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Anne Loron [a], Christian Gardrat [a], Nicolas Tabary [b], Bernard Martel [b], Véronique Coma [a,*]

[a] Laboratoire de Chimie des Polymères Organiques, Univ. Bordeaux, CNRS, Bordeaux INP, UMR 5629, 16 Avenue Pey-Berland, 33600 Pessac, France
[b] Université Lille, Sciences et Technologies, CNRS, ENSCL, INRAe, UMR 8207, Unité Matériaux Et Transformations (UMET), F-59655 Villeneuve D'Ascq, France

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ABSTRACT

The solubility of the scarcely water-soluble molecule tetrahydrocurcumin (THC), a promising curcumin derivative to be used as biopesticide, was enhanced through the formation of inclusion complexes with different cyclodextrins (CDs). Randomly methylated β-cyclodextrin (MeβCD) gave the best results among the different CDs whose size, substituent or structure was changing. Differential scanning calorimetry as well as ^{1}H- and 2D-Nuclear Magnetic Resonance (NMR) proved the inclusion of THC in MeβCD. Rotating-frame Overhauser effect spectroscopy NMR especially illustrated specific interactions of aromatic protons of THC and protons located inside the CD cavity. The complex formation between THC and MeβCD was studied using the Higuchi and Connor method, giving an association constant of 591 M^{-1}. THC-loaded MeβCD did not show any growth inhibition of the target fungus *Fusarium graminearum*. However, THC-loaded MeβCD polymers exhibited 25% inhibition of the fungal growth, thus making them promising material for solvent-free, aqueous and bio-based fungicide formulations.

1. Introduction

Plants are subject to attacks by fungi, nematodes and insects, which generate yield losses and several species in the genus *Fusarium* are generally considered the most important in Europe as regards crop contamination. Among them, *Fusarium graminearum* can infect cereals such as maize, wheat or barley, leading to yield decreases up to 50 % (Shah et al., 2017) but also the potential production of mycotoxins, that can cause health problems to humans and animals. Nowadays, pest control strategies and particularly regarding *Fusarium* species mainly rely on synthetic fungicides, which could be harmful to human health and detrimental to the environment (Gupta, 2011; Smart, 2003). As mentioned by Kumar and Singh (2015), the entry of synthetic pesticides into the food chain coupled with their bioaccumulation triggers several unforeseen consequences. It has become important now to develop alternative strategies to overcome the drawbacks of synthetic pesticides. Therefore, scientific research has shifted towards the use of sustainable and renewable products, exhibiting significant efficacy while being less harmful to people and environment friendly.

Phenolic compounds belong to one of the most studied classes of natural active substances to be potentially used in biopesticide formulations. They are present in almost all plants and possess antioxidant properties (Da, & Mumper, 2010). Essential oils (Avanço et al., 2017) or cereal extracts (Heidtmann-Bemvenuti et al., 2016) containing phenolic derivatives are already used to control fungal development. Pure phenolic compounds such as carvacrol, thymol and *o*-cresol have also shown ability to reduce the mycelium development (Teodoro, Ellepola, Seneviratne, & Koga-Ito, 2015; Wang et al., 2019). In this respect tetrahydrocurcumin (THC), obtained by hydrogenation of curcumin, one of the curcuminoids present in the rhizomes of *Curcuma longa L.* commonly called turmeric, has shown promising results for biopesticide formulations (Coma, Portes, Gardrat, Richard-Forget, & Castellan, 2011). THC consists of two guaiacyl subunits linked by a C7 aliphatic chain bearing a β-diketone group (THC, Fig. 1 A), and exhibits antioxidant, antifungal and antimycotoxinogenic properties. Unfortunately, its very low water-solubility strongly limits its potential applications in water-based formulations. Many dispersion techniques to increase the solubility of phenolic compounds are reported in the literature (Zhang, Xing, Zhao, &

* Corresponding author.
E-mail addresses: anne.loron@enscbp.fr (A. Loron), christian.gardrat@enscbp.fr (C. Gardrat), nicolas.tabary@univ-lille1.fr (N. Tabary), bernard.martel@univ-lille1.fr (B. Martel), veronique.coma@enscbp.fr (V. Coma).

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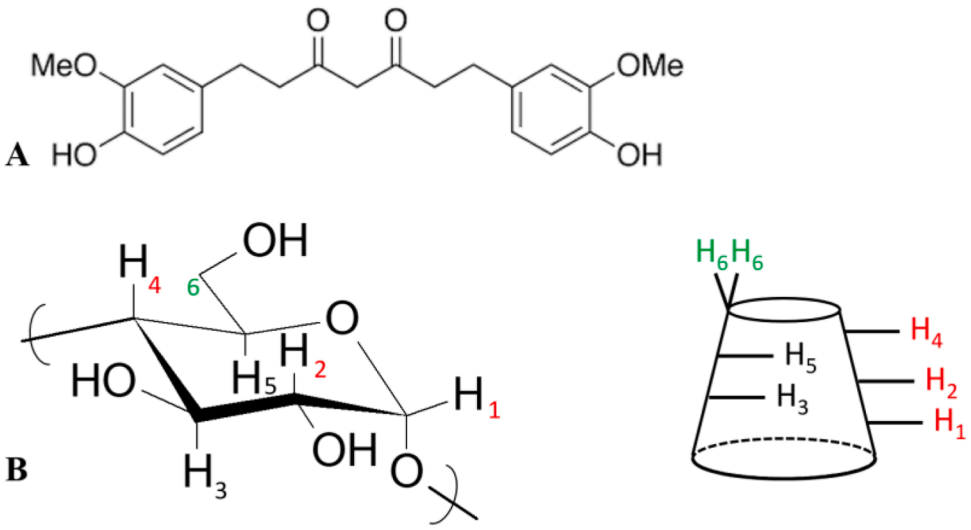

Fig. 1. Schematic representation of THC (A) and cyclodextrin with localization of their protons (B).

Ma, 2018.) and among others, encapsulation techniques such as spray-drying, solvent evaporation, freeze-drying, and liposome formation are attractive (Fang and Bhandari, 2010; Munin, & Edwards-Lévy, 2011). Because of their natural origin and their GRAS status (Braga, 2019), cyclodextrins (CDs) are particularly suitable carrier materials for such hydrophobic active agents (Cheirsilp, & Rakmai, 2017; Del Valle, 2004; Nardello-Rataj, & Leclercq, 2014; Pinho, Grootveld, Soares, & Henriquez, 2014). Briefly, CDs are biopolymers commonly composed of 6,7 or 8 β-linked glucose units, with a funnel-shaped structure composed of an external hydrophilic shell and an internal hydrophobic cavity (Fig. 1 B). The hydrophilic part makes them water-soluble whereas the lipophilic inside confers them the ability to trap hydrophobic molecules. CDs have been scarcely used in agriculture (Morillo, 2006) and inclusion complex systems have been yet reported to increase the solubility of poor water-soluble active agents (Singh et al., 2020) thus allowing a reduction of the applied dose (Morillo, 2006). For instance, CDs have been used to encapsulate synthetic and harmful fungicides like carbendazim (Wang et al., 2017) or chlorothalonil (Gao et al., 2019) and two plant extracts, carvacrol and linalool (Campos et al., 2018). All obtained inclusion complexes have been more efficient to control pest, leading to a reduction of the minimum active concentration.(((((

The aim of the current work was to investigate native and modified CDs to increase the apparent water solubility of THC in order to create new antifungal bio-based complexes potentially used for biopesticide applications.

The impact of some CD structures on the enhancement of the THC solubility was firstly investigated to select the most appropriate cyclodextrin. Described for the first time, THC-loaded MeβCD complexes were then deeply characterized in terms of physico-chemical properties by using differential scanning calorimetry, NMR spectroscopy and scanning electron microscopy. Finally, the bioactive properties of some THC-loaded cyclodextrins were compared to free THC against a model strain of *F. graminearum*.

2. Materials and methods

2.1. Materials

When the abbreviation CD is used, it refers either to the word "cyclodextrin" or to any type of cyclodextrins in this study (βCD, γCD, MeβCD, Poly-βCD and Poly-MeβCD).

β-Cyclodextrin Kleptose® (βCD) and Randomly-methylated-β-cyclodextrin Kleptose Crysmeb® (MeβCD) were provided by Roquette (Batch E0002, Lestrem, France). Citric acid (CTR), sodium dihydrogen hypophosphite ($NaH_2PO_2 \bullet H_2O$) and sodium carbonate (Na_2CO_3) used in the synthesis of polymer of βCD (Poly-βCD) were supplied from Sigma Aldrich (Saint-Quentin Fallavier, France).

MeβCD degree of substitution was 0.5, leading to a molecular mass of 1184 g/mol. γ-Cyclodextrin (γCD) was purchased from TCI Europe. FTIR and DSC spectra of CDs are available in supplementry information (Fig. S1 and Fig. S2).

