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Influence of oil phase composition on antifungal and mycotoxin inhibitory activity of clove oil nanoemulsions

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Abstract

The influence of oil compositions on the physical properties, antifungal and mycotoxin inhibitory activity of clove oil-in-water nanoemulsion were investigated. The physically stable clove oil-in-water nanoemulsions could be fabricated by incorporating either ≥ 75 wt% of corn oil, or ≥ 50 wt% of medium chain triacylglycerol (MCT) into clove oil before homogenization to prevent Ostwald ripening. The clove oil-in-water nanoemulsions with mean diameters <150 nm showed high physical stability over 30 days of storage time. The antifungal activity of physically stable clove oil nanoemulsions were further evaluated using effective concentration (EC) and inhibitory activity towards mycotoxin production in two chemotypes of *Fusarium graminearum* isolates. The composition of oil phase, i.e., ripening inhibitor type and concentration, in clove oil-in-water nanoemulsions had a remarkable impact on antifungal activity as well as inhibition of mycotoxin production. In general, under the same clove oil concentration in oil phase, the addition of MCT decreased the antifungal and mycotoxin inhibitory activity of clove oil more than corn oil. Compared with bulk clove oil, this study also indicated that mycotoxin inhibitory activity of clove was significantly enhanced when encapsulated in nanoemulsions. These results have important implications for the design of essential oil based nanoemulsions as effective antifungal and detoxification delivery system in food or other industries.

Keywords

Nanoemulsions; essential oil; Ostwald ripening; *Fusarium graminearum;* mycotoxins

1. Introduction

The Food and Agricultural Organization (FAO) has estimated that up to 25% of the world's cereal grains are contaminated by molds in the field and during storage, some of which are known to produce mycotoxins.[1] Toxigenic molds are produced by certain phytopathogenic and food spoilage fungi, such as *Aspergillus, Penicillium,* and *Fusarium* species, etc.[2] Mycotoxins are the secondary metabolites which are produced by fungi and exert detrimental toxic effects on animals and humans. Deoxynivalenol (DON, also known as vomitoxin) and its 3-acetyl and 15-acetyl derivatives (3-ADON and 15-ADON, respectively) are the most common mycotoxins found in *Fusarium* infected grains, such as wheat, rye, barley, corn, rice and oats, etc.[3] Deoxynivalenol can be produced not only during the development of the grains in the field but also in post-harvest and during storage. In general, DON is chemically stable to resist thermal processing, which can persist into the final food products (e.g., flour, bread, noodles, and beer) through contaminated grains.[4] The ingestion of DON has been reported to alter the intestinal, immune, endocrine, and nervous systems. The acute exposure of DON can cause severe illnesses associated with vomiting, anorexia, abdominal pain, diarrhea, malnutrition, headache and dizziness.[5] The reduction of such mycotoxins in food production is thus of primary importance and there is of great interest in developing efficient and safe prevention strategies in terms of food safety.

In recent years, the “clean-label” is on the rise in food industry which requires foods without artificial food additives including widely used chemical preservatives. Consequently, natural antimicrobial or antifungal agents could be used as potential alternatives to combat foodborne pathogens or fungal pathogens have received lots of attention.[6-7] Plant essential oils (EOs) have been shown to be effective in control of food spoilage and pathogenic bacteria in food safety

