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Research Paper

Organo-montmorillonite with biogenic compounds to be applied in antifungal coatings

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ABSTRACT

Antimicrobial surfaces are required to obtain more hygienic indoor environments. There are biogenic compounds like terpenoids, from plant essential oils, with potential antimicrobial activity. Besides, modified montmorillonites are promising nanoscale carriers for these compounds. The aim of this research was to prepare antimicrobial coatings with funtionalized montmorillonite hybrid. Three monoterpene compounds from plant essential oils such as citral, citronellol and linalool were assessed to obtain an eco-friendly and cheap antifungal hybrid filler for coatings. A natural surfactant such as soybean lecithin was used as an organic modifier of the clay mineral.

The synthetized hybrids were studied with X-ray diffraction (XRD) analysis, thermogravimetric Analysis (TG), Fourier-Transform Infrared (FTIR) spectroscopy, Zeta potential (ZP) and scanning electron microscopy (SEM). The modified montmorillonite with soybean lecithin and citronellol by 40% wt. showed higher antifungal activity. This bioactive hybrid achieved to impart its functionality into a waterborne paint formulated with 5% wt. in bioassays against three frequent and handful moulds *Chaetomium globosum* (KU936228), *Alternaria alternata* (KU936229) and *Aspergillus fumigatus* (KU936230).

1. Introduction

The design of intelligent buildings that integrate the use of functional materials such as hygienic coatings is necessary to control biofilms development. Infections in indoor environment represent a serious problem for health of urban populations which, in general, remain most of the time inside buildings ~16 h/day (Pigeot-Remy et al., 2013; WHO, 2009). It is well known that fungal species are widespread in the environment and are commonly isolated from indoor, including hospitals (Kousha et al., 2011). *Aspergillus fumigatus* is an example of an opportunistic pathogen which causes a wide spectrum of problems related to the respiratory system including life-threatening infections in immunocompromised patients (Kousha et al., 2011; Lee et al., 2013). Hygienic coatings are formulated to control indoor microbial growth in households and health care centers among other (Falkiewicz-Dulik et al., 2015; Johns, 2003). Their study is very active at the present, specially the seeking of new eco-friendly bioactive additives or microbiocides to replace those questioned due to their toxicity and low efficiency. Among the compounds intensely studied are: nanoparticles, quaternary ammonium salts, natural product and derivatives (Jaiswal et al., 2013; Negut et al., 2018; Siedenbiedel and Tiller, 2012; Weber and Rutala, 2013). In this sense, plant extracts and derivatives result promising because they come from renewable, abundant and low cost resource. Adding to this, some of these actives like terpenoids compounds could be obtained from industrial waste (Wu et al., 2017). Terpenoids are generally found in essential oil (EO) extracted from plants, for example, aromatic grasses (eg. Cymbopogon spices) which have enormous commercial value for agricultural, food, medical and perfumery applications (Chellappandian et al., 2018; Olugbenga et al., 2016; Sastry et al., 2014). EO are complex mixtures of several components, characterized by having one or two of them at high concentrations (20–70%) in some cases with antimicrobial potentialities (Olugbenga et al., 2016).

The efficient inclusion of this type of biogenic compounds in coatings requires the use of frameworks or carriers as siliceous matrix (Hendessi et al., 2016; López et al., 2007). There are published data of the use of this kind of frameworks with conventional organic biocides in waterborne paints, firstly reported by Edge et al., 2001. They proved

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prolonged efficiency over time of the coatings due to protect organic actives from degradation (Edge et al., 2001; Mardones et al., 2019; Sørensen et al., 2010). While in the aforementioned investigations synthetic siliceous matrix were reported, the present work has raised the use of a clay mineral as natural carrier chosen for being abundant, versatile and cheap. Clay minerals (e.g. montmorillonites, halloysite and sepiolite) have the property to exchange ions, being modified to a nanoscale level to work out as carriers for inorganic and organic compounds (Clegg et al., 2019; Nagy et al., 2013; Ramos et al., 2012). Soy lecithin could be used as a natural bio-surfactant and an environmentally friendly bio-modifier for the preparation of the organo-clays. In acidity conditions S presents a positively charged amino group that allows ion exchange (Nagy et al., 2013). In this sense, the clay surface changes from highly hydrophilic to hydrophobic, and in addition, with new sites of sorption, positive electrical charge (Bianchi et al., 2013). The hydrophobic characteristics of organo-clay samples could improve the adsorption of hydrophobic biogenic compounds, with respect to raw clays. Montmorillonite (Mt) is a promising carrier to biogenic compounds due to its low cost and abundance in Rio Negro, Argentina.

The aim of the present research was to obtain antimicrobial Mt hybrids with biogenic compounds to be integrated into functional coatings. Soy lecithin was used as organic modifier of the Mt by ionic exchange. The biogenic compounds evaluated were commercial terpenoids: citronellol (Col), citral (Cal) and linalool (Lol) which are generally found in oil extracted from species of Cymbopogon genus (eg. *C. winterianus* and *C. citratus*). The assessment of bioactivity of the terpenoids, hybrids synthesized and coatings was carried out by conventional agar plate assays. The strains used were: *Chaetomium globosum* (KU936228), *Alternaria alternata* (KU936229) and *Aspergillus fumigatus* (KU936230) isolated from biodeteriorated coatings in previous research work. The synthesized hybrids were characterized by scanning electron microscopy (SEM), fourier transform infrared spectroscopy (FTIR), zeta potential (ZP), differential thermal analysis (TGA) and x-ray diffraction (XRD).

2. Materials and methods

2.1. Materials

Na-montmorillonite (> 99%) employed was from Lago Pellegrini deposit, Río Negro, North Patagonia, Argentina, provided by Castiglioni Pes and Cia. Granular soy lecithin (food quality) was obtained from Melar S. A. (Buenos Aires, Argentina). Citronellol (Col), citral (Cal) and linalool (Lol) were probided by Alfredo Francioni S. A. (Buenos Aires, Argentina).

2.2. Antimicrobial potentialities of three biogenic compounds

In order to assess which of the three commercial terpenoids (Col, Cal and Lol) under study was the most active one, a screening of the antimicrobial potentialities was carried out by macrodilution method (Bellotti et al., 2013; Stupar et al., 2014). The chemical structure of the biogenic compounds studied can be seen in Fig. 1.

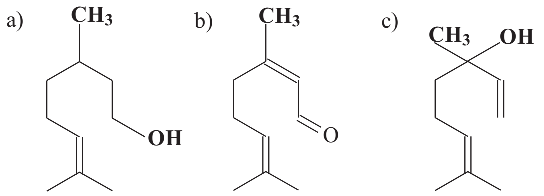

Fig. 1. Chemical structures of the monoterpenoids: Citronellol (a), Citral (b) and Linalool (c).

Spore suspensions of *Chaetomium globosum* (KU936228) and *Alternaria alternata* (KU936229) were prepared using malt extract agar (MEA) cultures of the strains incubated at 28 °C for 20–30 days (Deyá and Bellotti, 2017). The concentration was adjusted to 10^5 spores/mL by Neubauer chamber. MEA with different concentrations of Col, Cal and Lol (1.2, 2.5 and 5.0 mg/mL) were poured into Petri dishes. These were inoculated with 20 µL of spore suspensions in the center of each plate. The same procedure was carried out with controls without any biocides. Plates were incubated at 28 °C and the colony diameters were periodically measured over time. The percentage of inhibition (%I) was calculated was calculated bu. Eq. (1) as follow:

$$\%I = \frac{Control\ growth - growth\ with\ biocide}{Control\ growth} \times 100 \tag{1}$$

The assay was performed in triplicate. The most active terpenoid was selected to be used in the synthesis of the hybrids clays.

2.3. Bioactive Mt hybrids preparation

Mt was used as received, chemical analysis performed on purified Mt samples, have indicated that they are highly charged (0.41 eq/formula unit) with a structural formula: $[(Si_{3.89}\ Al_{0.11})\ (Al_{1.43}\ Fe_{0.26}\ Mg_{0.30})]\ M^{+}_{0.41}$ (Magnoli et al., 2008). Its main properties were: isoelectric point (IEP) pH = 2.7, total specific surface area (TSSA) = 621 m^2/g, and cationic exchange capacity (CEC) = 82.5 cmol (+)/Kg (Gamba et al., 2015).