Anionic water-soluble polymer of βCD (Poly-βCD) and MeβCD (Poly-MeβCD) were previously synthetized according to Junthip, Tabary, Leclercq, & Martel (2015) by using citric acid as crosslinking agent, sodium hypophosphite as catalyst and CD in molar ratio 59/40/1 and 61/40/1 for Poly-βCD and Poly-MeβCD, respectively. Briefly, after water removal, the solid mixture was cured at 140 °C during 30 min under vacuum. Water was then added, the resulting suspension filtered and the filtrate dialyzed during 72 h against water through 6–8 kDa membranes (SPECTRAPOR 1, Spectrumlabs). Finally, the water soluble anionic CD polymer was recovered after freeze drying. The number average molar mass (M_n) measured by aqueous size exclusion chromatography (SEC) were 11,800 g/mol and 15,600 g/mol, respectively for Poly-βCD and Poly-MeβCD (Polydispersity =1.7), using a DIONEX Ultimate 3000 equipped with a DAWN HELEOS II multi-angle light scattering and an OPTILAB rEX differential refractometer (4 columns Shodex OH-Pak 30 cm, flow rate = 0.5 mL/min, concentration of polymer = 3 g/L, mobile phase = 0.1 M $NaNO_3$ and NaN_3, dn/dc = 0.139). Based on the integrations values of H_1 (CD) and citrate methylene signals (CH_2-CTR) by proton NMR studies, Poly-βCD and Poly-MeβCD were estimated to contain 72.0 wt% in βCD moieties and 63.4 wt% in MeβCD moieties respectively. This percentage was employed to calculate the concentration of CD cavities. FTIR spectra of polymers are available in supplementary information (Fig. S3) and DSC spectra in the work of Tabary et al. (2016).

Tetrahydrocurcumin Tetrapure (THC) was purchased from Sabinsa Corporation SabiWhite® (USA).

Potato Dextrose Agar (PDA) medium (potato starch 4g/L, dextrose 20g/L, agar 15g/L) was provided from Biokar. For the Carboxymethylcellulose medium (CMC medium, CMC low viscosity 15 g/L; Yeast extract 1 g/L; $MgSO_4$; $7H_2O$ 0.5 g/L; NH_4NO_3 1 g/L; KH_2PO_4 1 g/L), products were provided by Aldrich (Saint-QuentinFallavier, France) and all salts used in media were in analytical grade.

The strain CBS 185.32 of *F. graminearum* belonging to INRAe MycSA laboratory collection (Centre INRA de Nouvelle-Aquitaine Bordeaux UR1264 MycSA, INRAe, Villenave d'Ornon, France) was used as the targeted strain for antifungal studies.

2.2. Solubility study of THC

2.2.1. Impact of cyclodextrins on THC solubility

Preliminary studies of the maximum solubility of THC with βCD, γCD, MeβCD and two cyclodextrin polymers (Poly-βCD and Poly-MeβCD) have been carried out. Two solutions of each cyclodextrin (15 mM) were prepared in distilled water at 20 °C. THC was added in excess to these solutions, which were stirred for 24 h at 180 rpm. After stirring, the solutions were centrifugated (Jouan CR 4–12) at 1200 *g* and the supernatant was collected. THC concentration in supernatant was determined with a calibration curve obtained by UV spectrophotometry (λ_{max}= 280 nm; Portes, Gardrat, & Castellan, 2007)(Agilent Cary 100 UV–visible).

2.2.2. Solubility curve of THC with randomly methylated β-cyclodextrin

An excess amount of THC (approx. 11 mg) was added to 8 mL of an aqueous MeβCD solution in varying concentrations (0, 5, 10, 15, 20, 30, 40 mM) in amber vials. After 24 h stirring, solutions were centrifuged for 5 minutes at 1200 *g* at room temperature (20°C). The supernatant was analyzed at 280 nm. A 10-times dilution was necessary for samples with highest concentrations of MeβCD to obtain an absorbance lower than 1.5. The THC amount in each sample was determined using calibration curves, previously established with MeβCD solutions at various concentrations. Indeed, the absorption coefficient of THC in the complex depends on ligand concentration within the solution. Solubility diagrams were obtained by plotting THC concentration *versus* MeβCD concentration. The association constant K is given by the Eq. (1) of Higuchi and Connors (Higuchi and Connors, 1965). Assuming that the stoichiometry of the inclusion complex is 1:1, the solubility constant is given by the following equation:

$$K^{1:1} = \frac{slope}{[THC]_0\ (1 - slope)} \quad (1)$$

2.3. Preparation of THC-loaded cyclodextrins and THC-loaded (poly) cyclodextrins

THC was added to a solution of CD adjusted at 15 mM of CD cavities. The amount of THC was given by the previous solubility study for each CD. Solutions were stirred at room temperature for 24 hours and then freeze-dried. The resulted white powder was stored at room temperature in the dark.

2.4. Physicochemical characterization of THC-loaded cyclodextrin

2.4.1. Differential Scanning Calorimetry (DSC)

DSC was performed with TA Instrument Q100 RCS. Approximately 4 mg of compound was placed in an aluminum pan and heated from 20°C to 110°C with a heating rate of 10°C/min under nitrogen (25 mL/min). A first cycle with a plateau at 90°C was necessary to eliminate water.

2.4.2. ^{1}H-NMR investigations

NMR was performed using a Liquid-state 400 MHz NMR spectrometer (Bruker AVANCE I). ^{1}H-NMR was performed in deuterated water with 16 scans or in deuterated methanol and water (20:80). A rotational Overhauser enhancement experiment (ROESY) was used to detect intermolecular nuclear Overhauser effects between MeβCD and THC. The number of scans was fixed at 32, temperature at 300 K and dwell time at 120.00 μsec.

2.4.3. Scanning electron microscopy (SEM)

SEM observations were performed at the Bordeaux Imaging Center (BIC) with a ZEISS Gemini SEM 300 equipment. Stubs with double-face tape were used as support for a thin layer of powder. Samples were metallized (Platine) during 5 minutes and observed in high vacuum mode under a 2 keV electron beam.

Table 1
THC maximum water solubility in various cyclodextrins. Standard deviations were calculated from two repetitions.

Cyclodextrins (concentration = 15 mM)	THC maximum solubility (mM)
No cyclodextrin	0.12 ± 0.02
β-cyclodextrin	0.10 ± 0.04
γ-cyclodextrin	0.32 ± 0.02
Randomly methylated β-cyclodextrin	1.10 ± 0.10
Poly-βCD	0.30 ± 0.05
Poly-MeβCD	0.28 ± 0.05

2.5. Microbiological assays against F.graminearum

Growth inhibition tests were performed on Potato Dextrose Agar, supplemented with MeβCD or THC-loaded MeβCD at 15 mM. For polymers of CD (Poly-βCD and Poly-MeβCD) concentration was set at 15 mM of CD cavities. THC concentration was 10 μM for free-THC, 1 mM with MeβCD and 0.3 mM with Poly-βCD and Poly-MeβCD. The blank consisted of PDA medium without any addition of THC or CDs.

For the inoculation of PDA medium with the target strain, a spore suspension was first prepared by using agar cubes from 7 days old *F. graminearum* culture incorporated in the CMC liquid medium. After 3 days of incubation at 25°C, the suspension was filtered through nylon filter (100 μm). One drop of 10 μL of the latter suspension containing approximately 100 spores was deposited on PDA before incubation at 25°C and 70 % of relative humidity (RH) for 5 days. Two perpendicular diameters were measured every day. All conditions were performed in triplicate. Percentages of inhibition were expressed as following:

$$\%Inh = \frac{Average\ diameter\ of\ blank - Average\ diameter\ of\ condition\ i}{Average\ diameter\ of\ blank} * 100 \quad (2)$$

3. Results and discussion

3.1. Selection of the most suitable cyclodextrin

This preliminary study was devoted to the selection of the cyclodextrin able of incorporating the most THC in the fixed experimental conditions. The study was only conducted at 20°C because the system is made to be applied outside in fields. Depending on their size (7 or 8 glucose units) and their substitution groups (methylation), cyclodextrins may behave differently as regards to the solubilization of THC in water. CD polymers like Poly-βCD and Poly-MeβCD, can promote solubilization of molecular species within CD cavities but also inside the 3D-network (Danel et al. 2013).

As shown in Table 1, excepted for βCD, all types of CDs improved the THC solubility in water with a proportional factor of 3 and 10 for γCD and MeβCD, respectively. Regarding polymeric CDs, the THC solubility with Poly-βCD was three times higher than with βCD but less THC has been dissolved by using Poly-MeβCD than MeβCD.