and preservation applications.[8] It has been reported that some EOs, such as clove oil, thyme oil, lemongrass oil, and cinnamon oil, have broad-spectrum antimicrobial and antifungal properties.[9-10] Plant essential oils are usually the mixtures of hundreds of chemical compounds. Phenolics, phenolic acids, quinones, saponins, flavonoids, tannins, coumarins, terpenoids, and alkaloids are the major compounds to display antimicrobial and antifungal activity.[10] For instance, eugenol, a phenolic component accounting for more than 80 % of clove oil, has been shown to exhibit antifungal activity against several fungi.[11-12] However, there are technological limitations with regards to the antimicrobial or antifungal efficacy of EOs in aqueous food products due to their low solubility in water and high volatility. In order to maintain antifungal activity, EOs should be restrained from interacting with food materials, and kept stable against environmental stress during food processing. Nanoemulsion based delivery systems, which have been widely applied in food and pharmaceutical industry to encapsulate lipophilic bioactive compounds such as vitamins, natural colors and antimicrobials, are a type of optimal system for essential oil protection.[13-15] Such delivery systems have two advantages[13]. The lipophilic antimicrobial or antifungal compounds, such as EOs, can be easily incorporated into aqueous foods after being encapsulated into nanoemulsion based delivery systems. In addition, the mass transfer efficacy of lipophilic bioactive compounds to certain sites of action is promoted by virtue of their increased water solubility in nanoemulsions.

Nanoemulsions are thermodynamically unstable systems that typically consist of oil, surfactant, and water. The small particle size (d < 200 nm) of nanoemulsions results in either a translucent or slightly turbid appearance. It is believed that nanoemulsions have a number of potential advantages over conventional emulsions for encapsulating lipophilic bioactive compounds.[16] In general, nanoemulsions have good stability against gravitational separation, flocculation and

coalescence due to their small particle size. Besides, the antimicrobial activity of the encapsulated EOs in nanoscale droplet might be increased when compared with the bulk essential oils due to an increased total surface to volume ratio.[13] However, nanoemulsions are more prone to encounter droplet growth with time due to Ostwald ripening.[16] The Ostwald ripening rate increases with the increase of oil solubility in aqueous phase. Smaller molar volume of relatively polar constituents in essential oils have appreciable solubility in water resulting in destabilization of essential oil-in-water nanoemulsions by Ostwald ripening. In contrast, larger molar weight of medium chain triacylglycerol (MCT) or long chain triglyceride type of oils such as corn oil are less water soluble, and therefore can be incorporated into oil phase and act as inhibitors to prevent Ostwald ripening in nanoemulsions.[17] However, the antifungal activity of EOs might be altered by the addition of ripening inhibitors.[18]

Over the last decade numerous studies on physiochemical stability and antimicrobial activity of essential oil nanoemulsions have been reported.[19-23] However, very few of the studies were aimed at investigating the effect of essential oil nanoemulsion compositions (e.g., Ostwald ripening inhibitors) on antifungal activities, and particularly the inhibition of mycotoxins production by *Fusarium graminearum*. In this study, clove oil was selected as a model essential oil to form food grade clove oil-in-water nanoemulsions using either MCT or corn oil as Ostwald ripening inhibitor. The impact of Ostwald ripening inhibitors (i.e., MCT and corn oil) on particle size and long term stability of clove oil nanoemulsions was assessed. Moreover, the role of oil phase composition (i.e., Ostwald ripening type and concentration) in clove oil nanoemulsions on antifungal activities against *Fusarium graminearum* isolates were evaluated. Finally, the effect of clove oil nanoemulsions on the inhibition of *Fusarium* mycotoxins production using rice culture was examined. The results of this study will provide useful

information for design and utilization of the essential oils as antifungal delivery systems in food industry.

2. Materials and methods

2.1 Materials

Polyoxyethylene (20) sorbitan monooleate (Tween 80), clove oil (purity≤100%), Mirex, and Bis(trimethylsilyl)acetamide (BSA)/trimethylchlorosilane (TMCS)/Trimethylchlorosilane (TSIM) kit were purchased from MilliporeSigma Co. (St. Louis, MO, USA). Corn oil mung beans and white basmati rice were obtained from a local supermarket (Fargo, ND, USA). Medium-chain triglyceride (MCT, NEOBEE M-5) was kindly provided by Stepan Company (Bordentown, NJ, USA). The manufacturer reported that the MCT used was mainly composed of 50-65 % caprylic acid (C8:0) and 30-45 % of capric acid (C10:0) in terms of its fatty acid profile. Potato dextrose agar (PDA) was purchased from AMRESCO (Solon, OH, USA). Potato dextrose broth was purchased from BD Biosciences (Franklin Lakes, NJ, USA). All solutions were prepared using ultrapure distilled de-ionized water (DDW, 18.2 MΩ cm, Barnstead ultrapure water system, Thermo Fisher Scientific, USA).

