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Polyhexamethylene guanidine as a fungicide, disinfectant and wound protector in lemons challenged with *Penicillium digitatum*

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ABSTRACT

Citrus green mold, a postharvest disease caused by *Penicillium digitatum*, provokes important economic losses on lemon production. Here, the effectiveness of polyhexamethylene guanidine (PHMG) to inhibit *P. digitatum* growth and to control green mold on artificially infected lemons was evaluated. At sublethal concentrations, PHMG inhibited conidia germination and infectivity (5 mg L^{-1}), and mycelial growth (50 mg L^{-1}). Viability of conidia was completely suppressed by treatment with 500 mg L^{-1} PHMG. In this condition, membrane integrity loss, cell wall disruption and ultrastructural alterations were detected, as well as conidia distortion, deformation and collapse. In artificially inoculated lemons, a 30 s-immersion in 500 mg L^{-1} PHMG completely inhibited green mold. PHMG also exhibited a high disinfectant activity, even in the presence of 1% organic matter, with a better performance than the standard NaClO disinfectant. In addition, 500 mg L^{-1} PHMG protected wounds against infection. Taken together, our results indicate that PHMG is a promising fungicide for the postharvest control of green mold in lemon packinghouses.

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1. Introduction

Penicillium digitatum is a necrotrophic wound pathogen that causes green mold, the most common postharvest disease in citrus fruit during transport and storage (Eckert and Brown, 1986; Marcet-Houben et al., 2012). To control green mold, synthetic fungicides such as imazalil (IMZ) and thiabendazole (TBZ) are routinely applied (Holmes and Eckert, 1999). Nevertheless, their continuous use at high doses leads to the emergence of resistant strains and leaves fungicide residues that exceed the limit levels allowed by fruit importing countries (Holmes and Eckert, 1995). Hence, there is an urgent need to find alternatives to control green mold in a safe and effective way (Gong et al., 2016; Palou et al., 2002; Venditti et al., 2005). The increasing costs of research and development of new active substances and the long timelines required to evaluate efficacy and safety interfere with their introduction into the market. The proposal of known molecules as new fungicides is an efficient strategy that simplifies this introduction.

Recently, polyhexamethylene guanidine (PHMG), a polycation member of the polymeric guanidine family, has received considerable attention as a novel disinfectant for different purposes. Currently, it is used as disinfectant in human eye infections and wound care (Das et al., 2010; Gerli et al., 2003; Welk et al., 2005), fabrics conditioning (Cazzaniga et al., 2002), water treatments in swimming pools (Kusnetsov et al., 1997), and disinfection of various inanimate surfaces (Allen et al., 2006). It has been found that PHMG has a broad *in vitro* antimicrobial activity against bacteria and fungi (Kratzer et al., 2006; Razzaghi-Abyaneh et al., 2006). Broxton et al. (1984) and Ikeda et al. (1985) suggested that guanidine-based antimicrobials, such as PHMG and polyhexamethylene biguanidine (PHMB), exert antimicrobial activity by disrupting bacteria cell walls through electrostatic interactions with the acidic phospholipids, causing irreversible loss of essential cellular components. It was also reported a cooperative binding between PHMB and DNA, indicating that antibacterial effect might be also related to this interaction (Allen et al., 2006).

Although PHMG has been proposed as a safe and highly effective antimicrobial agent for hospital and household facilities, there are

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few reports on PHMG antifungal effect against causal agents of diseases in horticultural crops (Feng et al., 2011; Koffi-Nevry et al., 2011; Razzaghi-Abyaneh et al., 2006). The aims of the present study were to evaluate the *in vitro* activity of PHMG against *P. digitatum*, to analyze its antifungal action in terms of morphological damage on the pathogen, and to assess its potential to control green mold in lemons.

2. Materials and methods

2.1. Chemicals and stock solutions

PHMG was obtained from Diransa San Luis S.A as the formulation Hygisoft V20, containing 20% PHMG as active ingredient. The compound was diluted in sterile distilled water. Imazalil (IMZ) was used as a sulfate formulation (75% imazalil, Fungaflor 75SP ®, Janssen PMP).