The different behavior of β- and γCD was problably due to the different diameter of cavities (0.60–0.65 nm and 0.75–0.83 nm, respectively) and the significant improvement of THC solubility by using MeβCD is explained by the occurence of the hydrophobic methyl groups leading to a higher hydrophobic character of such CDs compared to non-modified ones. Such methyl groups certainly play a role in the encapsulation process, either by helping to capture THC in the environment or by better retaining THC in the cavities. Tønnesen, Másson, and Loftsson (2002) and Çelik, Özyürek, Tufan, Güçlü, and Apak (2011) also found that modified cyclodextrins, with methyl or hydroxypropyl groups, present a better ability to trap hydrophobic compounds. With CD polymers, the THC solubility did not depend on the cyclodextrin structure, as the values are very close. One hypothesis might be that THC was mainly loaded into the network created by citric acid chains, which is

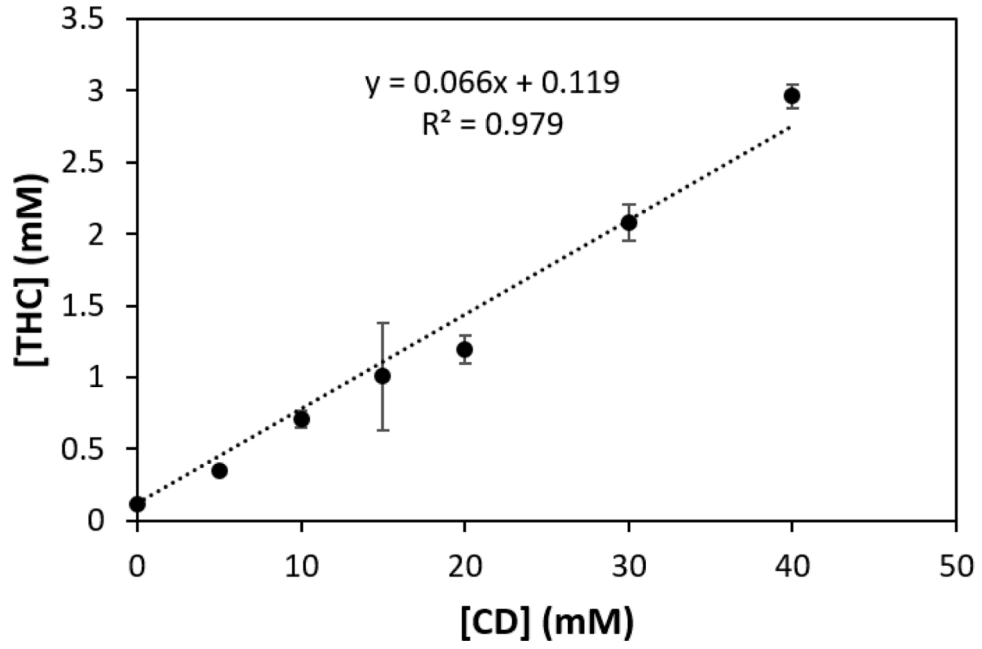

Fig. 2. THC concentration *versus* MeβCD concentration in water at 20°C. Standard deviation were calculated from two repetitions.

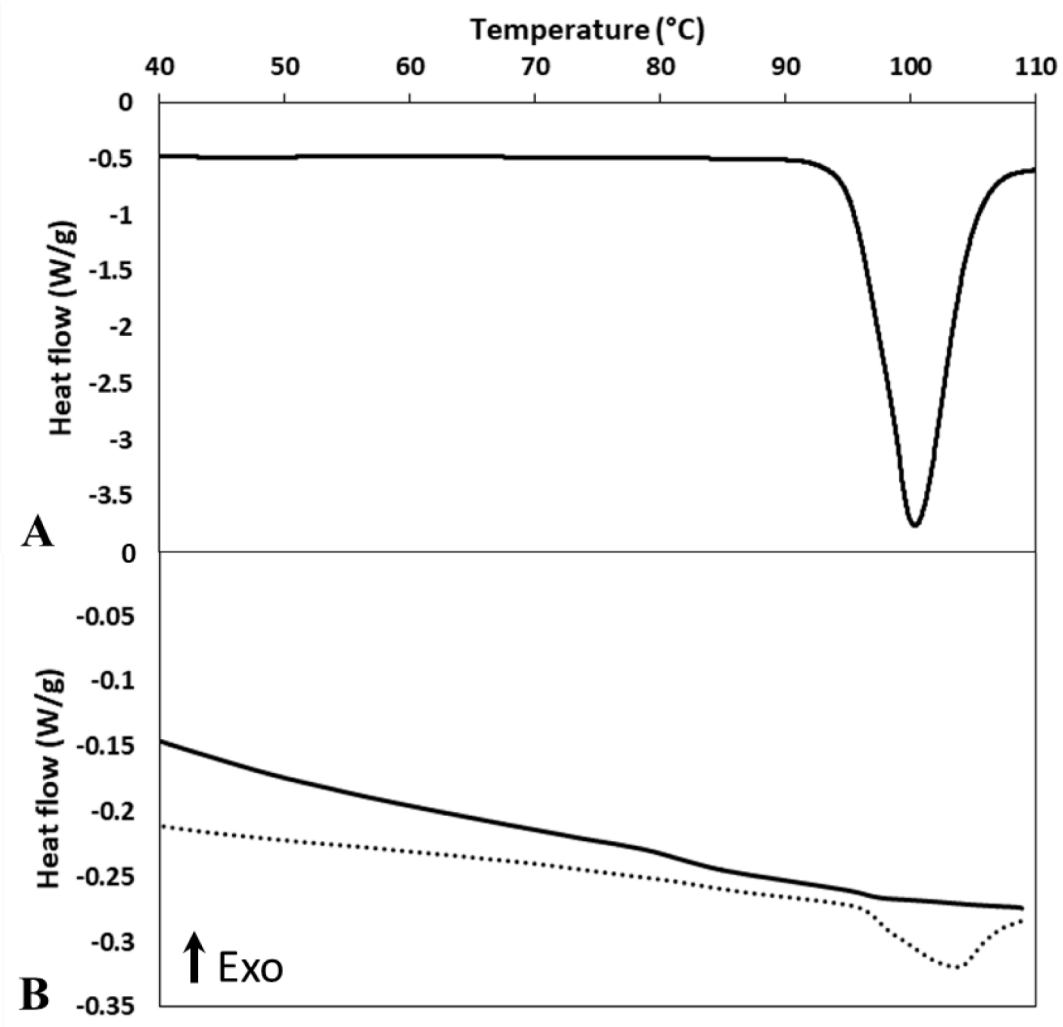

Fig. 3. Thermogram of DSC of (A) THC and (B) THC-loaded MeβCD complex (solid line), THC and MeβCD simple mixture (dash line).

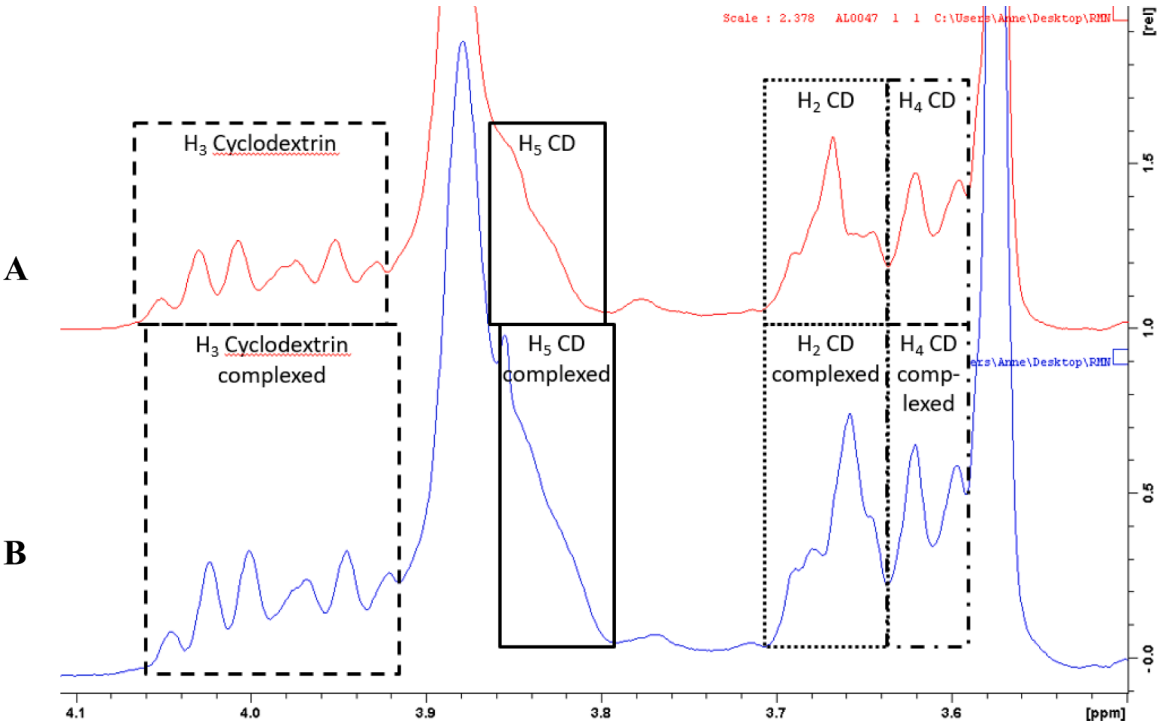

Fig. 4. NMR of MeβCD (A) and THC-loaded MeβCD complex (B) in D_2O.

the same for both polymers. Another hypothesis is that THC was still entrapped in the CD cavity, but thus suggesting that the complexation process is highly influenced by reticulation chains, which would act like substituents on CD. Regarding the huge solubility reduction with Poly-MeβCD compared to MeβCD, the high electronic density of phenolic rings of THC could lead to unfavorable interactions with carboxylate anions, thus preventing the molecule from entering the cavity. In addition, reticulation chains may hide methyl groups, which were thought to be of prime importance to enhance THC solubilization.