2.2 Nanoemulsion Preparation

The aqueous phase used to prepare clove oil nanoemulsions consisted of 0.5 wt% Tween 80 dispersing in 94.5 wt % of buffer solution (10 mM phosphate buffer, pH 7.0). Oil phase (5 wt%) was prepared by mixing different mass ratio of the clove oil and ripening inhibitors (MCT or corn oil, 0, 25, 50, 75, and 100 wt%) prior to homogenization. The oil phase was then mixed with the aqueous phase by a high-speed hand blender (M133/128-0, Biospec Products, Inc., ESGC, Switzerland) for 2 min. The mixture was further homogenized using a high pressure

homogenizer (LM 20-20 Microfluidizer Processor, Westwood, MA) at 15,000 psi for three pass. The nanoemulsions were kept on ice over the whole procedure. After homogenization, the nanoemulsions were collected and stored at 4 and 25 °C for long term storage stability study.

2.3 Particle Size Measurement

The mean particle diameters (Z-average) of nanoemulsions were measured at 0, 1, 2, 3, 4, 5, 6, 7, and 30-day using a dynamic light scattering instrument (Zetasizer Nano ZEN 3600, Malvern Instruments, Malvern, UK). The instrument determines the particle size from intensity-time fluctuations of a He–Ne laser beam (633 nm) scattered from a sample at a fixed angle of 173°. The data is reported as the mean droplet diameter and particle size distribution.

2.4 Determination of Antifungal Activity using effective concentrations (EC)

Fusarium graminearum isolates can be identified as one of three discrete chemotypes, i.e.,3-acetyl-deoxynivalenol (3-ADON), 15-acetly-deoxynivalenol (15-ADON), and nivalenol (NIV).[23] Two *F. graminearum* isolates (F8-1 and 10-124-1) were selected to evaluate the antifungal efficiency of clove oil nanoemulsions. Isolate F8-1 is a representative of deoxynivalenol (DON) and 3-ADON producers, and isolates 10-124-1 is a representative of DON and 15-ADON producers. These isolates were stored at -80°C and refreshed on PDA plates. The PDA cultures were incubated at 25°C avoiding light for 4 days prior to usage. The physically stable clove oil nanoemulsions with oil phase containing 50 wt% MCT (50M), 75 wt% MCT (75M), and 75 wt% corn oil (75C) were chosen to assess its antifungal activity using following methods (**See Section 3.3**). Firstly, the selected nanoemulsions were diluted by aqueous buffer to create desired gradient clove oil concentrations in the final nanoemulsions (0.125, 0.625, 1.250, 3.750, 6.250, 12.500, 25.000 mg/g nanoemulsions). Diluted

153 nanoemulsions were then filtered through a Whatman sterile filter (0.45 μm, 25 mm cellulose
154 acetate filtration medium, Catalog # 28138-406, GE Healthcare) to remove the microorganisms
155 before adding to PDA media. The PDA plates were prepared by pouring the autoclaved medium
156 to Petri dishes (10 cm diameter). After solidification of PDA, 500 μl nanoemulsions containing
157 a series concentration of clove oil were introduced to the surface of PDA media, whereas the
158 control dish was prepared by adding the same volume of distilled water. Then a square mycelial
159 plug (5 mm side length) of *Fusarium* isolates was placed at the center of media and incubated at
160 25°C avoiding light for 4 days prior to measurement of fungal growth. The diameter of mycelial
161 colony was measured and compared to control dish. The mycelial growth inhibition (MGI) rate
162 was calculated as MGI rate (%) =100 × (mycelial colony's diameter of control – mycelial
163 colony's diameter of treatment)/mycelial colony's diameter of control.[24] MGI rates were fitted
164 to cubic regression model and EC values were calculated by the regression equation. For
165 example, EC_{50} was calculated when MGI was observed in 50% inhibition.