2.2. Fungal isolate and fruit

The *P. digitatum* isolate used in this work was previously obtained from naturally infected lemons in Tucumán, Argentina (Cerioni et al., 2009). This strain has been deposited with code ICFC 842/15 in the IIB-INTECH collection of Fungal Cultures (ICFC, from the Laboratory of Mycology and Mushroom Cultivation, IIB-INTECH, Chascomús, Argentina; WDCM data base reference: 826). Some determinations were also performed using a fungicide-resistant isolate deposited as MMA-PD-16-3 in our lab collection.

Eureka lemons (*Citrus limon* (L.) Burm) were collected from commercial orchards in Tucumán and stored at 5 °C and 90% RH. Lemons used in the study were free from any postharvest applications. Before each treatment, fruit were selected according to their size and color and were randomized, superficially disinfected, rinsed with tap water, and allowed to air-dry at room temperature.

2.3. Fungal growth conditions and conidial suspension preparation

P. digitatum was grown on potato dextrose agar (PDA) at 22 ± 1 °C for 7–10 d. Preparation of conidial suspensions (10^6 conidia mL^{-1}) was performed as previously described (Cerioni et al., 2009). Dilution media was sterile distilled water, except for studies on conidia germination.

2.4. PHMG treatment on conidia

Conidial suspensions were exposed to different PHMG concentrations and incubated at 22 ± 1 °C for 24 h in the dark. After incubation, suspensions were centrifuged at 10000 g for 10 min, and pellets were washed and resuspended with sterile distilled water. Controls treated with water were included.

2.5. Determination of conidia germination and viability

Conidia germination was assessed following the protocol reported by Olmedo et al. (2017a). Briefly, conidial suspensions in potato dextrose broth (PDB) in the presence of PHMG were incubated at 22 ± 1 °C. Germination was calculated by counting at least 300 conidia. Viability of conidia after treatments was evaluated by spreading serial dilutions of suspensions on PDA medium. Cell survival was quantified as colony forming units (CFU) mL^{-1} after 4 d of incubation at 22 ± 1 °C.

2.6. Mycelial growth evaluation

PHMG activity against *P. digitatum* mycelium was evaluated following a method previously reported (Olmedo et al., 2017a). Conidial suspensions were added into 96-well microtiter plates and incubated for 24 h under static conditions at 22 ± 1 °C to allow mycelium formation, which was determined by OD_{420nm}. Then, different concentrations of PHMG were pipetted to the wells, and the mycelial growth was determined after further 24 h. Controls containing water without PHMG were run in parallel.

2.7. Evaluation of conidia ultrastructure

After treatments with PHMG during 24 h, conidia ultrastructure was studied. Permeability of plasmatic membrane and integrity of cell wall were evaluated using the fluorescent dyes SYTOX Green (SG) and Calcofluor White (CFW), respectively, following previously reported protocols (Olmedo et al., 2017a, 2017b). For ultrastructural characterization by transmission electron microscopy, conidia were processed as described by Cerioni et al. (2010). Observations were made with a Zeiss EM 109 transmission electron microscope located at CIME (Centro Integral de Microscopía Electrónica, CONICET-UNT). In all cases, water treated controls were performed in parallel.

2.8. Determination of infective capacity of treated conidia

To evaluate conidia infectivity after treatments, a previously described method was followed (Cerioni et al., 2009) with some modifications. Briefly, lemon inoculation was performed using a steel rod that was previously immersed in control and treated conidial suspensions. The rod tip was 1 mm wide and 2 mm long, which penetrated both flavedo and albedo tissues but not juice sacs of lemons. Fruit were stored at 20 °C and 95% relative humidity for 5 d, and incidence of infection was recorded at inoculation sites. The asymptomatic fruit were stored for 14 days before discharged.

2.9. Evaluation of PHMG curative and preventive activities

Curative treatments were performed according to a previously reported method (Cerioni et al., 2012). Briefly, fruit were inoculated using a stainless steel rod previously immersed into a freshly prepared conidial suspension. Lemons were maintained at 20 °C and 95% relative humidity for 24 h. After this storage, fruit were immersed for 30 s in aqueous solutions with PHMG at final concentrations of 500 and 1000 mg L^{-1}. After drying, lemons were placed into plastic cavity trays and stored at 20 °C and 95% relative humidity.

Preventive treatments were evaluated following a protocol reported by Usall et al. (2008) with some modifications. Fruit were immersed for 30 s in solutions containing 500 or 1000 mg L^{-1} PHMG. After a 24 h-incubation at 20 °C and 95% relative humidity, lemons were inoculated using a stainless-steel rod previously immersed into a conidial suspension. After drying, lemons were stored as explained above.