Finally, MeβCD gave the best results in terms of the amount of THC incorporated in CD and therefore was then selected for the rest of the study.

3.2. *Physicochemical characterization of the THC-loaded MeβCD complex*

Different techniques were used to fully characterize the host-guest interaction of THC with MeβCD. Solubility studies were undertaken to determine physicochemical parameters of the complex formation. The influence of temperature on THC interaction with MeβCD has been evaluated by DSC and NMR spectrometry was used to establish the geometry of the complex. Finally, SEM was performed to observe the potential impact of THC-loading on cyclodextrin external morphology.

3.2.1. *Study of THC solubility*

Solubility study by using the method of Higuchi and Connors (Higuchi and Connors, 1965) is a powerful tool to approximate the association constant and the stoichiometry of the complex (Kersani et al., 2020). The binding affinity between THC and hydroxypropyl βCD had already been studied by different authors (Goto et al., 2019; Thongyai, Kaewnopparat, & Songkro, 2016), whereas inclusion complexes with MeβCD were characterized by Çelik et al. (2011) or Duarte et al., 2015 but with other guest molecules.

As seen in Fig. 1, the plot of the THC concentration *versus* MeβCD content gave a linear curve, indicating a host/guest ratio of 1/1 for this domain of concentration. The association constant $K_{MeβCD}$, following the Eq. (1), was calculated from the slope of the curve and gave 591 ± 46 M^{-1}, which is consistent with literature data. Indeed, K_S values commonly found in literature are ranging from 10^1 to 10^5 M^{-1}, with most of the values ranging from 10^2 to 10^3 M^{-1} (Rekharsky & Inoue, 1998) especially from 100 to 1500 M^{-1} with, for example, curcumin-loaded βCD complexes (Jahed et al., 2014; Marcolino et al., 2011). Duarte et al., 2015 found an association constant of 1483 M^{-1} with a resveratrol-loaded MeβCD complex and Goto et al., 2019 obtained a value of 1200 M^{-1} with THC and hydroxypropyl βCD.

3.2.2. *DSC analysis*

To supplement the study, a complex of MeβCD and THC 2 % (w/w) and a simple mixture of THC and MeβCD in the same proportion were run on DSC. After a first cycle to eliminate water, the temperature was raised to a temperature above the THC melting point. In the mixture, a THC melting peak can be observed. No visible heat flow fluctuations could be seen (Fig. 2) with the complex whereas THC–MeβCD blend revealed a sharp melting peak. As a result, it could be assumed that THC was in an amorphous form, as was also concluded by Maharjan et al. (2019), or that THC in the complex was protected from melting within a cavity of MeβCD. (Fig. 3)

3.2.3. *^{1}H-NMR investigations*

To prove the inclusion of THC in MeβCD, ^{1}H and 2D NMR experiments were performed. A comparison of ^{1}H-NMR spectra of MeβCD in the presence or absence of THC was carried out to highlight chemical shift variations on the H_3 and H_5 protons located inside the CD cavity (Figs. 1 and 4). H_1, H_2 and H_4 protons remained at the same chemical shifts, but changes could be observed for H_3 (0.01 ppm) and H_5 (no value given due to a large signal width). On the contrary to Jahed et al., 2014 and Chao, Wang, Zhao, Zhang, & Zhang, (2012), these chemical shift variations have been estimated too small to conclude to the formation of an inclusion complex between MeβCD and THC).

A better approach was based on shift variations of aromatic protons of THC. First, HSQC was needed to attribute aromatic protons of THC (Fig. 5 A). After the loading of THC in MeβCD, notable shifts of protons a and c occurred (Fig. 5 B). The signal of proton c varied of -0.03 ppm while the proton a shifted of +0.04 ppm, which was explained by to the

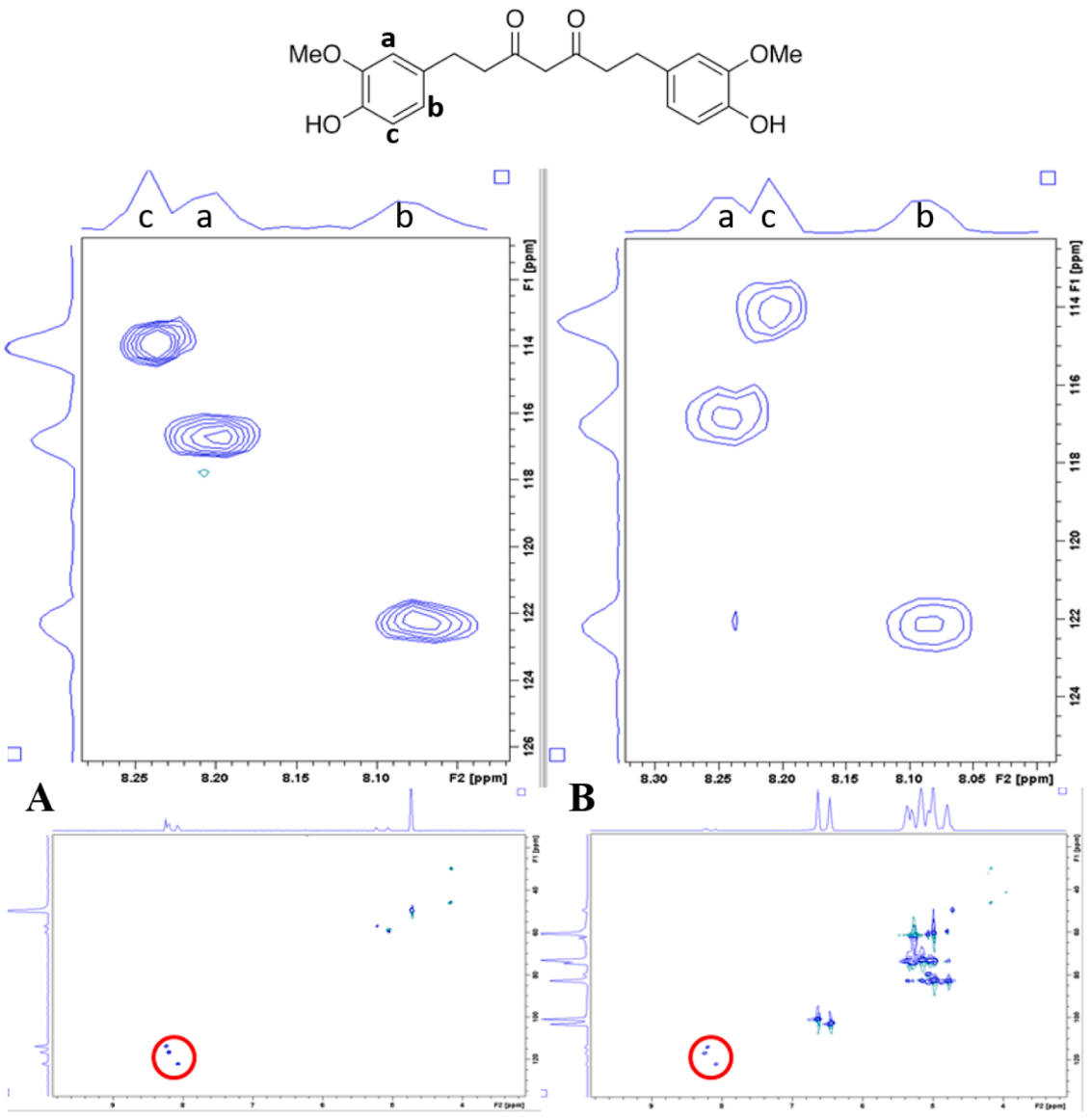

Fig. 5. HSQC of THC (A) and THC-loaded MeβCD complex (B) in MeOD/D_2O 1:4.

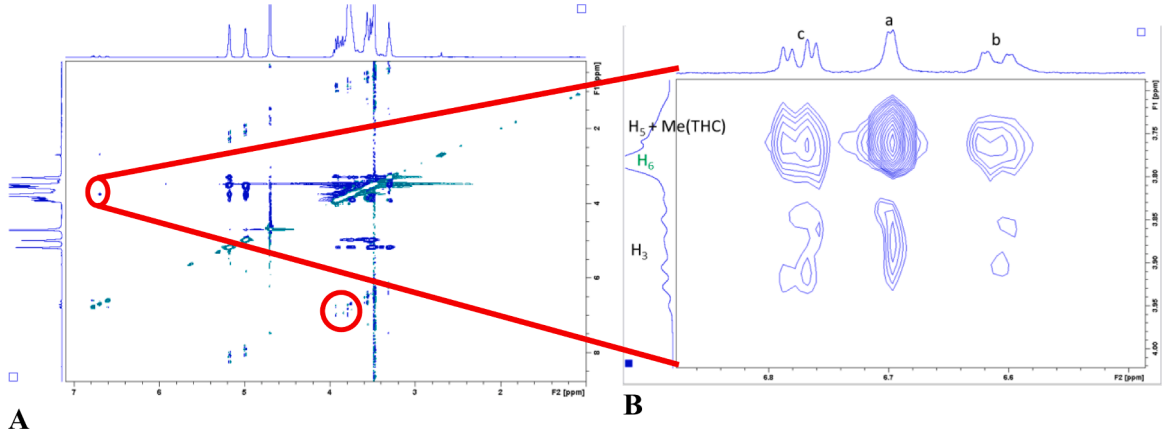

Fig. 6. ROESY of THC-loaded MeβCD complex in D_2O (A); Zoom of ROESY (B).

formation of inclusion complexes.