The modified Mt was obtained by a similar procedure to Nagy et al. (2013). The synthesis of the organo-modified Mt was performed by the following procedure: 30 g of Mt was pre-swollen in 2 L of distilled water (DW) for 24 h. Soy Lecithin was dissolved in ethanol, and the pH of the solution was adjusted to 2.3 by the addition of 0.1 M HCl. The S solution was then added to the Mt dispersion under constant stirring. After 30 min of stirring, the pH was re-adjusted to 2.3 by the addition of 0.1 M HCl. The DW:ethanol ratio was 1:2 (m/m), whereas the Soy lecithin-Mt ratio was 0.33:1. The system was stored at 45 °C for 24 h. The adsorbent was labeled as S—Mt

Therefore, hybrids clays (S—Mt) were attained incorporating different concentrations of Col suspensions (2.5, 5, 10 and 20% wt.) in 1:1 DW/ethanol. The products obtained were labeled as: S—Mt2.5, S—Mt5, S—Mt10 and S—Mt20, correspondingly. Raw Mt was treated with similar concentrations of Col too, following the same procedure but without the previous exchange with soy lecithin. Different concentrations of Col were performed to assess the minimum concentration with antifungal activity. In this case, the products obtained were labeled as: Mt2.5, Mt5, Mt10 and Mt20, respectivelly. Additionally, two more hybrids were synthesized with S—Mt and 40% wt. of Col, S-Mt40A and S-Mt40B, the last one differs in the sequence of the preparing procedure being soybean lecithin and Col suspensions (40% wt.) incorporated at the same time into the synthesis mix. Finally, the hybrids were separated by centrifugation and vacuum dried.

2.4. Characterization of Mt hybrids

Firstly, the bioactivity of the synthesized samples, which was fundamental to the functionality required for the hygienic coatings, was evaluated. The antimicrobial activity was assessed by agar well diffusion method (Fernández and Bellotti, 2017; Magnusson and Schnürer, 2001) a variation of Kirby-Bauer agar diffusion method with the same strains used previously: *C. globosum*, *A. alternata*. Petri dishes were prepared introducing 15 mL of inoculated MEA, 7 mm of diameter (D) wells were made in them and each one was filled with 20 mg of the tested hybrid solids; three replicates were made in each case. All the plates were incubated at 28 °C and after 48 h inhibition zones diameters were measured. In this sense, it was considered that samples with D < 7 mm had no activity and those with D ≥ 7 mm, were active.

The FTIR spectra were obtained by using a Perkin Elmer (Spectrum ONE) FTIR spectrometer equipped with KBr pellet holder in the

Table 1
Fungal inhibition assay: growth diameters (cm) of Citronellol (Col), Citral (Cal) and Linalool (Lol) against tested fungi at 28 °C.

		C. globosum								
		Col			Cal			Lol		
Time (days)		3	6	10	3	6	10	3	6	10
Concentrations (mg/mL)	1.2	0	0	0	0	0	2.8 ± 1.4	0.7 ± 0.4	4.1 ± 1.5	6.9 ± 1.5
	2.5	0	0	0	0	0	0	0	2.0 ± 0.2	6.3 ± 1.2
	5.0	0	0	0	0	0	0	0	0	5.1 ± 1.1

		A. alternata								
		Col			Cal			Lol		
Time (days)		3	7	10	3	7	10	3	7	10
Concentrations (mg/mL)	1.2	0	0	0	0	0	0	1.4 ± 0.3	7.3 ± 0.5	9.4 ± 0.1
	2.5	0	0	0	0	0	0	0	3.6 ± 0.4	7.4 ± 0.2
	5.0	0	0	0	0	0	0	0	0	0

Note: the growth diameters are expressed as means ± SD of three independent experiments. In the control plates without any biocide the coverage was total after ten days in both cases (diameter = 9.5 cm).

4000–500 cm^{-1} spectral range. For each sample, one scan per sample on transmission mode were recorded with a resolution of 4 cm^{-1}.

Crystallographic data of samples were obtained by X-ray diffraction (XRD) on semioriented (powder) samples with a Philips 3710 diffractometer using Cu Kα radiation 40 kV, 20 mA, Ni filter and patterns collected from 3 to 12° (2θ).

Thermogravimetric experiments (TG) were carried out using a Rigaku Thermo plus EVO instrument, with alumina as reference. The samples were placed into Pt crucibles and maintained at air throughout the heating period. The temperature was increased at a constant rate of 10 °C/min.

Zeta potential measurements (ZP) were carried out using the Brookhaven equipment (Zeta Potential function), at constant ionic strength of 10^{-3} M KCl. Samples were prepared at several pH values equilibrated for 24 h by dropwise addition of concentrated HCl or KOH solutions. Morphological observations of the samples were made by scanning electron microscopy (SEM) using a file emission gun scanning electron microscope JCM-6000.

2.5. Formulation and preparation of paints

The Mt hybrid with the higher antifungal activity was selected to integrate a waterborne acrylic paint formulation. The paints prepared corresponded with a conventional waterborne indoor formulation: pigments, acrylic resin, additives and DW as solvent (Deyá and Bellotti, 2017). The composition used was: 38.9% of DW (% by weight), 15.5% TiO_2, 30.2% talc, 6.4% Mt hybrid, 6.0% acrylic resin (1:1), 3.0% additives (antifoaming, cellulosic thickener, dispersants and surfactants). This paint was labeled as PMtH. Parallelly other paint was done with the similar composition but instead of Mt hybrid, Mt and free Col were added (PMtC). The amount of Col in this case was the same as that incorporated in PMtH. Controls paints without biocides were prepared: one with the base composition without Mt which was replaced by talc (paint P) and other with the raw Mt (paint PMt) replacing the Mt hybrid. The paints elaboration was carried out in a high speed disperser located in the pilot plant of CIDEPINT.

Taking into account, the importance of the water availability to fungal development in materials, water absorption of the paints was assessed. Acrylic panels were painted and cured for 15 days. Afterwards, they were placed in a dried environment for 3 days, weight in an analytical scale and placed into a closed environment with 100% of humidity at 23 ± 2 °C. Water vapor absorption was followed weighting the panels for 5 days. The reported experimental data is the average of three measurements each.

2.6. Bio-resistance test

The prepared paints were applied on slide glasses and cured under normal laboratory conditions. The bio-resistance test procedure was similar to that reported in previous published research (Bellotti et al., 2015). Coatings samples were disposed in plates with 8.5 mL of minimum mineral medium, six for each paint (P, PMt, PMtH and PMtC), inoculated with a same volume (50 μL) of spore suspension (10^5 spores/mL) and incubated at 28 °C during a month. The same strains were used: *Chaetomium globosum* (KU936228), *Alternaria alternata* (KU936229). Other strain was incorporated to this essay, *Aspergillus fumigatus* (KU936230), due to its importance to the human health in indoor environmental (Kousha et al., 2011; Lee et al., 2013).

Biofilm growth was assessed using the rating scale proposed by ASTM D5590 standard specification. In this way, the area covered are referred to percentages (%): none, 0%, trace of growth, < 10%, light growth, 10–30%, moderate growth, 30–60%, and heavy growth, 60–100% corresponding to a scale between 0 and 4, respectively.

At the end of the bio-resistance test, coatings were observed by SEM with a Philips FEI Quanta 200 microscope. The working conditions were low vacuum (10^{-2}–1 Torr).

3. Results

3.1. Assessment of the antimicrobial potentialities of three biogenic compounds

Fungal inhibitive performance of the terpenoids studied was assessed by measuring the diameters of the fungal growth in agar plates over time. Growth diameters after ten days are presented in the Table 1. The average diameters values were graphed with their respective standard deviation (SD). Among the three terpenoids studied, Lol was the least active since it could not prevent the growth of *C. globosum* in any of the tested concentrations. The compound that managed to inhibit 100% of the growth of both strains with the lowest concentrations (1.2 mg/mL) tested was Col which can be corroborated in the Fig. 2a and b. The photographic records resulted from plates are presented in the Fig. 2c. In general, *A. alternata* showed to be the most sensitive strain in this assay. Therefore, Col was the monoterpene selected to synthesize the Mt hybrids in the next stage.