In curative and preventive assays, green mold incidence was recorded after 5 d of storage and expressed as percentage of decayed fruit. The asymptomatic fruit were stored for 14 d before discharged. Controls consisted in fruit immersed either in water or 1000 mg L^{-1} IMZ.

2.10. Evaluation of PHMG as a disinfectant

Ten liters of *P. digitatum* conidial suspensions (10^6 conidia mL^{-1}) were exposed to 500 or 1000 mg L^{-1} PHMG during 10 min. Also, the disinfectant performance of 200 mg L^{-1} NaClO and of the combination of PHMG and NaClO were evaluated. In all cases, the effect of the presence of organic matter (1% powder milk) was tested.

Controls consisting of conidial suspensions only with water were included. After conidia treatment, wounded lemons were immersed in suspensions for 90 s. Fruit were stored and evaluated for disease incidence as explained above.

2.11. Evaluation of PHMG wound protection activity

A protocol previously reported was followed (Wild, 1993), with some modifications. Lemons were wounded and immersed in aqueous solutions of PHMG at different concentrations for 90 s. For controls, immersions in water or in 3% $NaHCO_3$ were performed. After a 24 h-storage, lemons were inoculated by their immersion in *P. digitatum* conidial suspensions. Fruit were stored and evaluated for disease incidence as previously explained.

2.12. Quality parameters of postharvest lemons after PHMG treatment

Lemons were treated with water (control) or with 1000 mg L^{-1} PHMG for 90 s and stored at 7 °C and 95% relative humidity. Measurements of quality parameters in control and PHMG treated fruit were performed after 45 d, according to Geng et al. (2011) as follows:

- *Weight loss:* lemons from each tray were weighted before treatment (A) and after storage (B); then, weight loss was calculated as (A−B)/A.
- *Fruit color:* color was determined at the end of the storage using a Minolta colorimeter (CR 300, D65, Hunter Lab system). L, a and b values were obtained and the citrus color index (CCI) was calculated as CCI = 1000 a/L*b (Jiménez-Cuesta et al., 1981).
- *Fruit firmness:* firmness values of each lemon were determined at the end of the storage by compression, using a GY-2 Texture Analyzer to the equatorial region of the fruit, and expressed in Newton (N).
- *Total soluble solids:* at the end of the storage, juice was obtained from control and treated lemons; total soluble solids (SS) were determined with a hand refractometer at room temperature and expressed as percentage.
- *Titratable acidity:* juice acidity was measured by titration with 0.1 N NaOH to pH 8.3 and results were expressed as citric acid percentage.

2.13. Statistical analysis

For *in vitro* assays, three replicates were performed for each condition, and the entire assays were done three times. *In vivo* assays included four replicates of 15 lemons for each condition and were repeated three times. In all cases, data were subjected to analysis of variance followed by Tukey's test with Infostat, 2013 version, for Windows. Differences of p value ≤ 0.05 were considered significant.

3. Results

3.1. Inhibition of conidia germination and mycelial growth by PHMG

The antimicrobial effect of the cationic polymer PHMG against the fungal phytopathogen *P. digitatum* was evaluated. Conidia germination was inhibited by the compound in a dose-dependent manner (Fig. 1A and B). A concentration of 5 mg L^{-1} was enough to completely prevent germination. Conidia remained ungerminated after 7 d of incubation in the presence of PHMG (data not shown). Nevertheless, this concentration was sublethal, as even 50 mg L^{-1} resulted in 2.2×10^2 viable conidia per mililiter (Table 1).