Finally, non-covalent interactions have been easily highlighted through ROESY (Fig. 6). Aksamija, Polidori, Plasson, Dangles, & Tomao (2016) observed interactions between the protons of their guest molecule (rosmarinic acid) and both H_3 and H_5 of βCD, thus proving the inclusion of aromatic rings in CD cavities. In the present study, the

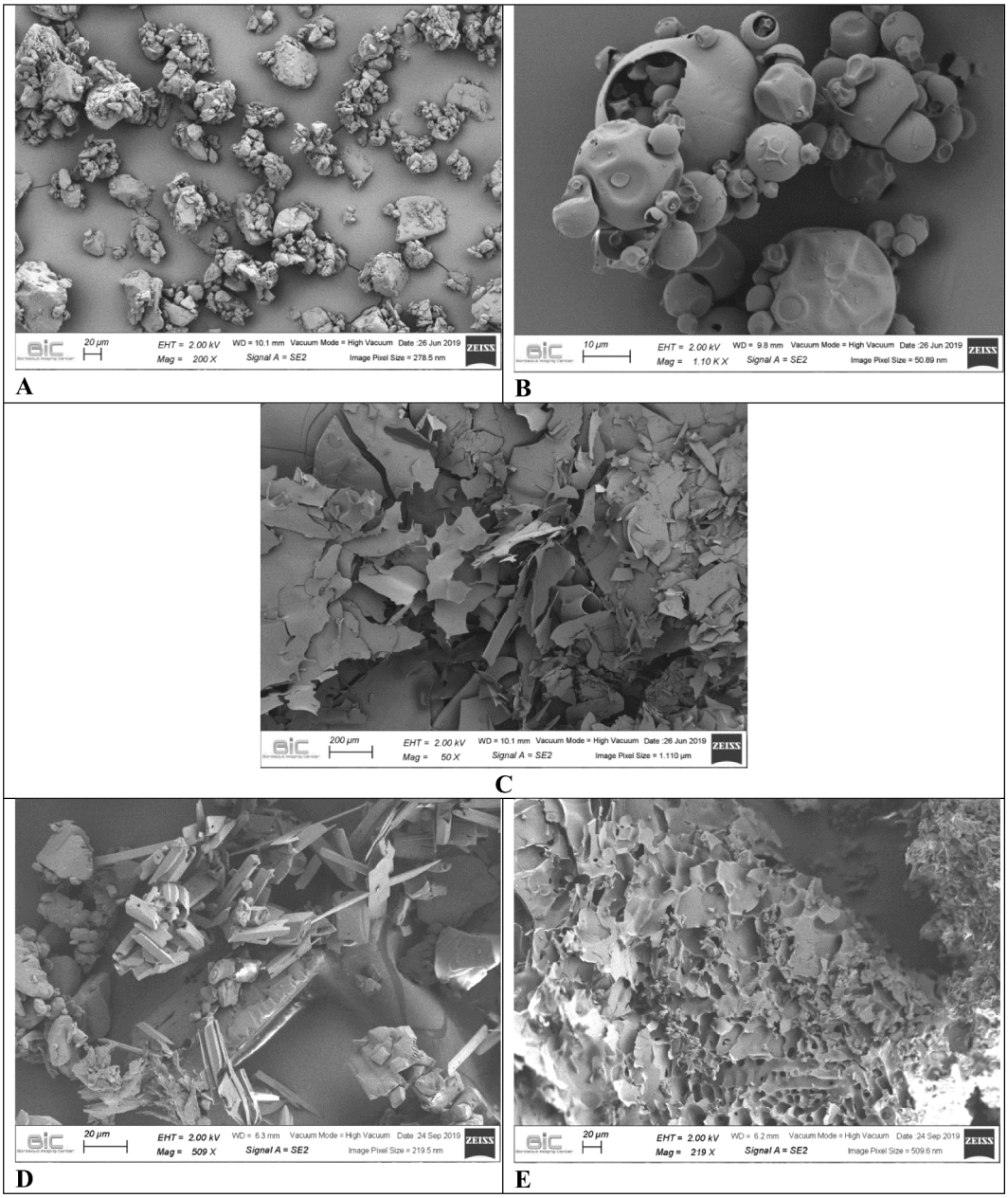

Fig. 7. SEM views of THC (A), MeβCD (B), THC/MeβCD complex (C), freezed-dried THC (D), freezed-dried MeβCD (E).

significant signal at 3.76 ppm corresponds to interactions between aromatic protons and protons of methyl group, both from THC. Unfortunately, protons H_5 of CD are also located at 3.76 ppm. As a result, potential interactions with aromatic protons of THC could not be clearly evidenced. However, the second signal at 3.87 ppm proved unambiguously the presence of interactions between protons H_3 of the inner cavity of CD and aromatic protons of THC, thus demonstrating the presence of inclusion complexes.

3.2.4. SEM observations

SEM was used to know if the powder of raw or complexed materials induced any changes in their own morphology. THC appears as coarse granules in raw powder and sharp needles in freeze-dried powder (Fig. 7 A and D). Strong differences between samples of MeβCDs were highlighted. In the raw powder, MeβCDs were round shaped, like golf ball or empty capsules, with a diameter from 3 to 30 μm, whereas in freeze-dried MeβCDs, thin sponge-like structures have been observed (Fig. 7 B and E). The morphology of the freeze-dried complexes presented thin sheets that were often broken (Fig. 7 C). It can be concluded that THC was trapped within MeβCDs. THC's spiked structures would have been visible among sheet-like MeβCDs if THC would have not been in the cavity of MeβCD. Furthemore, it can be assumed that the morphology seemed mainly determined by the drying process. Both lyophilized MeβCDs and complexes presented a similar structure. However, the MeβCDs furnished by the supplier were round-shaped. In parallel, some authors synthetized CD complexes with a spay-drying technique and observed round shaped products (Shan-Yang & Yuh-Horng, 1989). Freeze-dried powders looked like expanded sheets and spray-dried powders were ball-like. This is in contradiction with Duarte et al., 2015 who suggested that the new structure in leaf form they have observed was due to the formation of inclusion complexes.

3.3. Antifungal activity of THC-loaded cyclodextrins

Preliminary tests with THC at 10 μM did not show any activity against the *F. graminearum* growth. A higher THC concentration was

Table 2
Growth inhibition percentages of *F. graminearum* by CDs or CD complexes after 4 days of incubation at 25°C and 70 % RH. Standard deviations were calculated from three repetitions. Values with different letters in superscript are significantly different.

Tested compound	Growth inhibition (%)
THC 10 µM	$0^{d,e}$
MeβCD 15 mM	-12 ± 1^{f}
MeβCD 15 mM + THC 1.0 mM	$-2 \pm 1^{e,f}$
Poly-βCD 15 mM	$13 \pm 1^{c,d}$
Poly-βCD 15 mM + THC 0.3 mM	$23 \pm 1^{b,c}$
Poly-MeβCD 15 mM	$16 \pm 1^{a,b}$
Poly-MeβCD 15 mM + THC 0.3 mM	26 ± 1^{a}

tested *via* encapsulation in MeβCDs. Results are presented in Table 2 and pictures of the plates are available in supplementary information (Fig. S4). Surprizingly, MeβCDs at 15 mM activated the fungal growth by 12 %, which could be due to their metabolization by *F. graminearum* as carbon sources. To limit this phenomenon, a combination with other molecules like acarbose, an amylase inhibitor, could be an interesting way to explore (Abril-Sánchez, Matencio, Navarro-Orcajada, García--Carmona, & López-Nicolás, 2019). However, after THC loading, the growth activation was reduced by only 2 %, indicating light antifungal properties of THC and the fact that a large part of the active agent was probably released from CD.

