how of the staff.

CONCLUSIONS

Effective commercial friendly disinfectants based on plant extract are an economically viable alternative to chemical disinfectants for the postharvest agricultural sector.

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Figure captions

Fig. 1. *Monilinia fructicola* recovered with Rodac plates from artificially inoculated plastic (■) and wood (□) surfaces treated with different treatments. Water and untreated treatments were used as control. Plastic and wood surfaces were sampled at 2h (A) and 24 hours (B) after treatment. Means with the same letter are not significantly different (P<0.05) according to Tukey test when were compared treatments to plastic or wood. * Means significant differences between plastic and wood surfaces for each treatment.

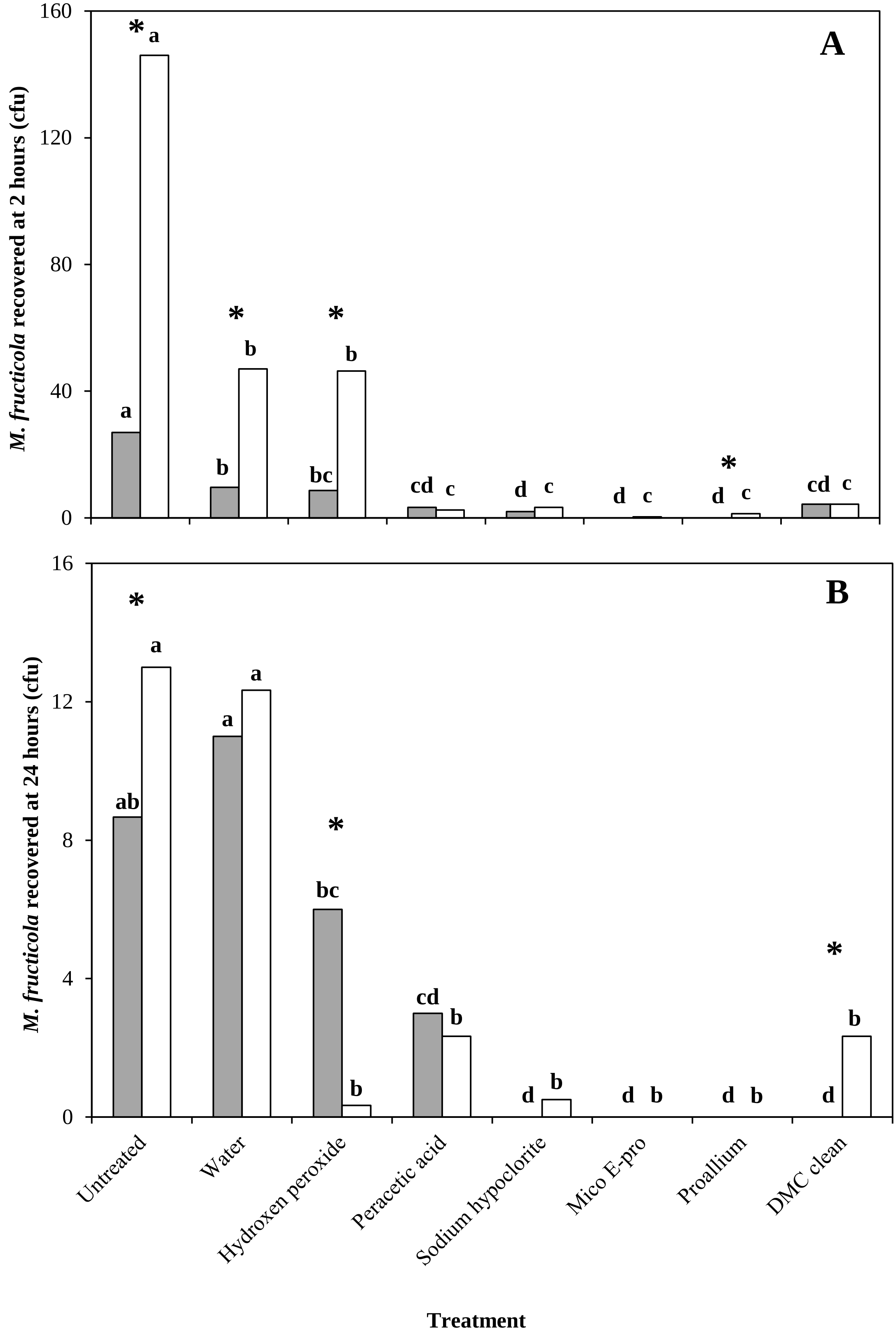
Fig. 2. *Penicillium expansum* recovered with Rodac plates from artificially inoculated plastic (■) and wood (□) surfaces treated with different treatments. Water and untreated treatments were used as control. Plastic and wood surfaces were sampled at 2h (A) and 24 hours (B) after treatment. Means with the same letter are not significantly different (P<0.05) according to Tukey test when were compared treatments to plastic or wood. * Means significant differences between plastic and wood surfaces for each treatment.