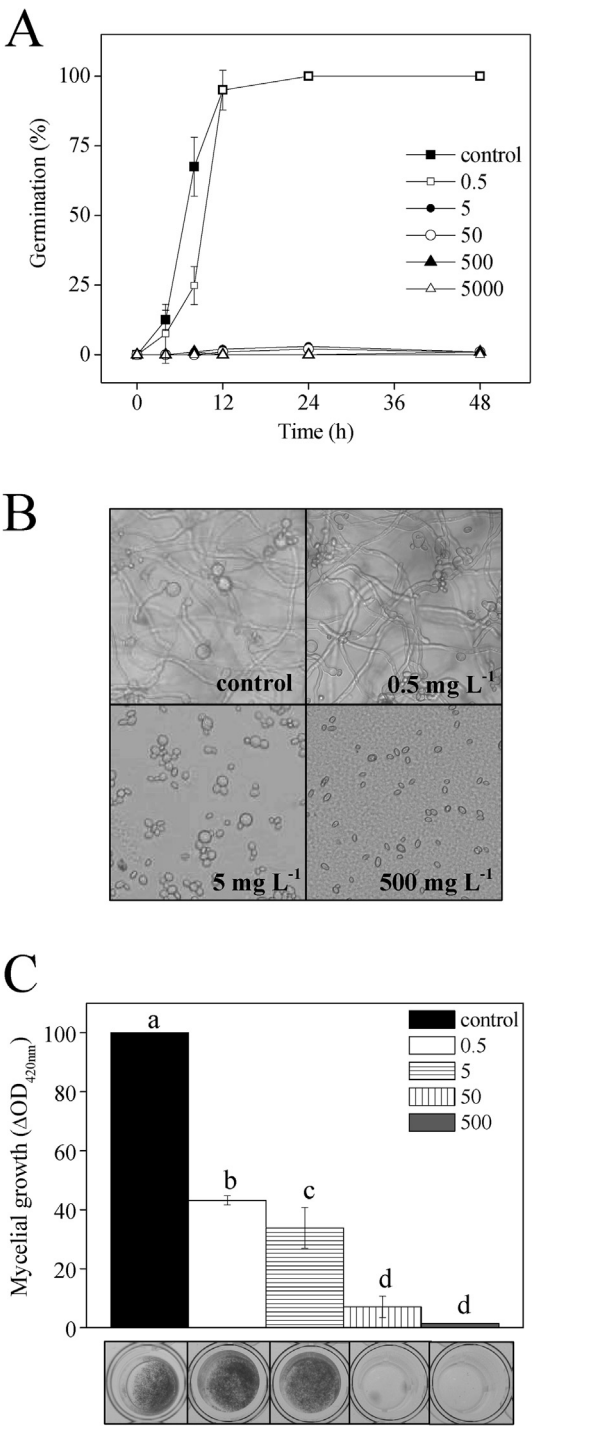

Fig. 1. Inhibition of conidia germination and mycelial growth by PHMG. (A) Percentage of germinated conidia exposed to PHMG (mg L^{-1}) at the indicated incubation times. Vertical bars indicate the standard deviation of the mean. (B) Representative micrographs of conidial suspensions exposed to the indicated PHMG concentrations during 24 h (40X). (C) Mycelial growth in the presence of different PHMG concentrations (mg L^{-1}). Growth is expressed as the difference between final and initial OD_{420nm} ± SD. Error bars represent standard deviation. Different letters indicate significant differences among treatments according to Tukey's test with a p value ≤ 0.05. Bottom panel shows representative images of mycelial growth after 7 d of incubation in each condition.

Table 1
Conidia viability after 24 h of incubation with PHMG.

PHMG (mg L^{-1})	CFU mL^{-1}
–	1.0×10^{6} [a]
0.5	8.5×10^{5} [a]
5	1.3×10^{4} [b]
50	2.2×10^{2} [b]
500	0 [c]

CFU mL^{-1}: colony forming units per milliliter. Values followed by different letters are significantly different at $p \leq 0.05$ according to Tukey's test.

The viability of ungerminated cells was completely inhibited in the treatment with 500 mg L^{-1} PHMG. Observations by light microscope revealed that the application of PHMG at this lethal concentration resulted in conidia with an evident condensate aspect (Fig. 1B). The same results were observed when PHMG was challenged against the isolate MMA-PD-16-3, resistant to IMZ and TBZ (data not shown).

Fig. 1C upper panel shows the effect of PHMG on *P. digitatum* mycelial growth at 24 h. Treatments with 50 and 500 mg L^{-1} significantly inhibited of mycelium development. PHMG applied at a concentration as low as 0.5 mg L^{-1} also exhibited a considerable effect, inhibiting growth by more than 50% as compared to water treated controls. After 7 d of exposure to concentrations of 0.5 and 5 mg L^{-1} PHMG, the pathogen kept its capacity to grow and sporulate (Fig. 1C bottom panel).