166 **2.5 Fungal Morphological Study**

167 This assay was aimed at the observation of potential morphological changes of *F. graminearum*
168 isolates when exposed to clove oil nanoemulsions. Mung bean agar (MBA) plates were
169 prepared by boiling and filtering of 40 g mung beans and mixing with 15 g agar in 1 L double
170 distilled water. After sterilization, the medium was poured into small Petri dishes (4 cm
171 diameter). EC_{50} concentration of nanoemulsions were added on the surface of the MBA plates
172 and a mycelial plug (3 mm side length) from the 4 day old *Fusarium graminearum* isolates was
173 placed at the center of medium for mycelia growth and conidia production. The plates were
174 incubated for 11 days at 25°C under an ultraviolet light. After eluting by double distilled water,

conidia were observed by phase contrast microscope (Olympus EX51TF, Olympus Optical CO, Japan) and images were taken at 400× magnification.

2.6 Determination of Mycotoxin Production in Rice Culture

Preparation of Fusarium graminearum conidial suspension. Mung bean agar (MBA) media were autoclaved and poured into Petri dishes (10 mm diameter). After cooling, mycelial plugs were cut from 4 day old cultures of *Fusarium graminearum* isolates (i.e., 10-124-1 and F8-1) and used to inoculate MBA plates by gently rubbing the plugs on the surface of the plates. All the inoculated MBA plates were stored under ultraviolet light (light on: light off=12h: 12h) at ambient temperature for 9 days. Then, conidial suspensions were made from the MBA plates and filtered through autoclaved Miracloth (pore size 22-25 μm, MilliporeSigma, St. Louis, MO, USA) to remove hyphae. The concentration of conidial suspensions was calculated using a Levy Ultraplane Hemocytometer (CA Hausser & Son, PA, USA) and diluted to 1×10^6 spore/ml.

Preparation of rice culture for mycotoxins production. The physically stable clove oil nanoemulsions with oil phase containing 50 wt% MCT (50M), 75 wt% MCT (75M), and 75 wt% corn oil (75C) were selected to evaluate the inhibitory effect of EO on mycotoxins production of *Fusarium graminearum* isolates *in vitro* (**See Section 3.3**). Rice (25 g) and water (10 ml) were added to a 125 ml Erlenmeyer flask, which was then autoclaved for 25 min. After cooling, a mixture of 700 μl conidia suspension (1×10^6 spore/ml) with 700 μl of series of clove oil nanoemulsions or 17.5 μl bulk clove oil was added to the rice culture, and then shaken for 10 s. For the control group, the 700 μl conidia suspension and 700 μl double distilled water were added. The final clove oil concentrations in the rice culture when treated with bulk clove oil, 50M, 75M and 75C were 700, 700, 350, and 350 μg/g rice, respectively. The rice cultures were

197 incubated in dark at 25 °C for 9 days.