Because less sensitive to fungal degradation, and potentially intrinsically bioactive, the polymers of CDs have also been tested, although they have a lower ability to solubilize THC. As shown in Table 2, the activation of MeβCD due to its degradation by the fungal strain was prevented thanks to the polymerization of MeβCD. Results showed that CD polymers with and without THC inhibited the mycelium growth of *F. graminearum* by 13 % and 16 %, respectively. This could be due to the antifungal effect of free carboxylic acid functions of citric acid used in the synthesis of Poly-CD (Shokri, 2011). After THC-loading, an improvement of 10 % was obtained, leading to an inhibition percentage of about 23 % and 25 % for Poly-βCD and Poly-MeβCD, respectively. Encapsulation of 0.3 mM of THC improved the inhibition percentage, thus showing once again the THC's ability to reduce the fungal development. In the literature, antifungal properties of natural compounds are mainly attributed to lipophilic interactions. Indeed, hydrophobic moieties of such active molecules could interact with the phospholipid bilayers of fungal membranes (Valette, Perrot, Sormani, Gelhaye, & Morel-Rouhier 2017) and membrane damages have often been reported (Hammer, 2004; Peng et al., 2012; Yutani et al., 2011), leading to the leakage of cellular content and thus to the cell death (Peng et al., 2012; Valette et al. 2017). In two different studies, phenolic compounds with one or more methoxy group(s) on their ring –which is also the case for THC– were more active than analogues bearing hydroxyl group(s) (Bastos et al., 2009; Fitzgerald et al., 2005). Moreover, a previous work on THC (Coma et al., 2011) has demonstrated that a *F. proliferatum* inhibition of 67 % was achievable using, a higher THC concentration (13.6 mM), nevertheless requiring tetrahydrofurane during the solubilization process. In comparison, a significant inhibition was achieved herein study without any organic solvent and by using a bio-based encapsulation system.

4. Conclusions

Among different studied CDs, MeβCDs enhanced the most the THC water-solubility. A detailed solubility study showed that a 1:1 complex was formed in our conditions and ^{1}H- and 2D-NMR investigations proved an inclusion of THC within CD cavity. Indeed, aromatic protons of THC underwent chemical shift variations when complexation occurred. Moreover, a SEM study finally revealed no free THC. Regarding the atimicrobial activity of such complexes, the mycelium growth of the model strain *F. graminearum* was, at the best, reduced by a quarter with THC-loaded MeβCD polymers. As a result, these solvent-free and bio-based inclusion complexes of THC with CD derivatives are promising for the development of new aqueous biofungicide.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.carpta.2021.100113.

References

Abril- Sánchez, C., Matencio, A., Navarro-Orcajada, S., García-Carmona, F., & López-Nicolás, J. M. (2019). Evaluation of the properties of the essential oil citronellal nanoencapsulated by cyclodextrins. *Chemistry and Physics of Lipids, 219*, 72–78. https://doi.org/10.1016/j.chemphyslip.2019.02.001.

Aksamija, A., Polidori, A., Plasson, R., Dangles, O., & Tomao, V. (2016). The inclusion complex of rosmarinic acid into beta-cyclodextrin : A thermodynamic and structural analysis by NMR and capillary electrophoresis. *Food Chemistry, 208*, Article 258263. https://doi.org/10.1016/j.foodchem.2016.04.008.

Avanço, G. B., Ferreira, F. D., Bomfim, N. S., Santos, P. A. S. R.dos, Peralta, R. M., Brugnari, T., Mallman, C. A., de Abreu Filho, B. A., Michka, J. M. G., & Machinski Jr., M. (2017). *Curcuma longa* L. essential oil composition, antioxidant effect, and effect on *Fusarium verticillioides* and fumonisin production. *Food Control, 73*, Article 806813. https://doi.org/10.1016/j.foodcont.2016.09.032.

Bastos, M., Lima, M., Conserva, L. M., Andrade, V. S., Rocha, E. M., & Lemos, R. P. (2009). Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Vell.) Bur. (Bignoniaceae) extracts and their main constituents. *Annals of Clinical Microbiology and Antimicrobials, 8*(1), 16. https://doi.org/10.1186/1476-0711-8-16.

Braga, S. S. (2019). Cyclodextrins: Emerging Medicines of the New Millennium. *Biomolecules, 9*(12), 801. https://doi.org/10.3390/biom9120801.

Campos, E. V. R., Proença, P. L. F., Oliveira, J. L., Melville, C. C., Della Vechia, J. F., de Andrade, D. J., & Fraceto, L. F. (2018). Chitosan nanoparticles functionalized with β-cyclodextrin: A promising carrier for botanical pesticides. *Scientific Reports, 8*(1), 2067. https://doi.org/10.1038/s41598-018-20602-y.

Çelik, S. E., Özyürek, M., Tufan, A. N., Güçlü, K., & Apak, R. (2011). Spectroscopic study and antioxidant properties of the inclusion complexes of rosmarinic acid with natural and derivative cyclodextrins. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 78*, Article 16151624. https://doi.org/10.1016/j.saa.2011.02.017.

Chao, J., Wang, H., Zhao, W., Zhang, M., & Zhang, L. (2012). Investigation of the inclusion behavior of chlorogenic acid with hydroxypropyl-β-cyclodextrin. *International Journal of Biological Macromolecules, 50*, Article 277282. https://doi.org/10.1016/j.ijbiomac.2011.11.008.

Cheirsilp, B., & Rakmai, J. (2017). Inclusion complex formation of cyclodextrin with its guest and their applications. *Biology, Engineering and Medicine, 2*, 1–6. https://doi.org/10.15761/BEM.1000108.

Coma, V., Portes, E., Gardrat, C., Richard-Forget, F., & Castellan, A. (2011). In vitro inhibitory effect of tetrahydrocurcuminoids on *Fusarium proliferatum* growth and fumonisin B $_1$ biosynthesis. *Food Additives & Contaminants: Part A, 28*, Article 218225. https://doi.org/10.1080/19440049.2010.540721.

Dai, J., & Mumper, R. J. (2010). Plant phenolics : Extraction, analysis and their antioxidant and anticancer properties. *Molecules, 15*, Article 73137352. https://doi.org/10.3390/molecules15107313.

Danel, C., Azaroual, N., Chavaria, C., Odou, P., Martel, B., & Vaccher, C. (2013). Comparative study of the complex forming ability and enantioselectivity of cyclodextrin polymers by CE and ^{1}H NMR. *Carbohydrate Polymers, 92*, Article 22822292. https://doi.org/10.1016/j.carbpol.2012.11.095.

Del Valle, E. M. M. (2004). Cyclodextrins and their uses : A review. *Process Biochemistry, 39*, Article 10331046. https://doi.org/10.1016/S0032-9592(03)00258-9.

Duarte, A., Martinho, A., Luís, Â., Figueiras, A., Oleastro, M., Domingues, F. C., & Silva, F. (2015). Resveratrol encapsulation with methyl-β-cyclodextrin for antibacterial and antioxidant delivery applications. *LWT - Food Science and Technology, 63*, Article 12541260. https://doi.org/10.1016/j.lwt.2015.04.004.

Fang, Z., & Bhandari, B. (2010). Encapsulation of polyphenols – A review. *Trends in Food Science & Technology, 21*, Article 510523. https://doi.org/10.1016/j.tifs.2010.08.003.

Fitzgerald, D. J., Stratford, M., Gasson, M. J., & Narbad, A. (2005). Structure−Function analysis of the vanillin molecule and its antifungal properties. *Journal of Agricultural and Food Chemistry, 53*(5), 1769–1775. https://doi.org/10.1021/jf048575t.

Gao, S., Liu, Y., Jiang, J., Ji, Q., Fu, Y., Zhao, L., Li, C., & Ye, F. (2019). Physicochemical properties and fungicidal activity of inclusion complexes of fungicide chlorothalonil

with β-cyclodextrin and hydroxypropyl-β-cyclodextrin. *Journal of Molecular Liquids, 293*, Article 111513. https://doi.org/10.1016/j.molliq.2019.111513.

Goto, M., Ifuku, S., Azuma, K., Arima, H., Kaneko, S., Iohara, D., Hirayama, F., & Anraku, M. (2019). Preparation and evaluation of freeze dried surface-deacetylated chitin nanofiber/sacran pellets for use as an extended-release excipient. *International Journal of Biological Macromolecules, 124*, Article 888894. https://doi.org/10.1016/j.ijbiomac.2018.11.225.

Gupta, P. K. (2011). Herbicides and fungicides. In R. C. Gupta (Ed.), *Reproductive and developmental toxicology* (1st ed., pp. 503–521). Amsterdam: Elsevier Acad. Press. ISBN: 978-0-12-382032-7.

Hammer, K. A. (2004). Antifungal effects of *Melaleuca alternifolia* (tea tree) oil and its components on *Candida albicans, Candida glabrata* and *Saccharomyces cerevisiae*. *Journal of Antimicrobial Chemotherapy, 53*(6), 1081–1085. https://doi.org/10.1093/jac/dkh243.

Heidtmann-Bemvenuti, R., Tralamazza, S. M., Ferreira, Jorge, C., F., Corrêa, B., & Badiale-Furlong, E (2016). Effect of natural compounds on *Fusarium graminearum* complex. *Journal of the Science of Food and Agriculture, 96*, Article 39984008. https://doi.org/10.1002/jsfa.7591.

Higuchi, T., & Connors, K. A. (1965). Phase solubility techniques. *Advances in Analytical Chemistry and Instrumentation, 4*, 117–212.

Jahed, V., Zarrabi, A., Bordbar, A. K., & Hafezi, M. S. (2014). NMR (^{1}H, ROESY) spectroscopic and molecular modelling investigations of supramolecular complex of β-cyclodextrin and curcumin. *Food Chemistry, 165*, Article 241246. https://doi.org/10.1016/j.foodchem.2014.05.094.