3.2. Modifications in conidia structure by PHMG

The integrity of the conidia membrane after exposure to PHMG was evaluated using the SG dye (Fig. 2, left panels). While fluorescence was not detected in control cells, a 24 h-exposure to 500 mg L^{-1} PHMG generated an evident fluorescence emission in 100% of the conidia. Treatments with 0.5 and 5 mg L^{-1} PHMG also caused loss of membrane integrity (data not shown). Fig. 2 central panels show fluorescence and light microscopy images of CFW stained cells. Conidia treated with PHMG exhibited a stronger CFW fluorescence pattern on cell surface compared to untreated controls. The enhancement in fluorescence emission is indicative of cellular disorganization and exposure of some polysaccharide residues in PHMG treated samples. In addition, Fig. 2 right panels illustrate the morphological changes detected by TEM in fungal ultrastructure of PHMG-treated samples in comparison with water treated controls. Almost all conidia treated with 50 mg L^{-1} PHMG exhibited unclear nuclear structures and disordered cytoplasm, revealing severe cellular damage. In several cases, distortion of cell shape and/or loss of intracellular content were observed. The presence of empty conidial envelopes was frequent. In contrast, control conidia showed a well-organized cytoplasm, organelles with normal appearance, and nuclei and vacuoles surrounded by well-defined envelopes.

3.3. Decrease in conidia infectivity by exposure to PHMG

As an indicator of conidia infectivity, lemons were inoculated with PHMG treated conidia and disease incidence was recorded 5 d post inoculation (Fig. 3). A treatment with 5 mg L^{-1} reduced green mold incidence by 75% compared to water controls. As expected, after treatment with 500 mg L^{-1} PHMG (lethal condition), no infected lemons were detected. It is worth noting that when suspensions were treated with 50 mg L^{-1} PHMG, a 100% reduction in fruit decay was observed, despite 10^{2} conidia mL^{-1} were still viable (Table 1).

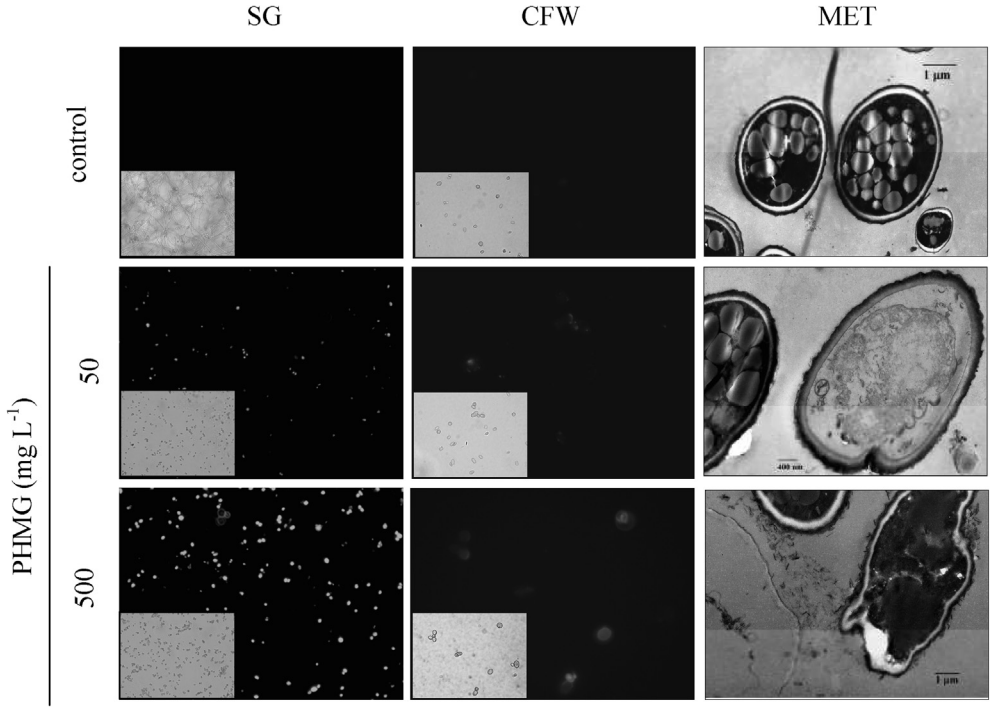

Fig. 2. PHMG effect on membrane permeability, cell wall integrity and ultrastructural organization of conidia. (Left panels) PHMG treated conidia were stained with SG and visualized by fluorescence microscopy ($\lambda_{exc} = 450–490$ nm/$\lambda_{em} = 515–565$ nm, 40X). (Central panels) PHMG treated conidia were stained with CFW and visualized by fluorescence microscopy ($\lambda_{exc} = 330\text{-}385$/$\lambda_{em} = 440$ nm, 100X). Inserts in fluorescent images show the corresponding bright fields. (Right panels) PHMG treated conidia were visualized by TEM. In all cases, water treated controls are shown in the upper panels.