198 **2.7 Extraction and detection of mycotoxins in rice culture by GC-MS**

199 The procedure to extract mycotoxins including DON, 15-ADON, and 3-ADON in rice culture
200 was conducted using the method described by Rishi et al. with some modifications.[25] The
201 inoculated rice cultures were frozen at -80℃ prior to freezing drying (Lyophilizer, SP scientific,
202 Gardiner, New York) for two days. The dried rice cultures were ground with a Perten laboratory
203 mill (model 3600, Perten Instruments, Hagersten, Sweden), and 2 g of rice flour were extracted
204 using 20 ml of acetonitrile:water (84/16, v/v) solution by shaking at 180 rpm (Eberbach
205 Corporation, Ann Arbor, MI, USA) for 1 h. Then, 4 ml of supernatant was filtered through a
206 siliaprep C18/alumina solid phase extraction column (Chrom Tech Inc, MN, USA). After
207 filtration, 2 ml solution was transferred to sample tube (15×150 mm) and concentrated by
208 drying in an evaporator at 50 ℃ along with air flush for 1 h. Then, 100 µl of BSA:TMCS:TMSI
209 (3:2:3, v:v:v) was added into each sample tube and derivatized for 30 min. One milliliter of
210 isooctane consisting of 0.5 µg/ml Mirex as internal standard was added into the sample tube
211 before the termination of derivatization by adding 1 ml $NaHCO_3$ (3 %) solution. The derivatized
212 mycotoxins were extracted into the supernatant by shaking for 10 min, and then transferred to 2
213 ml GC vial. Tricothecene mycotoxins were measured by GC-MS as previously described.[26] The
214 system consisted of an Agilent 6890N gas chromatography coupled with 5973 mass selective
215 detector and a 35% phenyl siloxane column (30.0 m × 250 µm × 0.25 µm film) (Agilent HP-35).
216 Two microliters of the derivatized extract were injected and carried out in splitless mode at
217 300℃. The oven temp was initially kept at 150℃ for 1 min, then raised to 280℃ at a rate of
218 10 ℃/min, further ramped to 310 ℃ at a rate of 30 ℃/min, and finally maintained for 5 min at

219 310 □. The energy was −70 eV in electron impact mode. The following fragment ions (m/z)
220 were used for the qualification of trimethylsilyl ether derivatives of mycotoxins, as well as
221 Mirex: 295.20 for DON; 392.20 for 15-ADON; 377.20 for 3-ADON; and 271.90 for Mirex. The
222 limits of quantitation (LOQ) and detection (LOD) for all the mycotoxins were 0.20 and 0.10
223 μg/g, respectively.

224 **2.8 Statistical analysis**

225 All measurements were performed at least triplicate using freshly prepared samples (*i.e.,* new
226 samples were prepared for each series of experiments) and were reported as mean ± standard
227 deviation. One-way analysis of variance (ANOVA) was conducted, and significant difference of
228 mean value was defined at $p < 0.05$ by Tukey's test (IBM SPSS 24).

229 # 3. Results and discussions

230 **3.1 Pure Clove Oil-in-Water Nanoemulsions Formation**

231 The primary goal of this research is to fabricate food-grade clove oil nanoemulsions as
232 antifungal and detoxification agents in food systems. Consequently, it is important to ensure that
233 clove oil can be encapsulated in the nano-size range emulsions with diameter less than 200 nm
234 and good initial physical stability as well.

235 Initially, 5 wt% of pure clove oil, as the solo oil phase, was dispersed into the aqueous phase
236 containing 0.5 wt% Tween 80 before homogenizing using microfludizer. However, the resulting
237 fresh emulsions with the mean particle diameter of 784 nm were highly unstable to droplet
238 growth. Prompt phase separation of emulsions was visualized after 1.5 h preparation and the
239 mean particle diameter was around 876 nm (**Fig. 1a**). The measurements of the evolution of
240 particle size showed that there were two main size classes with peaks around 100 and 1000 nm

241 in diameter in pure clove oil-in-water emulsions soon after homogenization, suggesting that
242 droplet growth occurred very rapidly in this system (**Fig. 1b**). The oil droplets continued to
243 grow very fast during storage and after 1 h, the population of small-sized droplets had
244 disappeared and only a population of larger droplets was observed (**Fig. 1b**). The instability of
245 bulk clove oil-in-water emulsions and the growth of large size population can be explained by
246 the occurrence of Ostwald ripening, the process whereby large droplets grow at the expense of
247 smaller ones through the intervening continuous phase. Ostwald ripening is a common problem
248 responsible for the instability of EOs emulsions or nanoemulsions due to the relatively high
249 water solubility of EOs leading to the mass transport of dispersed phase from one droplet to
250 another. The phenomenon observed in the current study was in good agreement with other
251 published papers.[18-19, 27] For example, emulsion made by pure peppermint oil exhibited very
252 larger droplet around 4 μm right after homogenization.[18]