3.2. Characterization of Mt hybrids

In the first place, the bioactivity of the samples was assessed by the agar-diffusion test. The diameters of the inhibition zones obtained are

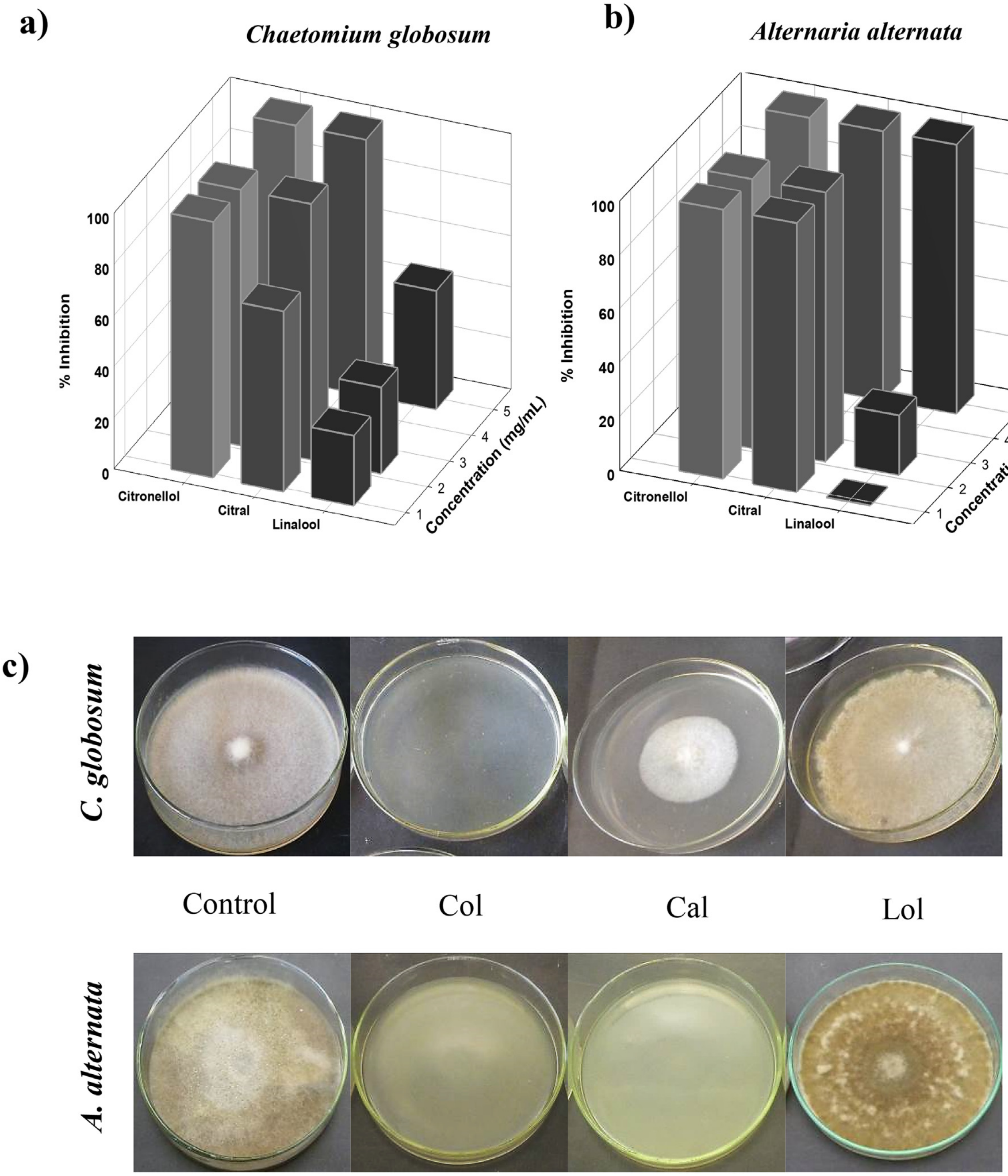

Fig. 2. Fungal inhibition assay: (a and b) % Inhibition calculated after ten days of incubation; c) plates after ten days of incubation with the lowest concentration (1.2 mg/mL) of Citronellol (Col), Citral (Cal) and Linalool (Lol) against tested fungi. Controls correspond with plates without biocides.

Table 2
Agar-diffusion test: diameters of inhibition zone (mm).

Samples	Mt	S-Mt	Mt2.5	Mt5	Mt10	Mt20	S-Mt2.5	S-Mt5	S-Mt10	S-Mt20	S-Mt40A	S-Mt40B
C. globosum	< 7	< 7	< 7	< 7	7*	7*	12.2 ± 0.9	10.9 ± 0.9	10.7 ± 0.8	10.9 ± 0.6	11.0 ± 1.0	14.0 ± 1.0
A. alternata	< 7	< 7	< 7	< 7	< 7	7*	< 7	< 7	11.5 ± 0.9	11.1 ± 1.4	14.5 ± 0.9	16.7 ± 1.2

Note: D < 7 and 7* mean: no active sample and inhibition of microbial growth over the sample, respectively.

presented in Table 2, data are expressed as mean ± SD of three experiments. The result was promising and showed that in general the modified Mt with soy lecithin and Col was more active than the correspond Mt without treatment with S. In that sense, while S—Mt2.5 and S—Mt10 were active against *C. globosum* and *A. alternate*, Mt-2.5 and Mt-10 did not show such activity, respectively. Mt and S—Mt without Col were not active. S-Mt40A and S-Mt40B showed higher inhibition zones especially when both organic compounds were added at the same time (S-Mt40B) which can be corroborated in Fig. 3a. In addition, it could be noted that this hybrid maintained the antifungal activity over time showing no growth after 6 days in the diffusion test (see graphical abstract).