Fig. 3. *Rhizopus* spp. recovered with Rodac plates from artificially inoculated plastic (■) and wood (□) surfaces treated with different treatments. Water and untreated treatments were used as control. Plastic and wood surfaces were sampled at 2h (A) and 24 hours (B) after treatment. Means with the same letter are not significantly different (P<0.05) according to Tukey test when were compared treatments to plastic or wood. * Means significant differences between plastic and wood surfaces for each treatment

Fig. 4. *Alternarias* spp. recovered with Rodac plates from artificially inoculated plastic (■) and wood (□) surfaces treated with different treatments. Water and untreated treatments were used as control. Plastic and wood surfaces were sampled at 2h (A) and 24 hours (B) after treatment. Means with the same letter are not significantly different

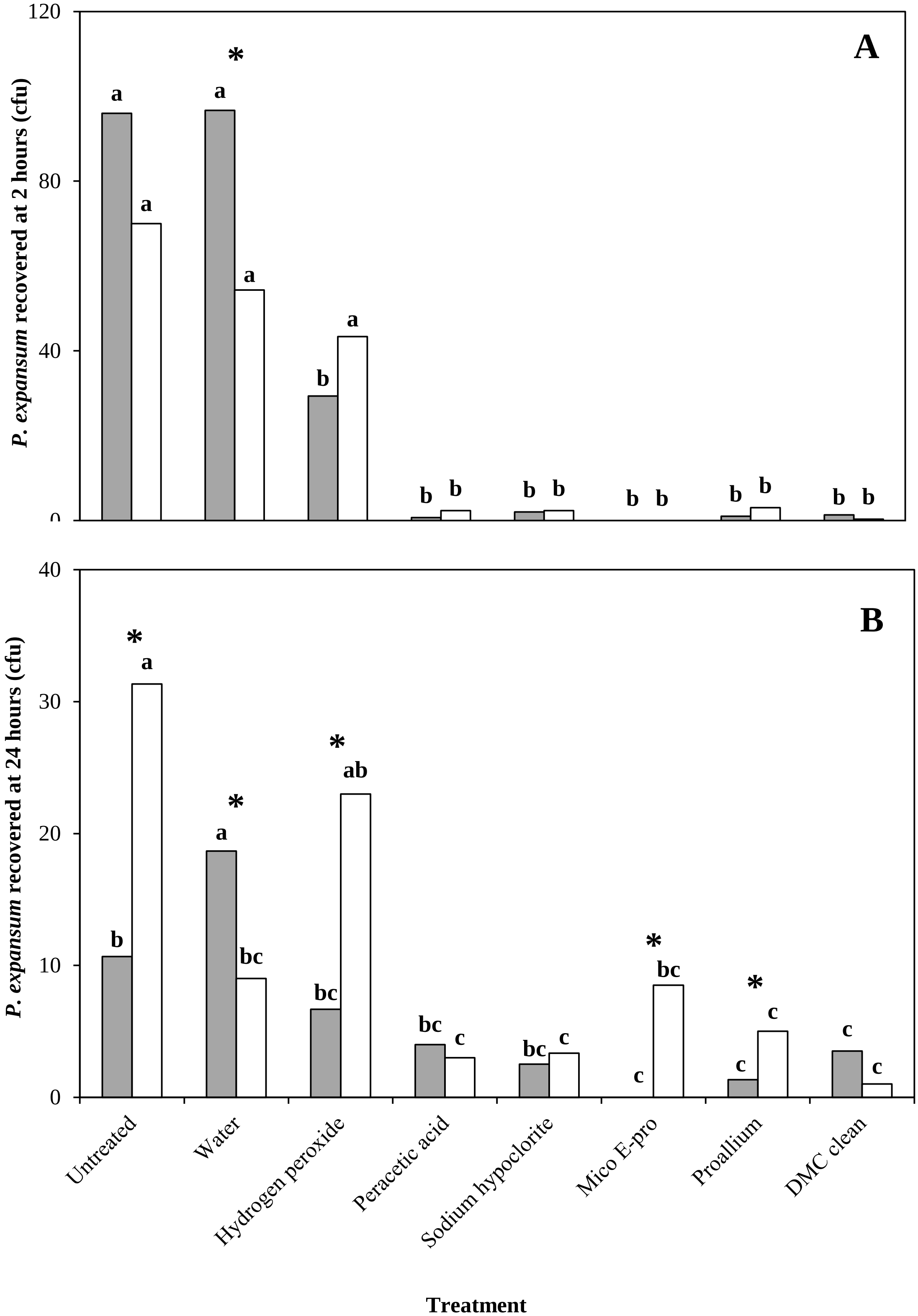
(*P*<0.05) according to Tukey test when were compared treatments to plastic or wood. * Means significant differences between plastic and wood surfaces for each treatment.