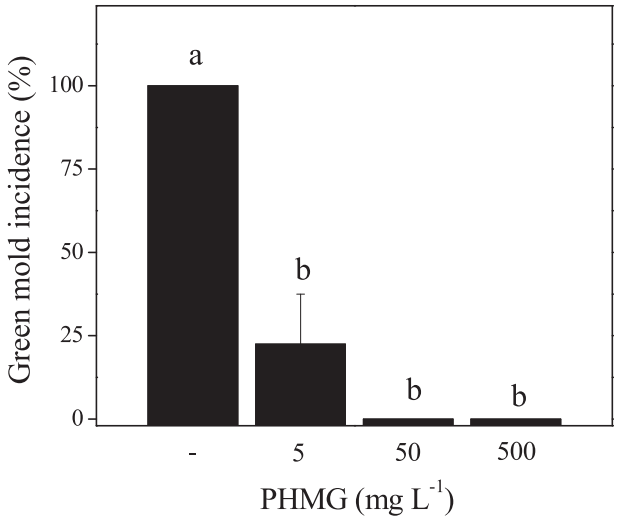

Fig. 3. Conidia infectivity after PHMG treatment. Green mold incidence in lemons inoculated using conidia treated with water or PHMG was evaluated after 5 d of storage. Values are the means of the percentages of three independent assays; error bars represent standard deviation. Different letters indicate significant differences among treatments according to Tukey's test with a p value ≤ 0.05.

3.4. Curative and preventive actions of PHMG

The curative effect of PHMG was evaluated on inoculated lemons, simulating infections that might take place during harvest or transit of fruit to the packinghouse. PHMG curative treatments resulted in 10-fold reductions in green mold incidence compared to water treated lemons, achieving a complete control of primary infections in treatments with either 500 mg L^{-1} PHMG or 1000 mg L^{-1} IMZ (Fig. 4A). In assays to evaluate PHMG preventive action, lemons were treated with the polymer 24 h before inoculation with the pathogen. Results indicate a lack of preventive activity in PHMG, since decay incidence was not reduced when compared with water treated controls (Fig. 4B). In this approach, IMZ was highly effective, as green mold incidence was 0%.

3.5. Disinfectant and wound protective activities of PHMG

Disinfection assay was designed to compare PHMG and NaClO performances, simulating the initial fruit sanitization in packinghouse soak tanks. In both PHMG treatments tested (500 and 1000 mg L^{-1}), green mold incidences were below 15% (Fig. 4C). It should be noted that the disinfection potential of PHMG was significantly higher than that of NaClO. When both chemicals were combined, disinfection efficacy was the same of PHMG alone. The presence of organic matter had an adverse effect on NaClO disinfectant action, in agreement with previous reports (Gelinas and Goulet, 1983; Harrison and Hand, 1981). However, PHMG performance was not altered by this factor. In the wound protection experiment, green mold incidence was significantly lower in fruit treated with PHMG compared to those treated with water (Fig. 4D). The decrease of fungal infections with PHMG at the lowest concentration tested (500 mg L^{-1}) was similar to the one achieved with 3% $NaHCO_3$, with decay incidences below 5% in both cases.