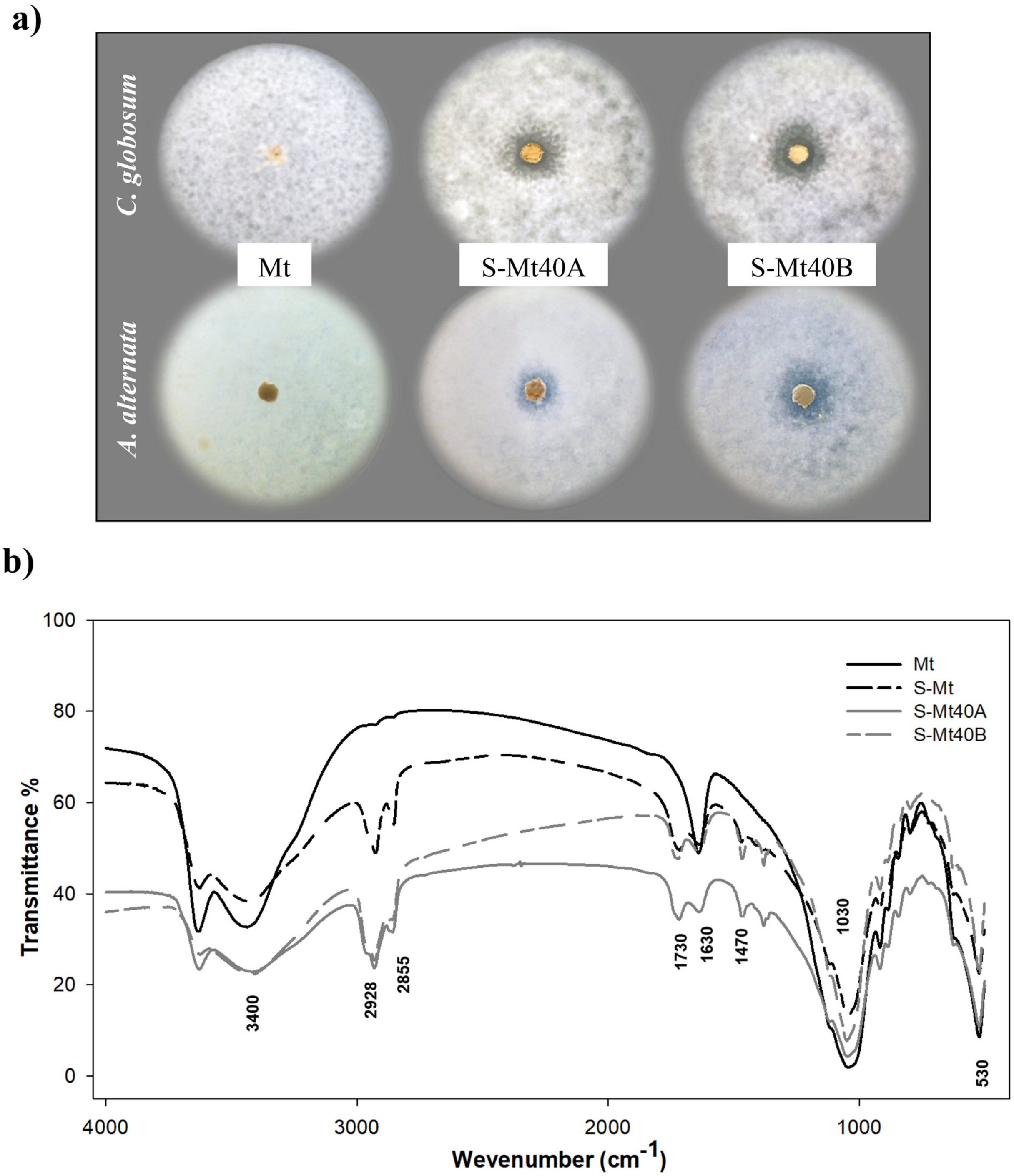

Fig. 3. a) Agar-diffusion test: plates after 48 h of incubation with Mt and S-Mt40A/B; b) FTIR spectra and c) XRD patterns of Mt, S—Mt (Mt modified with soy lecithin) and S-Mt40A/B (S—Mt with Citronellol); d) TGA curves of indicated samples; e) ZP values as a function of pH being: (■) S-Mt, (◊) S-Mt40A and (○) S-Mt40B; f and e) SEM micrographs from S-Mt40A and S-Mt40B, respectively.

Taking into account these results, S-Mt40B was selected to integrate the formulation of the experimental waterborne paint and to be more intensely studied.

FTIR of Mt, S-Mt, S-Mt40A and S-Mt40B spectra is presented in Fig. 3b. The modified Mt has some new peaks, 2928 cm^{-1} and 2855 cm^{-1}, compared with the raw Mt which would correspond with aliphatic CH (Chen et al., 2019). Adding to this, it can be seen a peak at 1730 cm^{-1} which appear by the modification with the soy lecithin due to C=O groups presented. All samples presented a peak ~1630 cm^{-1} due to O—H stretching from Mt (Nagy et al., 2013). The Mt hybrids presented a peak at 1470 cm^{-1} corresponding with amonium salts. The broadband at ~ 3400 cm^{-1} region was wider in the case of hybrids with Col which is an aliphatic alcohol. All the samples presented peaks at 1040 cm^{-1} that correspond with stretching vibration of Si—O (Giannakas et al., 2017).

X-ray diffractograms of raw Mt, S—Mt, S-Mt40A and S-Mt40B samples are presented in Fig. 3c. The X-ray diffraction pattern of the Mt sample shows a reflection (001) peak at 1.27 nm (7° 2θ) corroborating the predominance of a one-layer hydrate in the interlayer space (Lagaly et al., 2006). The adsorption of the organic modified expanded the interlayer space 0.12 nm (to 1.39 nm) for the Mt sample in S—Mt This basal spacing is close to that calculated by Nagy et al. (2013) to Mt

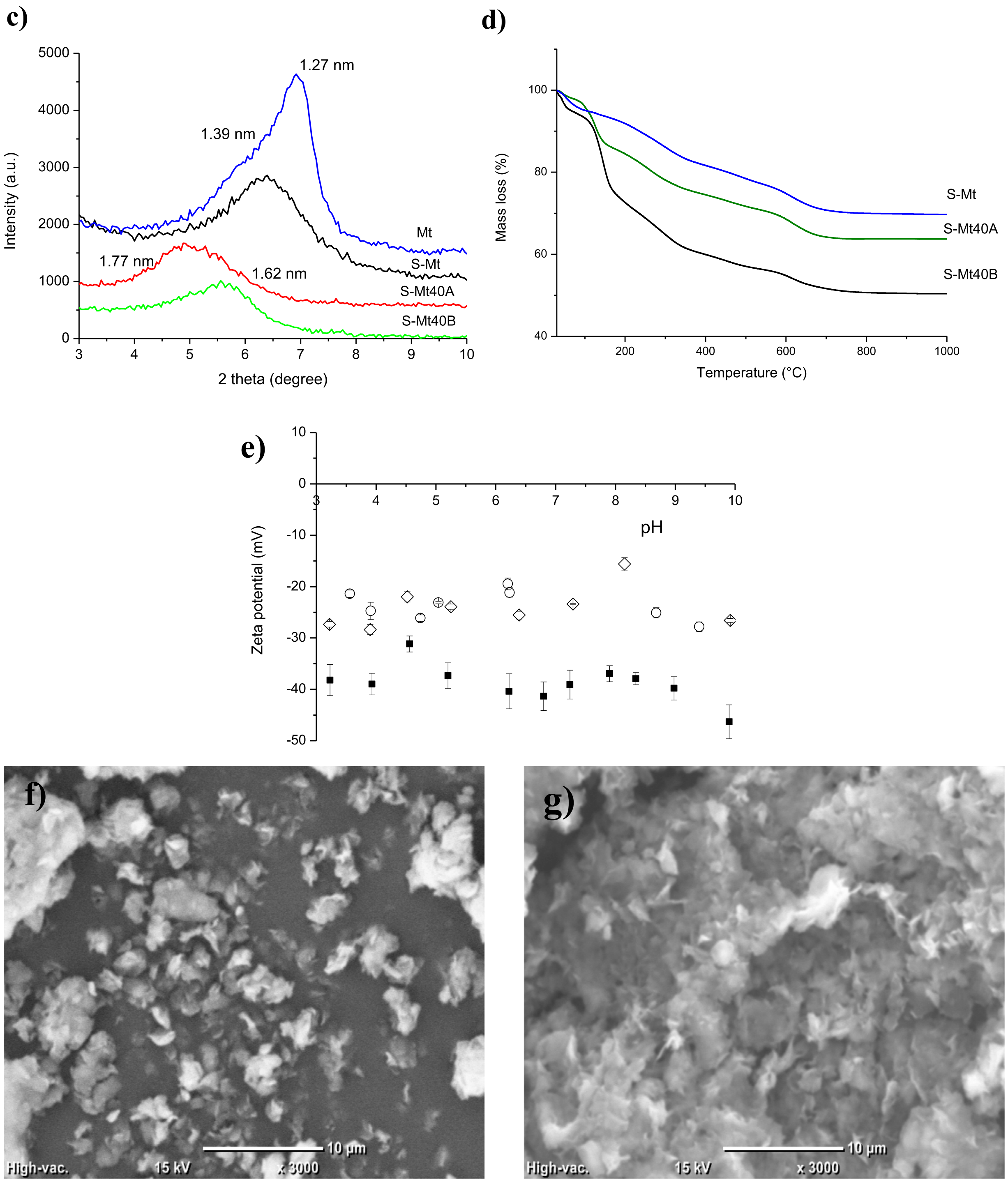

Fig. 3. (*continued*)

hybrids with lysolecithin (1.36 nm) if the lysolecithin molecule is supposed to adopt a flat-lying position between the layers for monolayer orientation.

The increase of the basal spacing (0.12 nm) coincided with the value obtained by Merino et al. (2016) for a bentonite (1.32 nm) and the hybrid soy lecithin-bentonite (1.44 nm) with a reaction time of 30 min. In the case of Nagy et al. (2013) determined that the basal spacing of Mt (1.23 nm) shifted to 1.51 nm to the modified Mt with lysolecithin, revealing an increase of 0.28 nm for air-drying sample. These differences in the basal spacing could be due to different drying methodologies in the case of the present study hybrids were dried under vacuum.