253 Fig 1 inserted here

254 **3.2 Influence of Ostwald Ripening Inhibitors on Clove Oil Nanoemulsions Formation**

255 Previous studies have evidenced that Ostwald ripening can be retarded or inhibited by
256 incorporating highly hydrophobic component, such as medium chain triacylglycerol (MCT) and
257 long chain triacylglycerol (LCT), in the essential oil phase prior to homogenization.[22, 28] It is
258 corroborated that molecules with low water solubility might not only inhibit Ostwald ripening
259 by generating entropy of mixing effect to counterbalance the interfacial curvature effect, but
260 also facilitate the size reduction of droplets to the desired nano-size range.[16] In this study, we
261 examined whether food grades corn oil as a LCT representative or MCT was more effective to
262 mitigate Ostwald ripening in clove oil-in-water nanoemulsion systems. A series of

nanoemulsions with different clove oil concentrations (100, 75, 50, 25 wt%) mixed with different type and amounts of ripening inhibitor (corn oil or MCT) were prepared to examine the effect of ripening inhibitor on the stability of clove oil nanoemulsions. The ripening inhibitors were mixed with clove oil prior to homogenization. After homogenization, the nanoemulsion samples were stored for 24 h at 25 °C prior to measure the particle size (**Fig. 2**).

Fig. 2 inserted here

For the MCT mixed with clove oil system, the trend of decreasing droplet diameter was found with increasing concentration of MCT (**Fig. 2a**). The droplet size decreased dramatically to 118 nm when 50 wt% of MCT was mixed with clove oil in oil phase, which can be attributed to the ability of MCT to inhibit Ostwald ripening. A further increase in MCT concentration did not change the mean droplet diameter steeply. The smallest mean droplet diameter of 94 nm was obtained in the system produced by 75 wt% of MCT in oil phase. For the system containing more than 50 wt% of MCT, it could be considered as nanoemulsions, that is, $d \leq 200$ nm. In the system containing corn oil in the oil phase, there was a slightly decrease in droplet diameter (972 nm) of clove oil emulsion when 25 wt% of corn oil was incorporated in oil phase. Surprisingly, as corn oil increased to 50 wt%, a highly unstable dispersion system was appeared accompanied with a visible oiling off soon after homogenization (**Fig. 2b**). Further increase corn oil content to 75 wt% in oil phase yielded a stable clove oil nanoemulsions with mean droplet diameter of 86 nm. The turning point as 50 wt % of corn oil was present can be explained by the formation of relatively small droplets under high pressure homogenization, followed by a quick droplet growth, presumably because of the entropy of mixing in 50 wt% of corn oil is lower than that of interfacial curvature. Our results demonstrated that 50 wt% MCT is

285 the threshold to retard Ostwald ripening, whereas 75 wt% of corn oil was needed to prevent
286 droplet growth in clove oil nanoemulsions.
287 Interestingly, one would expect that MCT is less effective to prevent Ostwald ripening since the
288 water solubility of MCT is somewhat higher than that of corn oil. However, our results indicated
289 that MCT is a highly effective ripening inhibitor in the performance of enhancing physical
290 stability of clove oil-in-water nanoemulsions than corn oil. Similar findings were also reported
291 by Chang et al. [17], whose results also demonstrated that the addition of corn oil could inhibit
292 Ostwald ripening in thyme oil-in-water nanoemulsions at pH 3.5 more efficient than MCT. The
293 discrepancy implies that solubility is not the only factor to determine the efficacy of inhibitors
294 to prevent Ostwald ripening. Overall, mixing sufficient amount of MCT or corn oil with clove
295 oil phase before high pressure homogenization was a useful tool to inhibit Ostwald ripening of
296 clove oil nanoemulsions.