Fig. 1



A
B
160
120
80
40
0
16
12
8
4
0
M. fructicola recovered at 2 hours (cfu)
M. fructicola recovered at 24 hours (cfu)
Untreated
Water
Hydroxen peroxide
Peracetic acid
Sodium hypoclorite
Mico E-pro
Proallium
DMC clean
Treatment

Fig. 2



A
B
120
80
40
0
40
30
20
10
P. expansum recovered at 2 hours (cfu)
P. expansum recovered at 24 hours (cfu)
Untreated
Water
Hydrogen peroxide
Peracetic acid
Sodium hypoclorite
Mico E-pro
Proallium
DMC clean
Treatment

Fig. 3

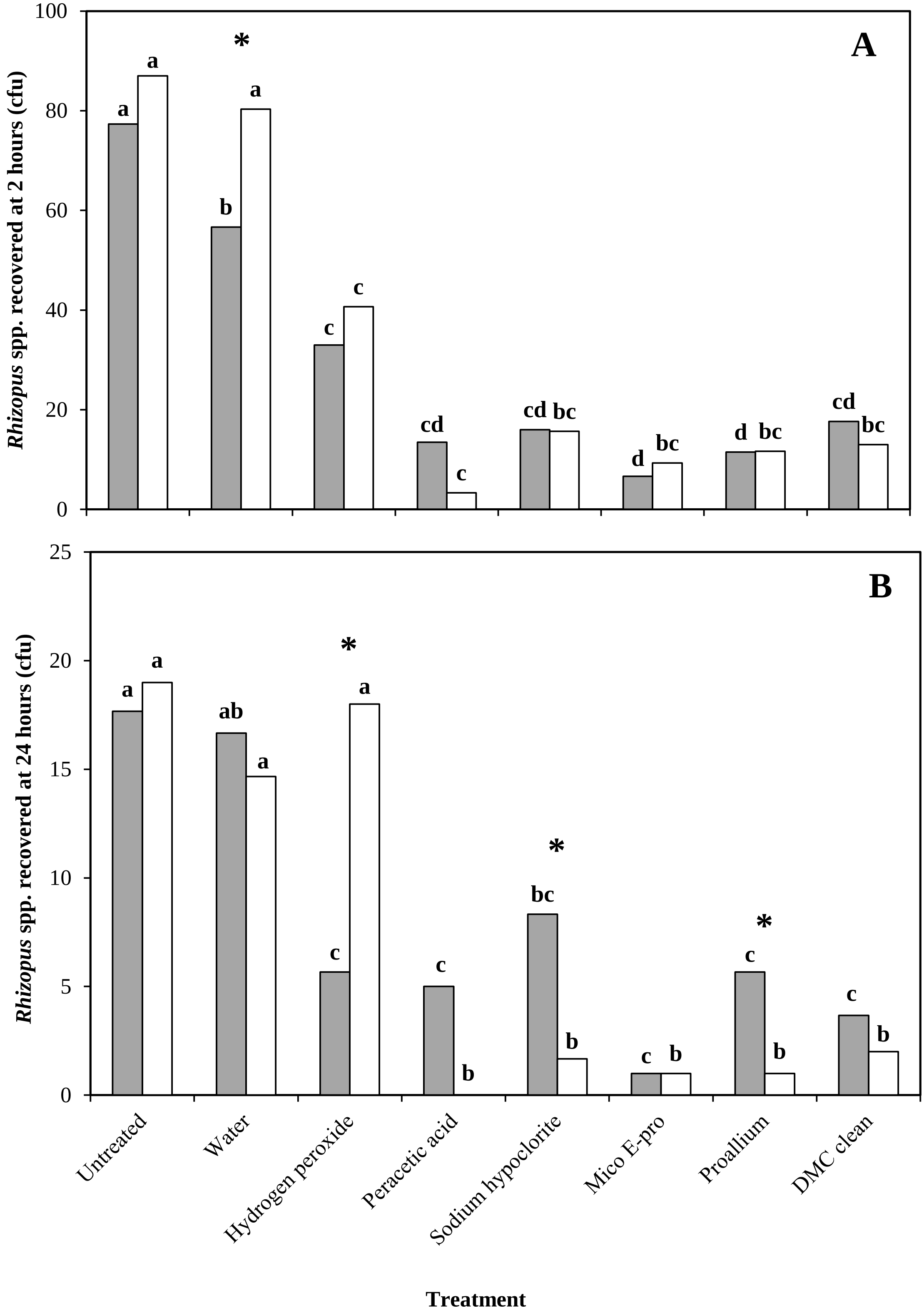
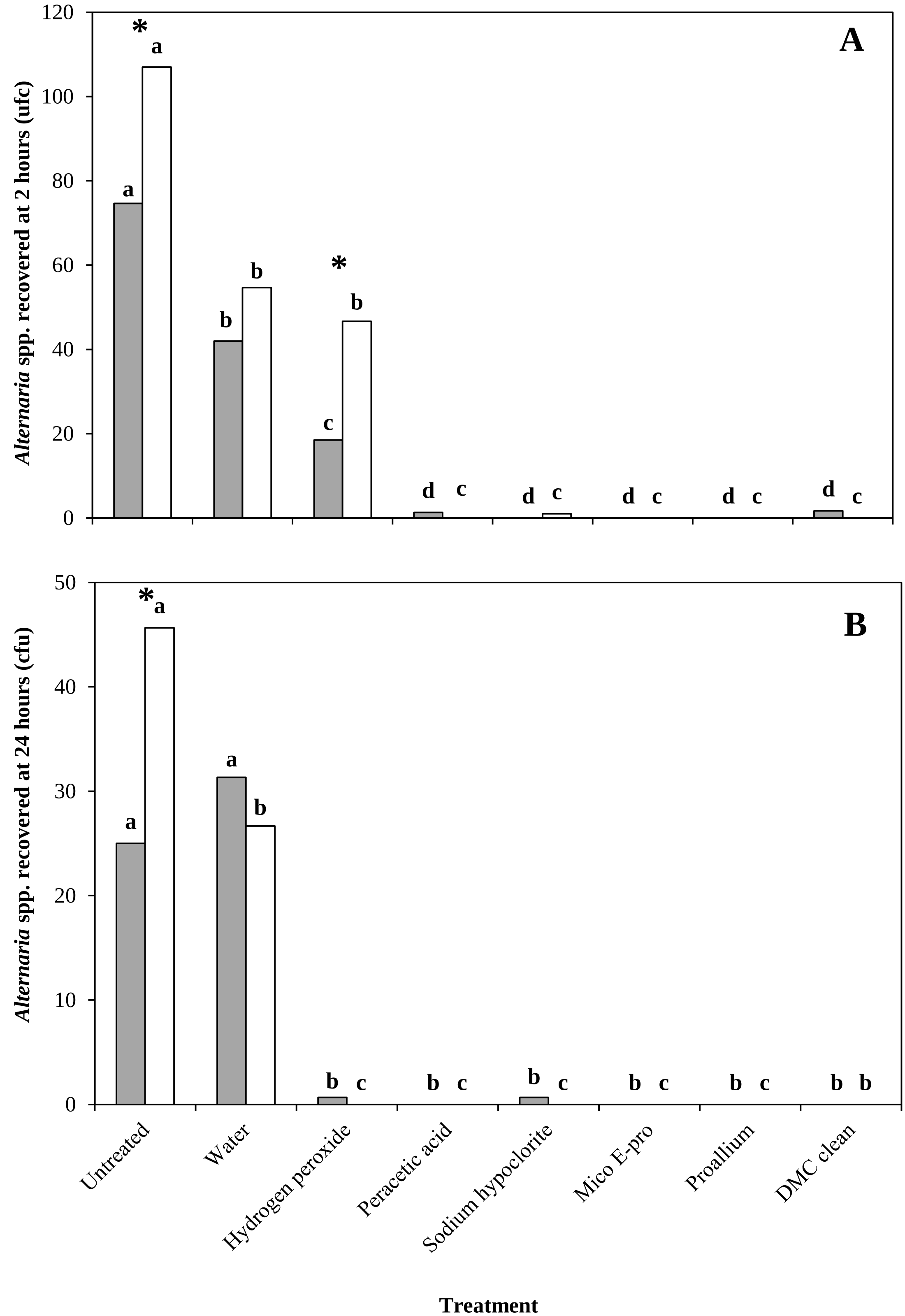

A
B
Rhizopus spp. recovered at 2 hours (cfu)
Rhizopus spp. recovered at 24 hours (cfu)
100
80
60
40
20
0
25
20
15
10
5
0
*
a
a
b
a
c
c
cd
c
cd
bc
d
bc
d
bc
cd
bc
a
a
ab
a
c
a
c
b
bc
b
c
b
c
b
c
b
Untreated
Water
Hydrogen peroxide
Peracetic acid
Sodium hypoclorite
Mico E-pro
Proallium
DMC clean
Treatment

Fig. 4


A
B
Alternaria spp. recovered at 2 hours (ufc)
Alternaria spp. recovered at 24 hours (cfu)
120
100
80
60
40
20
0
50
40
30
20
10
0
*
a
b
c
d
Untreated
Water
Hydrogen peroxide
Peracetic acid
Sodium hypoclorite
Mico E-pro
Proallium
DMC clean
Treatment