On the other hand, taking into acount that 001 value of dehydrated Mt is 0.97 nm (Barraqué et al., 2018) the interlayer space thicknesses of S—Mt simple would be of 0.42 nm which could be indicating a posible bilayer arrangment of lecithin molecules in the Mt interlayer.

The analysis of the reflection (001) for the Mt hybrids (S-Mt40A and S-Mt40B) indicated an increase of the basal spacing of 0.50 nm and 0.35 nm, respectively to the same reflection (001) position found for the Mt sample. This was attributed to soy lecithin and Col intercalation. The difference in the basal spacing increase to S-Mt40A and S-Mt40B could be due to the difference in the arrangement in the interlayer obtained to the biosurfactant and the monoterpene studied due to the difference in the preparation process. The basal spacing strongly depends on the packing density of surfactant within the montmorillonite

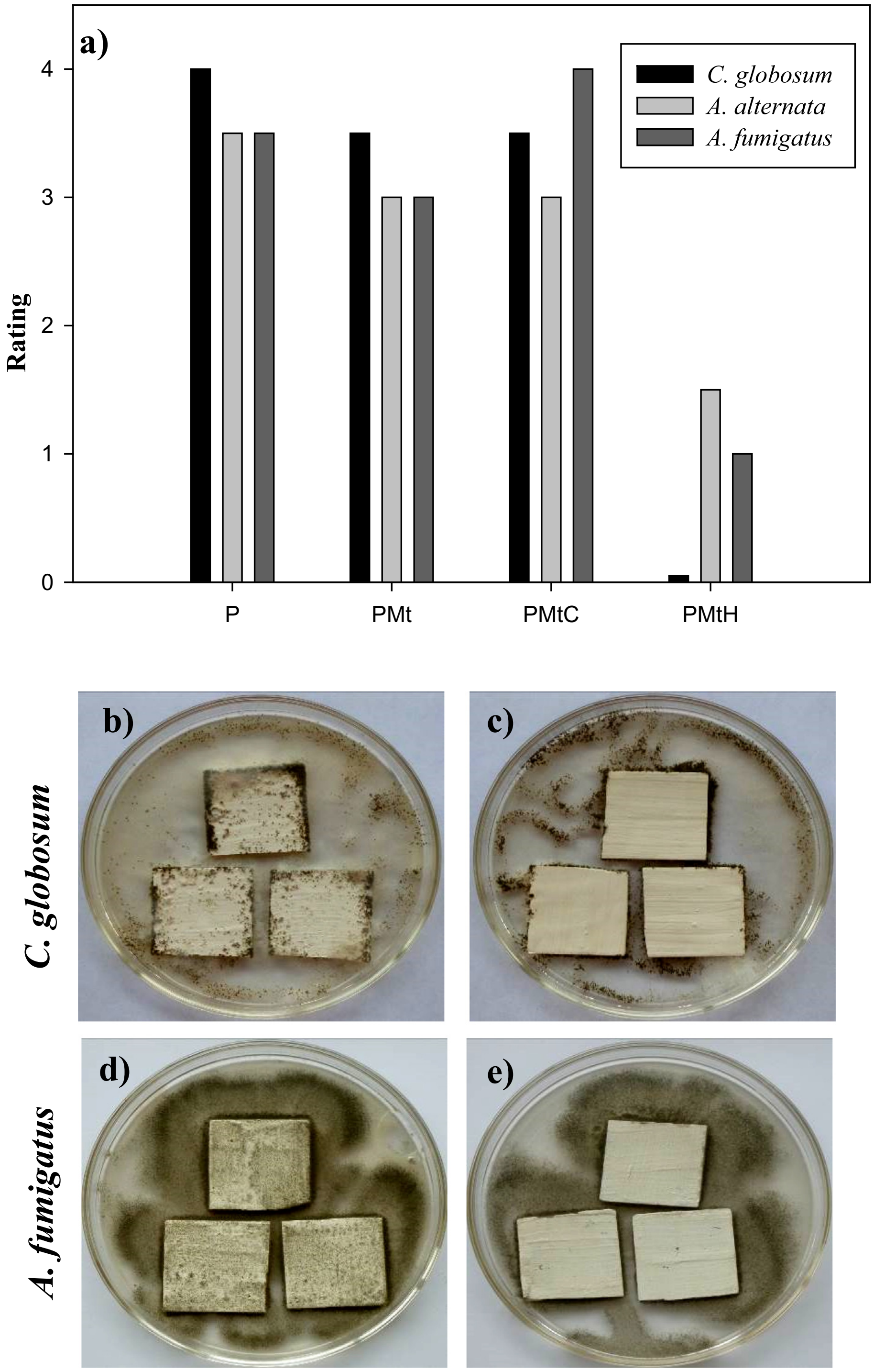

Fig. 4. Coating bio-resistance tests: (a) fungal growth rating by ASTM D5590 standard specification; (b,c) Images from PMtC (control paint with citronellol) and (d,e) PMtH, (paint with the Mt hybrid) plates incubated at 28 °C during after 4 weeks.

interlayer space (He et al., 2006).

Fig. 3d illustrates the TG analysis curves of the modified Mt (S—Mt, S-Mt40A and S-Mt40B). In the case of S-Mt the curve showed distinct stages of mass loss percentage. The corresponding temperature ranges coincided with the well-defined stages of the decomposition of lecithin (Nagy et al., 2013). The TG curve of S-Mt reveals a mass loss of 4.91% at 62.3 °C corresponding to the desorption of the water adsorbed on the surface and in the interlayer space. The water adsorved for the S-Mt resulted lower that the reported for unmodified Mt which resulted of 13.3% (at 123 °C) (Fernández Solarte et al., 2019). The mass loss at 200–1000 °C was 25.4% and is attributable to the descomposition of lecithin and dehydroxilation layered (Zhou et al., 2007). In the case of Mt, 2.6% has been reported as the mass loss due to the structural deshydroxilation of a Na-montmorillonite with the same origin (Fernández Solarte et al., 2019).

In the case of S-Mt40A (sample containing Col) a further increase in mass loss is observed with respect to S-Mt sample, indicating that Col was bound to the sample. This effect was favored with soy lechitin presence. The Col content, derived from the TG, curves was ~ 7%. It would be adsorbed in the free pores between the particles and in the