Junthip, J., Tabary, N., Leclercq, L., & Martel, B. (2015). Cationic β-cyclodextrin polymer applied to a dual cyclodextrin polyelectrolyte multilayer system. *Carbohydrate Polymers, 126*, Article 156167. https://doi.org/10.1016/j.carbpol.2015.02.064.

Kersani, D., Mougin, J., Lopez, M., Degoutin, S., Tabary, N., Cazaux, F., Janus, L., Maton, M., Chai, F., Sobocinski, J., Blanchemain, N., & Martel, B. (2020). Stent coating by electrospinning with chitosan/poly-cyclodextrin based nanofibers loaded with simvastatin for restenosis prevention. *European Journal of Pharmaceutics and Biopharmaceutics, 150*, 156–167. https://doi.org/10.1016/j.ejpb.2019.12.017.

Kumar, S., & Singh, A. (2015). Biopesticides: present status and the future prospects. *Journal of Fertilizers & Pesticides, 6*, e129. https://doi.org/10.4172/2471-2728.1000e129.

Maharjan, P., Jin, M., Kim, D., Yang, J., Maharjan, A., Shin, M. C., Kim, M. S., Cho, K. H., & Min, K. A. (2019). Evaluation of epithelial transport and oxidative stress protection of nanoengineered curcumin derivative-cyclodextrin formulation for ocular delivery. *Archives of Pharmacal Research, 42*, Article 909925. https://doi.org/10.1007/s12272-019-01154-9.

Marcolino, V. A., Zanin, G. M., Durrant, L. R., Benassi, M. D. T., & Matioli, G. (2011). Interaction of curcumin and bixin with β-cyclodextrin : complexation methods, stability, and applications in food. *Journal of Agricultural and Food Chemistry, 59*, Article 33483357. https://doi.org/10.1021/jf104223k.

Morillo, E. (2006). *Cyclodextrins and their complexes: Chemistry, analytical methods, applications 16.3 Application of cyclodextrins in agrochemistry* (Helena Dodzivk).

Munin, A., & Edwards-Lévy, F. (2011). Encapsulation of natural polyphenolic compounds; a review. *Pharmaceutics, 3*, Article 793829. https://doi.org/10.3390/pharmaceutics3040793.

Nardello-Rataj, V., & Leclercq, L. (2014). Encapsulation of biocides by cyclodextrins: Toward synergistic effects against pathogens. *Beilstein Journal of Organic Chemistry, 10*, Article 26032622. https://doi.org/10.3762/bjoc.10.273.

Peng, L., Yang, S., Cheng, Y. J., Chen, F., Pan, S., & Fan, G. (2012). Antifungal activity and action mode of pinocembrin from propolis against Penicillium italicum. *Food Science and Biotechnology, 21*(6), 1533–1539. https://doi.org/10.1007/s10068-012-0204-0.

Pinho, E., Grootveld, M., Soares, G., & Henriques, M. (2014). Cyclodextrins as encapsulation agents for plant bioactive compounds. *Carbohydrate Polymers, 101*, Article 121135. https://doi.org/10.1016/j.carbpol.2013.08.078.

Portes, E., Gardrat, C., & Castellan, A. (2007). A comparative study on the antioxidant properties of tetrahydrocurcuminoids and curcuminoids. *Tetrahedron, 63*, Article 90929099. https://doi.org/10.1016/j.tet.2007.06.085.

Rekharsky, M. V., & Inoue, Y. (1998). Complexation thermodynamics of cyclodextrins. *Chemical Reviews, 98*, Article 18751918. https://doi.org/10.1021/cr970015o.

Shan-Yang, L., & Yuh-Horng, K. (1989). Solid particulates of drug-β-cyclodextrin inclusion complexes directly prepared by a spray-drying technique. *International Journal of Pharmaceutics, 56*, Article 249259. https://doi.org/10.1016/0378-5173(89)90022-7.

Shokri, H. (2011). Evaluation of inhibitory effects of citric and tartaric acids and their combination on the growth of Trichophyton mentagrophytes, Aspergillus fumigatus, Candida albicans, and Malassezia furfur. *Comparative Clinical Pathology, 20*, 543–545.

Singh, A., Dhiman, N., Kar, A. K., Singh, D., Purohit, M. P., Ghosh, D., & Patnaik, S. (2020). Advances in controlled release pesticide formulations: Prospects to safer integrated pest management and sustainable agriculture. *Journal of Hazardous Materials, 385*, Article 121525. https://doi.org/10.1016/j.jhazmat.2019.121525.

Smart, N. A. (2003). Fungicides. *Encyclopedia of food sciences and nutrition* (2nd ed., pp. 2832–2842). Amsterdam: Academic Press (Caballero, B., Trugo, L. C., Finglas Eds, P. M.)ISBN: 987-0-12-227055-0.

Tabary, N., Garcia-Fernandez, M. J., Danède, F., Descamps, M., Martel, B., & Willart, J.-F. (2016). Determination of the glass transition temperature of cyclodextrin polymers. *Carbohydrate Polymers, 148*, 172–180. https://doi.org/10.1016/j.carbpol.2016.04.032.

Teodoro, G. R., Ellepola, K., Seneviratne, C. J., & Koga-Ito, C. Y. (2015). Potential use of phenolic acids as anti-candida agents: A review. *Frontiers in Microbiology, 6*, 1420. https://doi.org/10.3389/fmicb.2015.01420.

Thongyai, S., Kaewnopparat, N., & Songkro, S. (2016). *Naresuan University Journal: Science and Technology, 24*, 34–42.

Tønnesen, H. H., Másson, M., & Loftsson, T. (2002). Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: Solubility, chemical and photochemical stability. *International Journal of Pharmaceutics, 244*, Article 127135. https://doi.org/10.1016/S0378-5173(02)00323-X.

Valette, N., Perrot, T., Sormani, R., Gelhaye, E., & Morel-Rouhier, M. (2017). Antifungal activities of wood extractives. *Fungal Biology Reviews, 31*(3), 113–123. https://doi.org/10.1016/j.fbr.2017.01.002.

Wang, D., Jia, M., Wang, L., Song, S., Feng, J., & Zhang, X. (2017). Chitosan and β-cyclodextrin-epichlorohydrin polymer composite film as a plant healthcare material for carbendazim-controlled release to protect rape against sclerotinia sclerotiorum (Lib.) de Bary. *Materials, 10*(4), 343. https://doi.org/10.3390/ma10040343.

Wang, K., Jiang, S., Pu, T., Fan, L., Su, F., & Ye, M. (2019). Antifungal activity of phenolic monoterpenes and structure-related compounds against plant pathogenic fungi. *Natural Product Research, 33*, Article 14231430. https://doi.org/10.1080/14786419.2017.1419232.

Yutani, M., Hashimoto, Y., Ogita, A., Kubo, I., Tanaka, T., & Fujita, K. (2011). Morphological changes of the filamentous fungus *mucor mucedo* and inhibition of chitin synthase activity induced by anethole: Morphological changes of *Mucor Mucedo* induced by anethole. *Phytotherapy Research, 25*(11), 1707–1713. https://doi.org/10.1002/ptr.3579.

Zhang, X., Xing, H., Zhao, Y., & Ma, Z. (2018). Pharmaceutical dispersion techniques for dissolution and bioavailability enhancement of poorly water-soluble drugs. *Pharmaceutics, 10*, 74. https://doi.org/10.3390/pharmaceutics10030074.