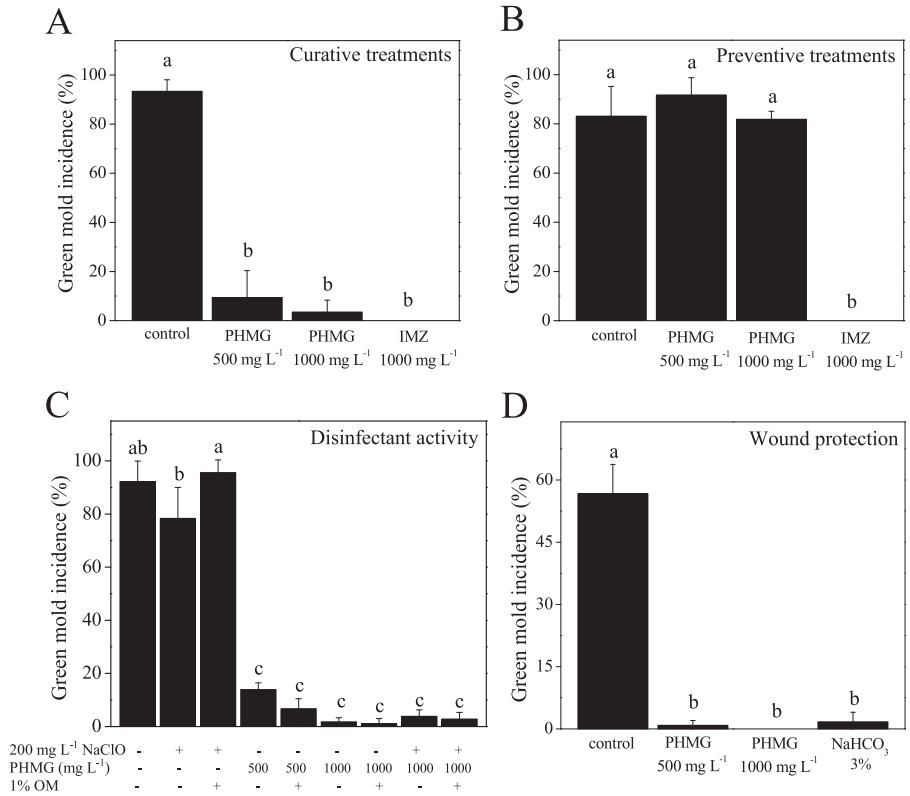

Fig. 4. PHMG as fungicide, disinfectant and lemon wound protector. Green mold incidence was evaluated: (A) after treatment of inoculated fruit with PHMG or IMZ (curative treatments); (B) after inoculation of fruit previously treated with PHMG or IMZ (preventive treatments); (C) after inoculation of fruit with conidial suspensions exposed to PHMG alone or in combination with 200 mg L^{-1} NaClO and 1% organic matter (OM) (disinfectant activity); (D) after inoculation of fruit previously wounded and treated with PHMG or 3% $NaHCO_3$ (wound protection). In all cases, negative controls consisted in lemons treated with water, and incidence was expressed as percentage values recorded after 5 d of storage. Error bars indicate the standard error of the mean. Different letters indicate significant differences among treatments according to Tukey's test with a p value ≤ 0.05.

Table 2
Effect of the immersion in PHMG on postharvest lemons quality parameters.

	Weight loss (%)	CCI	Firmness (N)	TSS (%)	Titratable acidity (%)
control	0.99 ± 0.32^a	-2.41 ± 0.04^a	0.83 ± 0.17^a	7.55 ± 0.11^a	7.01 ± 0.13^a
PHMG	1.11 ± 0.22^a	-2.38 ± 0.02^a	0.84 ± 0.21^a	7.67 ± 0.17^a	7.20 ± 0.16^a

Lemons were immersed for 90 s either in water (control) or in 1000 mg L^{-1} PHMG, and stored at 7 °C for 45d. For each parameter, the same letter indicates that there are no significant differences between treatments at $p \leq 0.05$ according to Tukey's test.

3.6. Effect of PHMG on postharvest lemons quality

Table 2 shows that PHMG treatment had no significant effect on fruit weight loss when compared to control. Color and firmness were similar in fruit treated with PHMG and with water. In addition, there were no differences in soluble solids content and titratable acidity of juice obtained from both samples.

4. Discussion

The present study demonstrates the effectiveness of PHMG to inhibit *P. digitatum in vitro* growth and to control lemon green mold as a disinfectant, fungicide and wound protector.

The polymer efficiently inhibited conidia germination and mycelial growth at 5 and 50 mg L^{-1}, respectively. PHMG acted as a fungistatic compound at these concentrations, while viability suppression was achieved only at a concentration of 500 mg L^{-1}. Similarly to our results, it was demonstrated that in *Escherichia coli* the polymer was bacteriostatic at low concentrations (1–10 mg L^{-1}), but bactericidal at higher concentrations (Allen et al., 2006). Moreover, Feng et al. (2011) reported the antifungal activity of PHMG against *Geotrichum citri-aurantii*, other citrus postharvest phytopathogen, observing inhibitory effect at comparable concentrations to those of the present study.