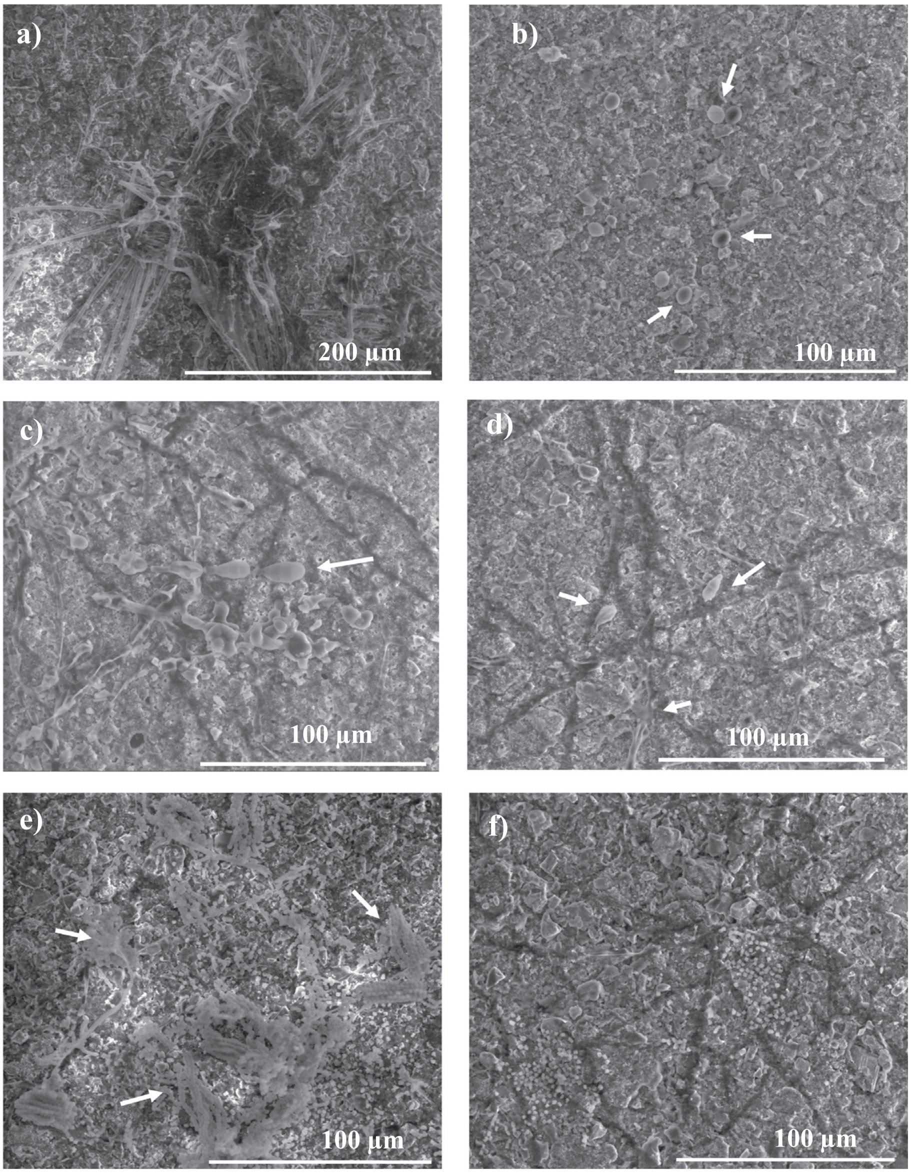

Fig. 5. SEM micrographs from coating bio-resistance test: coatings inoculated with (a, b) *C. globosum*, (c, d) *A. alternaria* and (e, f) *A. fumigatus*; (a, c and e) PMtC (control paint with citronellol) and (b, d and f) PMtH, (paint with the Mt hybrid). Coatings samples incubated at 28 °C for four weeks.

basal spacing of S-Mt, because an increase interlayer structure of clay with alkyl chains was seen (Fig. 3c). At 200–1000 °C the mass loss resulted ~ 25%. To S-Mt40B sample, a mass loss of 6.78% at 51.5 °C corresponding to desorption of the water adsorbed was noted. The removal of physically adsorved and intercalated water, shifted to lower temperatures compared with S-Mt (62.3 °C) and Mt (123 °C), this would be explained by the water molecules that weakly interact with the Mt hybrid with lower surface energy when it incorporate the monoterpene (Merino et al., 2016). Adding to this the incorporation of the Col was corroborated by XRD due to the increce of the basal spicing determined. A mass loss of 42.81% in the range of 200–1000 °C was observed. In this case, the loss of soy lecithin and Col was greater, so it could be inferred that this method of synthesis was more efficient because more quantity of components were incorporated. Therefore, a greater amount of the terpenoid compound was incorporated since it is evidenced for the latter sample greater antifungal activity. (see Fig. 3a).

The ZP values of the different samples as a function of pH are showed in Fig. 3e. The ZP of samples with citronellol was negative. As illustrated in Fig. 3e, the values of negative zeta potential on the particles ranged from −20 to −30 mV for S-Mt40A and S-Mt40B. The negative charge on the surface shows that the association is superficial since all the components have a negative charge when they are separated (Septiyanti et al., 2017). These values are in agreement with those found for lecithin micelles that take negative surface charge values (Boelter and Brandelli, 2016). The adsorbate interacts with the adsorbent surface more frequently with hydrogen bonds, electrostatic and hydrophobic interactions. In fact, hydrogen bonding can occur by the interaction of hydroxyl groups in clay molecules with an unsaturated carbon atom or the aliphatic ring of terpenoid molecule. The hydrophobic interaction may appear between the hydrophobic terpenoids and the hydrophobic character of the lecithin molecules adsorbed in the clay (Nakhli et al., 2018).

The ZP values of the samples were not significantly different that S-Mt Zeta potential is an indicator of the charge presence on the surface of the particle, thereby indicating the degree of stability.

SEM images of S-Mt40A and S-Mt40B (Fig. 3f and g), show a uniform size of about 1 μm and higher agglomeration than that obtained for Mt sample (Fernández et al., 2014). The micrographs did not revealed structural differences between S-Mt40A and S-Mt40B.

3.3. Formulation and preparation of paints

Taking into account, that S-Mt40B showed higher fungal inhibition than the rest of the hybrids, it was selected to integrate an indoor waterborne acrylic paint formulation (PMtH). The paint prepared corresponded with a conventional formulation where the $CaCO_3$ was replaced by another extender pigment (talc) (Roselli et al., 2014). The replacement was done due to the paint with $CaCO_3$ was not homogeneous making its applicability impossible this can be seen in the Fig. 1 of supplementary material. The water absorption of the paints showed (Fig. 2 of supplementary material) that in the first hours the paint with raw Mt (PMt) is the one that absorbs more water compared with the others. Control paint (P) absorbs water continuously while paints with clay have discontinuities, probable due to absorption-desorption processes of the clay itself. In the case of the paint with Mt and free Col (PMtC), the absorption of water is less than in the case of the control maybe due to the loss of oil during the assay.

3.4. Bio-resistance test

Bio-resistance test of paints prepared (P, PMt, PMtC and PMtH) was performed by exposing the films during four weeks to fungal growth in plates. After that, coating samples were rated using ASTM D5590 standard scale. The results of this test can be seen in Fig. 4a. PMtH inoculated with *C. globosum* presented no growth, rating (R) as 0 which contrasts greatly with that obtained with the paint containing Col in free-form (PMtC). In this sense, PMtC presented heavy fungal growth ($R = 4$) with a high development. These observations can be corroborated in the Fig. 4b and c. These results coincided with those found by coatings inoculated with *A. fumigatus* which can be seen in the Fig. 4d and e.

The volatile nature of the monoterpenoids as Col could explain the reason that it cannot be retained in the PMtC films and therefore it would not be available to impart its antifungal activity. In addition, the inclusion of the Col in a free-form could lead to reactions with some of the components contained in the paint which would decrease its bioactivity. On the other hand, the good performance of the Mt hybrid as part of the coating beside to improve the retention of Col, could be related with a more efficient arrangement achieved by monoterpenoid molecules in the nano-carrier (Hendessi et al., 2016).

Coatings inoculated with *A. alternata* yielded similar results but with a smaller difference between PMtC and PMtH with a R assigned were 3 and ~2, respectively. InIn general the control paints P and PMt, presented high fungal development with coverage of the area > 60% with the three strains.

SEM micrographs in Fig. 5. show the contrast between the fungal development between PMtC and PMtH. In this sense, PMtC sample inoculated with *C. globosum* presented a thick hyphae network unlike what seen in PMtH where only ungerminated spores were observed (marked with white arrows) as can be seen in the Fig. 5a and b, respectively. In the Fig. 5c which corresponds to the control paint, an active growth of *A. alternata* with a higher degree of development than in the case of the paint that contained the hybrid (PMtH) in the Fig. 5d, is showed. Coatings inoculated with *A. fumigatus* presented appreciable differences by SEM. In this sense, micrograph in Fig. 5e from PMtC show a highly developed biofilm with great quantity of reproductive structures (conidiophores, marked with white arrows) while in PMtH the development was much lower and a lot of ungerminated spores can be seen in Fig. 5f. These results are highly promising due to the importance of *A. fumigatus* in infections in indoor environments as was stated in the introduction of the present research work.