Supplementary information

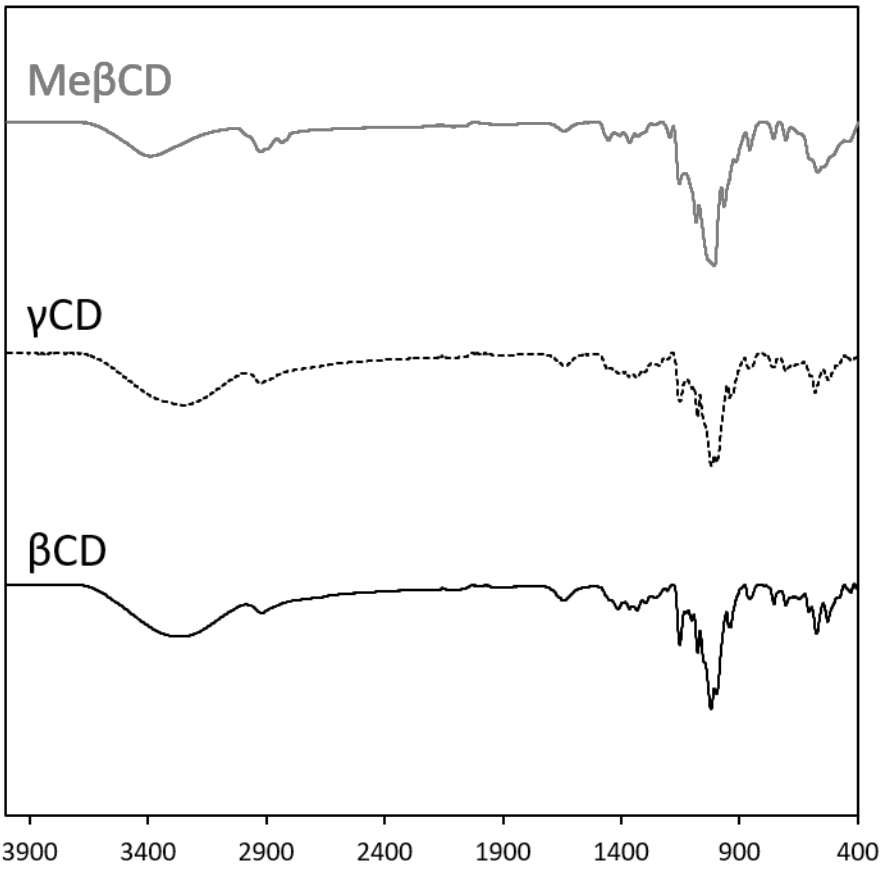

Fig. S1 FT-IR spectra of MeβCD; γCD; βCD

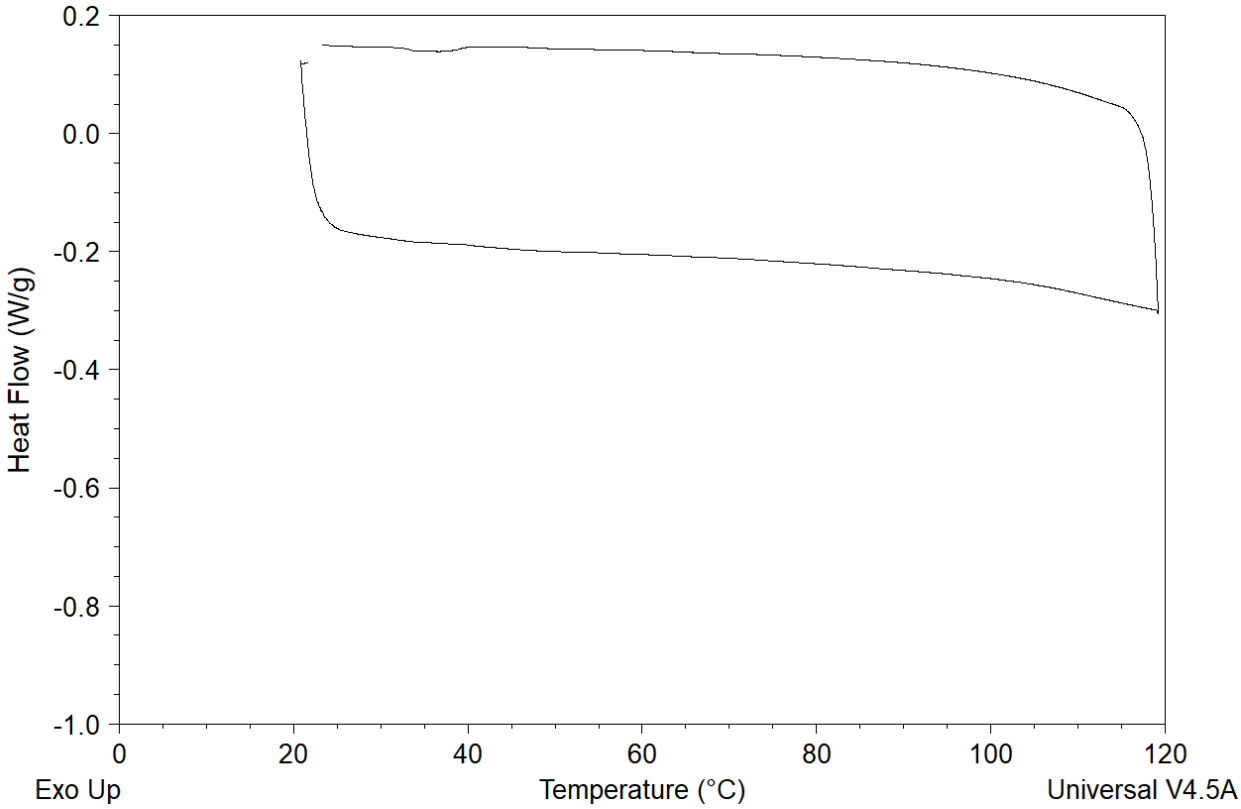

Fig. S2 DSC spectra of γCD

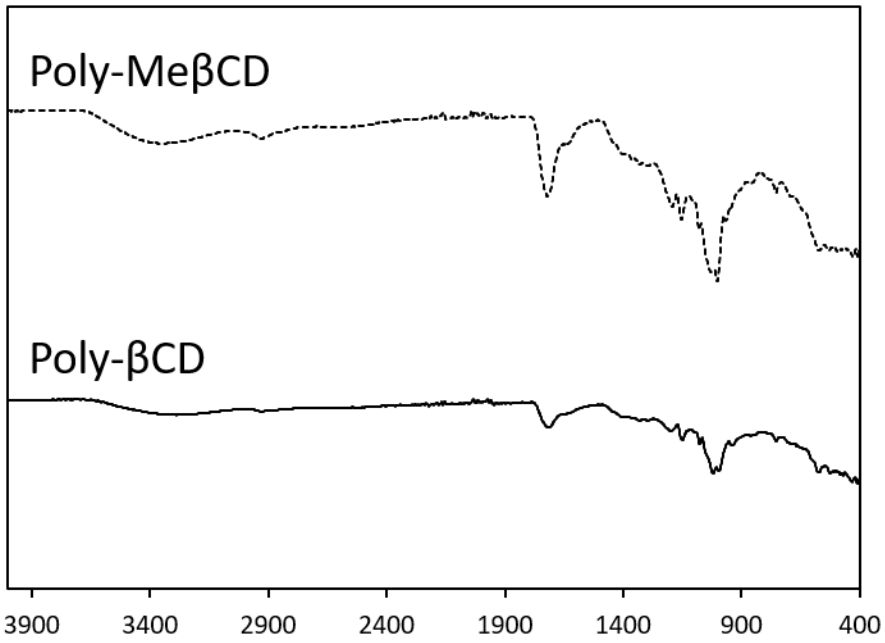

Fig. S3 FT-IR spectra of Poly-MeβCD; Poly-βCD

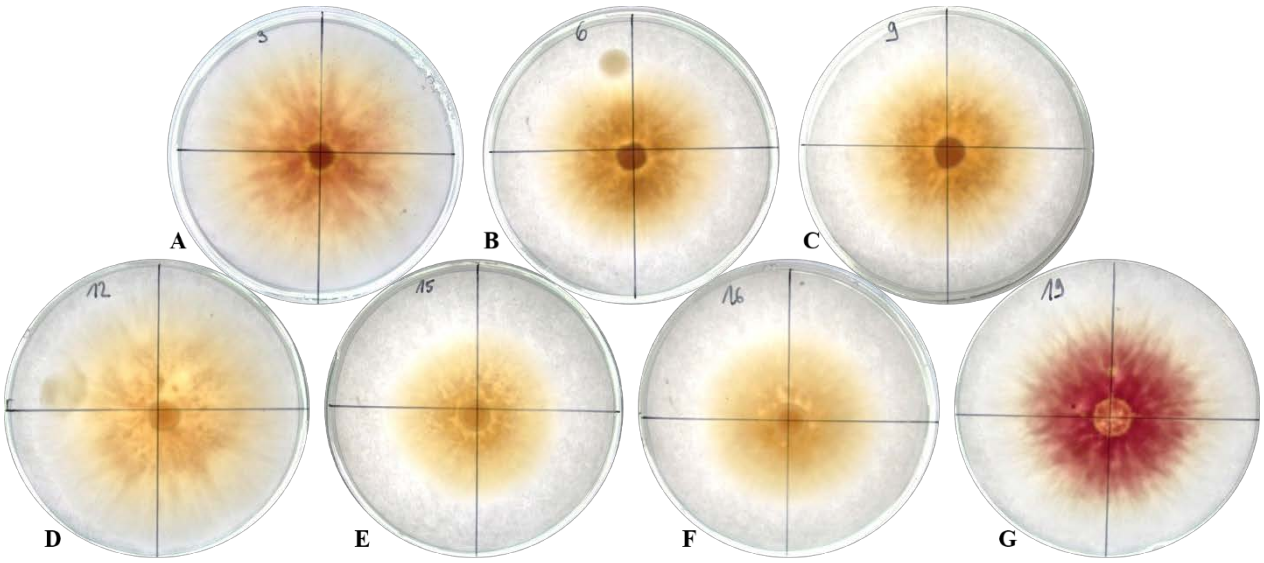

Fig. S4 Petri dishes of *F. graminearum* after 3 day of incubation. Medium supplemented with CD at 15 mM and THC when mentioned. MeβCD (**A**); Poly-βCD (**B**); Poly-MeβCD (**C**); MeβCD and THC at 1 mM (**D**); Poly-βCD and THC at 0.3 mM (**E**); Poly-MeβCD and THC at 0.3 mM (**F**); Control (**G**)