Damage provoked by PHMG on different cell structures of *P. digitatum* was investigated. For instance, fluorescence and TEM microscopic images showed structural alterations in conidia treated with PHMG, including distortion of cell shape, plasma membrane permeabilization, and wall disruption. In agreement, guanidine-based polymeric disinfectants provoked a clear cytoplasm disorganization and plasma membrane permeabilization in *Aspergillus parasiticus* (Razzaghi-Abyaneh et al., 2006) and *E. coli* cells (Zhou et al., 2010). The mode of action of polyguanidines is supposed to be similar to that of polybiguanides (e.g., PHMB), which has been extensively studied in bacteria. In the presence of the polymer, the homogeneous distribution of phospholipids in biological membranes is severely modified, leading to an irreversible loss of essential cellular components, first of small cationic materials, such as potassium ions, and later of intracellular pool materials (Broxton et al., 1984). Our results indicate that PHMG mechanism of action in filamentous fungi might be also dependent on envelope damage. It should be noted that guanidine based polymers interact only superficially with the lipid bilayer, therefore their antimicrobial effect is not susceptible to resistance mechanisms mediated through multidrug efflux pumps (Gabriel et al., 2007). In addition, neutral phospholipids are not affected by the polymer (Ikeda et al., 1983, 1985), reason for the low toxicity of the polymer against human cells (Kramer and Roth, 2008).

Conidia infectivity was severely affected by PHMG sublethal treatments. The amount of viable conidia after treatment with 5 mg L^{-1} PHMG was 10^4 cfu mL^{-1}, which should be sufficient to develop disease on fruit, as previously stated (Vilanova et al., 2012). Nevertheless, the low incidences observed when lemons were inoculated with these treated conidia could be attributable to a reduction in the pathogen virulence.

Disinfection of fresh fruit and vegetables after harvest is an essential step of postharvest handling (Feliziani et al., 2016). In citrus packing lines, fruit residence time and disinfectant concentration in dump tanks should be sufficient to kill spores in water and on fruit. Otherwise, they could contaminate subsequent processing steps and increase decay incidence (Smilanick et al., 2002). In our assays, PHMG presented outstanding properties as a fruit disinfectant, especially when compared with NaClO, the traditional disinfectant used in drencher tanks. The remarkable disinfectant efficiency observed for PHMG is reinforced by its high water solubility and the lack of pH control needed for its application (pKa = 13.5 (Guzenko et al., 2011)). On the contrary, the activity of chlorine, a corrosive and volatile compound, is known to be dependent on pH. In addition, our results show that PHMG activity is not altered by the presence of organic matter, while it is known that NaClO leads to the formation of toxic organic by-products (Brown and Chambers, 1999), in detriment to its disinfecting action.

Our studies showed that PHMG significantly reduced green mold incidences in curative treatments. At the lowest concentration tested, the polymer controlled green mold in the same extent than IMZ. PHMG application in the fungicide blend would be feasible due to its high water solubility (Rogalsky et al., 2016), unlike synthetic fungicides that usually require continuous agitation (Sepulveda et al., 2015). Also, PHMG has low toxicity for humans and animals (Wei et al., 2013), which represents an advantage over harmful conventional fungicides (Valencia-Chamorro et al., 2009).

PHMG exhibited the capacity to protect the wound site against *P. digitatum*, in a similar way than a treatment with 3% $NaHCO_3$. This behavior suggests that the control of green mold by PHMG could be explained not only by the direct antimicrobial action of the compound on the pathogen, but also by other events that might occur during the interaction between the polymer and the skin of the wounded lemon. It has been reported that wound protection observed in treatments with carbonates and bicarbonates (pH between 8 and 10) was related to tissue alkalinization (Prusky et al., 2004), salt residue retention (Smilanick et al., 1999), and scoparone accumulation (Venditti et al., 2005) at the wound site. All these effects evidenced a positive correlation with decay reduction. PHMG treatment solutions are pH 8, which might partially explain the wound protection observed in our work. Nevertheless, further studies should be conducted to elucidate this protective mechanism.

5. Conclusions

The present work reports for the first time that PHMG has significant antimicrobial properties against *P. digitatum*. The exposure of the pathogen to this compound provoked germination inhibition, conidia infectivity reduction, and severe cellular damage. The polymer has outstanding properties as fruit disinfectant and wound protector, being also effective in the control of *P. digitatum* primary infections. In addition, PHMG did not modify or impair lemon quality parameters. Taken together, our results indicate PHMG is a promising new antifungal compound to control postharvest fungal diseases in citrus packinghouses.

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