There are reports about modified clays, bentonite, halloysite and sepiolite, used to be applied in functional coatings with antimicrobial and self-cleaning properties (Chen et al., 2019; Clegg et al., 2019; Hendessi et al., 2016). Currently regulation agencies and consumers demands are tending towards utilization of natural compounds such as essential oils and their derivatives (Bellotti and Deyá, 2018; Shemesh et al., 2015). In this sense, carvacrol is the monoterpene that registers more published works regarding to antimicrobial films and coatings (Alkan Tas et al., 2019; Hendessi et al., 2016; Shemesh et al., 2015). In all the cases to obtain halloysite nanotubes loaded with carvacrol, ultrasonication with a microprobe is used which would seem difficult to take to a larger scale of production. On the other hand, as in the present study, montmorillonite has been modified with compounds derived from plants such as essential oils, monoterpenes and lecithin to obtain hybrids for functional materials applications (Giannakas et al., 2017; Merino et al., 2016; Nagy et al., 2013). In this sense, the present investigation is the first that obtain an affective hybrid with citronellol and Mt to be applied in a antifungal coating formulation. Adding to this has the advantage of employ natural and removable modifiers to obtain the functional bioactive hybrids.

4. Conclusions

The present work shows the feasibility of use modified montmorillonite as bioactive filler material which count with a promising wide field of applications besides coating technology. Therefore, the exploitation of local deposits of these minerals is highly favorable due to they can be subject to an interesting variety of possible modifications to which they may be subject.

Monoterpenes compounds, from plant extracts, can be used as antifungal agents. Citronellol proved to be the most active one. Montmorillonite from Rio Negro (Argentina) an abundant and inexpensive resourses, can be modified with soy lecithin and citronellol to obtain hybrid materials that could be used as antimicrobial component for functional waterborne coatings. The use of an organic surfactant as soy lecithin showed to be more effective to achieve the stated objective. Therefore, for the first time the use of citronellol for application in antifungal waterborne coatings was reported. The most active hybrid prepared was that in which soy lecithin and citronellol (40% wt.) were added at the same time. The X-ray diffraction measumerents reveled a basal spacing of 1.62 nm for this hybrid showed a shifted respect to the clay modified with soy lecithin (1.39 nm) which was attributed to soy lecithin and Col intercalation. The incorporation of Col in the process of preparation of the organo-clay was reflected in the TG curves and the mass loss showed a significative increase at temperature $> 200^\circ$ from 25% for S-Mt to 42% for the hybrid. This bioactive hybrid achieved to impart its functionality into a waterborne paint formulated with 5% wt. in bioassays against three frequent fungal strains. Therefore the method used has the advantage of being easily scalable and low cost.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clay.2019.105369.

References

Alkan Tas, B., Sehit, E., Erdinc Tas, C., Unal, S., Cebeci, F.C., Menceloglu, Y.Z., Unal, H.,

2019. Carvacrol loaded halloysite coatings for antimicrobial food packaging applications. Food Packag. Shelf Life 20, 100300. https://doi.org/10.1016/j.fpsl.2019.01.004.

Barraqué, F., Montes, M.L., Fernández, M.A., Mercader, R.C., Candal, R.J., Torres Sánchez, R.M., 2018. Synthesis and characterization of magnetic-montmorillonite and magnetic-organo-montmorillonite: surface sites involved on cobalt sorption. J. Magn. Magn. Mater. 466, 376–384. https://doi.org/10.1016/j.jmmm.2018.07.052.

Bellotti, N., Deyá, C., 2018. Natural products applied to antimicrobial coatings. Stud. Nat. Prod. Chem. https://doi.org/10.1016/B978-0-444-64181-6.00014-0.

Bellotti, N., Salvatore, L., Deyá, C., Del Panno, M.T., del Amo, B., Romagnoli, R., 2013. The application of bioactive compounds from the food industry to control mold growth in indoor waterborne coatings. Colloids Surf. B: Biointerfaces 104. https://doi.org/10.1016/j.colsurfb.2012.11.037.

Bellotti, N., Romagnoli, R., Quintero, C., Domínguez-Wong, C., Ruiz, F., Deyá, C., 2015. Nanoparticles as antifungal additives for indoor water borne paints. Prog. Org. Coat. 86. https://doi.org/10.1016/j.porgcoat.2015.03.006.

Bianchi, A.E., Fernández, M., Pantanetti, M., Viña, R., Torriani, I., Sánchez, R.M.T., Punte, G., 2013. ODTMA+ and HDTMA+ organo-montmorillonites characterization: new insight by WAXS, SAXS and surface charge. Appl. Clay Sci. 83–84, 280–285. https://doi.org/10.1016/j.clay.2013.08.032.

Boelter, J.F., Brandelli, A., 2016. Innovative bionanocomposite films of edible proteins containing liposome-encapsulated nisin and halloysite nanoclay. Colloids Surf. B: Biointerfaces 145, 740–747. https://doi.org/10.1016/j.colsurfb.2016.05.080.

Chellappandian, M., Vasantha-srinivasan, P., Senthil-nathan, S., Karthi, S., Thanigaivel, A., Ponsankar, A., Kalaivani, K., Hunter, W.B., 2018. Botanical essential oils and uses as mosquitocides and repellents against dengue. Environ. Int. 113, 214–230. https://doi.org/10.1016/j.envint.2017.12.038.

Chen, B., Jia, Y., Zhang, M., Li, X., Yang, J., Zhang, X., 2019. Facile modification of sepiolite and its application in superhydrophobic coatings. Appl. Clay Sci. 174, 1–9. https://doi.org/10.1016/j.clay.2019.03.016.

Clegg, F., Breen, C., Muranyi, P., Schönweitz, C., 2019. Antimicrobial, starch based barrier coatings prepared using mixed silver/sodium exchanged bentonite. Appl. Clay Sci. 179, 105144. https://doi.org/10.1016/j.clay.2019.105144.

Deyá, C., Bellotti, N., 2017. Biosynthesized silver nanoparticles to control fungal infections in indoor environments. Adv. Nat. Sci. Nanosci. Nanotechnol. 8. https://doi.org/10.1088/2043-6254/aa6880.

Edge, M., Allen, N.S., Turner, D., Robinson, J., Seal, K., 2001. The enhanced performance of biocidal additives in paints and coatings. Prog. Org. Coat. 43, 10–17. https://doi.org/10.1016/S0300-9440(01)00244-2.

Falkiewicz-Dulik, M., Janda, K., Wypych, G., 2015. Biodegradation, biodeterioration, and biostabilization of industrial products. Handb. Mater. Biodegrad. Biodeterior. Biostabl. 99–132. https://doi.org/10.1016/b978-1-895198-87-4.50008-6.

Fernández, M.A., Bellotti, N., 2017. Silica-based bioactive solids obtained from modified diatomaceous earth to be used as antimicrobial filler material. Mater. Lett. 194. https://doi.org/10.1016/j.matlet.2017.01.144.

Fernández Solarte, A.M., Villarroel-Rocha, J., Morantes, C.F., Montes, M.L., Sapag, K., Curutchet, G., Torres Sánchez, R.M., 2019. Insight into surface and structural changes of montmorillonite and organomontmorillonites loaded with Ag. Comptes. Rendus Chim. 22, 142–153. https://doi.org/10.1016/j.crci.2018.09.006.

Fernández, M., Curutchet, G., Torres Sánchez, R.M., 2014. Removal of humic acid by organo-montmorillonites: Influence of surfactant loading and chain length of alkylammonium cations. Water Air Soil Pollut. 225. https://doi.org/10.1007/s11270-014-1987-9.

Gamba, M., Flores, F.M., Madejová, J., Sánchez, R.M.T., 2015. Comparison of imazalil removal onto montmorillonite and nanomontmorillonite and adsorption surface sites involved: an approach for agricultural wastewater treatment. Ind. Eng. Chem. Res. 54, 1529–1538. https://doi.org/10.1021/ie5035804.

Giannakas, A., Tsagkalias, I., Achilias, D.S., Ladavos, A., 2017. A novel method for the preparation of inorganic and organo-modified montmorillonite essential oil hybrids. Appl. Clay Sci. 146, 362–370. https://doi.org/10.1016/j.clay.2017.06.018.

He, H., Frost, R.L., Bostrom, T., Yuan, P., Duong, L., Yang, D., Xi, Y., Kloprogge, J.T., 2006. Changes in the morphology of organoclays with HDTMA+ surfactant loading. Appl. Clay Sci. 31, 262–271. https://doi.org/10.1016/j.clay.2005.10.011.

Hendessi, S., Sevinis, E.B., Unal, S., Cebeci, F.C., Menceloglu, Y.Z., Unal, H., 2016. Antibacterial sustained-release coatings from halloysite nanotubes/waterborne polyurethanes. Prog. Org. Coat. 101, 253–261. https://doi.org/10.1016/j.porgcoat.2016.09.005.

Jaiswal, S., Bhattacharya, K., Sullivan, M., Walsh, M., Creaven, B.S., Laffir, F., Duffy, B., McHale, P., 2013. Non-cytotoxic antibacterial silver-coumarin complex doped sol-gel coatings. Colloids Surf. B: Biointerfaces 102, 412–419. https://doi.org/10.1016/j.colsurfb.2012.07.047.

Johns, K., 2003. Hygienic coatings: the next generation. Surf. Coat. Int. Part B Coat. Trans. 86, 101–110. https://doi.org/10.1007/BF02699620.

Kousha, M., Tadi, R., Soubani, A.O., 2011. Pulmonary aspergillosis: a clinical review. Eur. Respir. Rev. 20, 156–174. https://doi.org/10.1183/09059180.00001011.

Lagaly, G., Ogawa, M., Dékány, I., 2006. Chapter 7.3 Clay mineral organic interactions. Dev. Clay Sci. 1, 309–377. https://doi.org/10.1016/S1572-4352(05)01010-X.

Lee, S.Y., Kim, S.H., Hong, C.Y., Park, M.J., Choi, I.G., 2013. Effects of (−)-borneol on the growth and morphology of Aspergillus fumigatus and Epidermophyton floccosom. Flavour Fragr. J. 28, 129–134. https://doi.org/10.1002/ffj.3138.

López, P., Sánchez, C., Batlle, R., Nerín, C., 2007. Development of flexible antimicrobial films using essential oils as active agents. J. Agric. Food Chem. https://doi.org/10.1021/jf071737b.

Magnoli, A.P., Tallone, L., Rosa, C.A.R., Dalcero, A.M, Chiacchiera, S.M., Torres Sanchez, R.M., 2008. Commercial bentonites as detoxifier of broiler feed contaminated with aflatoxin. Appl. Clay Sci. 40, 63–71. https://doi.org/10.1016/j.clay.2007.07.007.

Magnusson, J., Schnürer, J., 2001. The Carnegie stages34. Appl. Environ. Microbiol. 67, 1–5. https://doi.org/10.1128/AEM.67.1.1.

Mardones, L.E., Legnoverde, M.S., Monzón, J.D., Bellotti, N., Basaldella, E.I., 2019. Increasing the effectiveness of a liquid biocide component used in antifungal waterborne paints by its encapsulation in mesoporous silicas. Prog. Org. Coat. 134. https://doi.org/10.1016/j.porgcoat.2019.04.058.

Merino, D., Ollier, R., Lanfranconi, M., Alvarez, V., 2016. Preparation and characterization of soy lecithin-modified bentonites. Appl. Clay Sci. 127–128, 17–22. https://doi.org/10.1016/j.clay.2016.04.006.

Nagy, K., Bíró, G., Berkesi, O., Benczédi, D., Ouali, L., Dékány, I., 2013. Intercalation of lecithins for preparation of layered nanohybrid materials and adsorption of limonene. Appl. Clay Sci. 72, 155–162. https://doi.org/10.1016/j.clay.2012.11.008.

Nakhli, A., Mbouga, M.G.N., Bergaoui, M., Khalfaoui, M., Cretin, M., Huguet, P., 2018. Modeling of essential oils adsorption onto clays towards a better understanding of their interactions. J. Mol. Liq. 249, 132–143. https://doi.org/10.1016/j.molliq.2017.11.012.

Negut, I., Grumezescu, V., Ficai, A., Grumezescu, A.M., Holban, A.M., Popescu, R.C., Savu, D., Vasile, B.S., Socol, G., 2018. MAPLE deposition of *Nigella sativa* functionalized Fe 3 O 4 nanoparticles for antimicrobial coatings. Appl. Surf. Sci. 455, 513–521. https://doi.org/10.1016/j.apsusc.2018.05.202.

Olugbenga, E., Oladele, A., Esho, O., 2016. Journal of applied research on medicinal and aromatic plants effects of storage time on the antimicrobial activities and composition of lemon grass oil. J. Dermatol. Sci. 3, 105–111. https://doi.org/10.1016/j.jarmap.2016.02.005.

Pigeot-Remy, S., Real, P., Simonet, F., Hernandez, C., Vallet, C., Lazzaroni, J.C., Vacher, S., Guillard, C., 2013. Inactivation of Aspergillus Niger spores from indoor air by photocatalytic filters. Appl. Catal. B Environ. 134–135, 167–173. https://doi.org/10.1016/j.apcatb.2013.01.023.

Ramos, M., Jim??nez, A., Peltzer, M., Garrig??s, M.C., 2012. Characterization and antimicrobial activity studies of polypropylene films with carvacrol and thymol for active packaging. J. Food Eng. 109, 513–519. https://doi.org/10.1016/j.jfoodeng.2011.10.031.

Roselli, S., Bellotti, N., Deyá, C., Revuelta, M., Amo, B., Romagnoli R., 2014. Lanthanum-exchanged zeolite and clay as anticorrosive pigments for galvanized steel. J. Rare Earths 32, 352–359. https://doi.org/10.1016/S1002-0721(14)60078-8.

Sastry, P., Srinivas, K.V.N.S., Kumar, J.K., Kumar, A.N., Rajput, D.K., Maheswara, V.U., 2014. Variation in the essential oil content and composition of Citronella (*Cymbopogon winterianus* Jowitt.) in relation to time of harvest and weather conditions. Ind. Crop. Prod. 61, 240–248. https://doi.org/10.1016/j.indcrop.2014.06.044.

Septiyanti, M., Meliana, Y., Agustian, E., 2017. Effect of citronella essential oil fractions as oil phase on emulsion stability. AIP Conf. Proc. 1904. https://doi.org/10.1063/1.5011927.

Shemesh, R., Krepker, M., Natan, M., Danin-Poleg, Y., Banin, E., Kashi, Y., Nitzan, N., Vaxman, A., Segal, E., 2015. Novel LDPE/halloysite nanotube films with sustained carvacrol release for broad-spectrum antimicrobial activity. RSC Adv. 5, 87108–87117. https://doi.org/10.1039/c5ra16583k.

Siedenbiedel, F., Tiller, J.C., 2012. Antimicrobial polymers in solution and on surfaces: Overview and functional principles. Polymers (Basel). 4, 46–71. https://doi.org/10.3390/polym4010046.

Sørensen, G., Nielsen, A.L., Pedersen, M.M., Poulsen, S., Nissen, H., Poulsen, M., Nygaard, S.D., 2010. Controlled release of biocide from silica microparticles in wood paint. Prog. Org. Coat. 68, 299–306. https://doi.org/10.1016/j.porgcoat.2010.03.009.

Stupar, M., Grbić, M.L., Džamić, A., Unković, N., Ristić, M., Jelikić, A., Vukojević, J., 2014. Antifungal activity of selected essential oils and biocide benzalkonium chloride against the fungi isolated from cultural heritage objects. South African J. Bot. 93, 118–124. https://doi.org/10.1016/j.sajb.2014.03.016.

Weber, D.J., Rutala, W.A., 2013. Self-disinfecting surfaces: review of current methodologies and future prospects. Am. J. Infect. Control 41, S31–S35. https://doi.org/10.1016/j.ajic.2012.12.005.

WHO, 2009. WHO Guidelines for Indoor air Quality: dampness and Mould.

Wu, F., Jin, Y., Xu, X., Yang, N., 2017. Electrofluidic pretreatment for enhancing essential oil extraction from citrus fruit peel waste. J. Clean. Prod. 159, 85–94. https://doi.org/10.1016/j.jclepro.2017.05.010.

Zhou, Q., Frost, R.L., He, H., Xi, Y., 2007. Changes in the surfaces of adsorbed Paranitrophenol on HDTMA organoclay-the XRD and TG study. J. Colloid Interface Sci. 307, 50–55. https://doi.org/10.1016/j.jcis.2006.11.016.