297 **3.3 Storage Stability of Clove Oil Nanoemulsions**
298 As mentioned earlier, clove oil nanoemulsions are anticipated to be used as effective antifungal
299 and detoxification agents in food systems. Therefore, a good long term stability of
300 nanoemulsion is critical to ensure the activity of encapsulated clove oil to be retained during
301 storage. However, the successful fabrication of clove oil nanoemulsions with good initial
302 physical stability cannot guarantee a long term storage stability, especially under different
303 storage temperatures. In this case, three clove oil nanoemulsions that were found to be stable to
304 droplet growth during the first 24 h storage, i.e., the oil phase containing 50 wt% MCT (50M),
305 75 wt% MCT (75M), and 75 wt% corn oil (75C), were selected for the long term storage study.
306 The change of particle size within 30 days storage at different storage temperature (4 and 25 □)

were measured as shown in **Fig. 3**.

Fig 3 inserted here

Under both storage temperature, there was a slight increase in mean particle diameter of clove oil nanoemulsions from ~84 and ~ 94 nm to ~90 and ~102 nm, respectively, with oil phase containing either 75 wt% corn oil or 75 wt% MCT in first 7 days storage, after which maintained constantly over the course of 30 days storage (**Fig. 3a & b**). The mean particle diameter of nanoemulsions containing 50 wt% MCT in oil phase was increased from ~101 nm to ~118 nm after 7 days storage and subsequently remained constant over storage time when stored at 4□; however, higher storage time promoted the growth of particle size to 144 nm, still remaining in nanometric range, after 30 days storage (**Fig. 3c**). In the meantime, no phase separation or oiling off was observed after 30 days storage at both storage temperatures, strongly manifesting its good long term stability.

The particle size distribution, rather than just the mean particle diameter, is an important factor for monitoring stability of nanoemulsions. We therefore plotted particle size distribution of nanoemulsion during storage time (**Fig. 4**).

Figure 4 inserted here

The particle size distribution of clove oil nanoemulsions (75C & 50M) had no shift and maintained monomodal pattern within 30 days, again indicating that Ostwald ripening has been largely inhibited (**Fig. 4a & c**). However, a slightly difference among size distribution were observed in the nanoemulsions prepared by different concentration (50M & 75M) of Ostwald ripening inhibitor, with the higher concentration one in the oil phase generating longer stability (**Fig. 4b & c**). The particle size distribution of 50M nanoemulsions had a slightly shift towards

larger region after 7 days storage; still, no phase separation was observed upon 30 days storage (**Fig. 4b**).

Overall, the consistent mean diameter of the three clove oil nanoemulsions over storage time implies that the nanoemulsions are highly stable against droplet growth across the whole measurement temperature and storage time.

3.4 Influence of Oil Phase Composition on Antifungal Activity of Clove oil Nanoemulsions

The antifungal activity and inhibition of mycotoxins production of clove oil might be affected by the oil composition in nanoemulsion systems. The antifungal activity of the three clove oil nanoemulsions (MCT or corn oil ≥ 50 wt% in oil phase) that exhibited good long term physical stability was then evaluated against two common chemotypes (3-ADON and 15-ADON) of *Fusarium graminearum* isolates in USA using agar dilution method. The mycelial growth inhibition (MGI) was used to compare the antifungal activity of nanoemulsions carrying different concentrations of clove oil (0.125 to 12.500 mg/g) and higher MGI rate represents greater activity.

Emulsions in the absence of clove oil (i.e., 100 wt% of corn oil or MCT in oil phase) against *Fusarium graminearum* was also examined and neither of them exhibited any antifungal activity (data not shown), indicating that it was the clove oil which exclusively generates antifungal activity against *Fusarium graminearum*. We did not examine the antifungal activity of nanoemulsions prepared by bulk clove oil due to its extremely physical unstable nature. The mycelial growth inhibition (MGI) rate of nanoemulsions loaded with different concentrations of clove oil and ripening inhibitors (corn oil or MCT) in oil phase was shown in **Fig. 5**.

The results clearly showed that MGI rate increased with increasing the concentration of clove

oil in nanoemulsions. At the lower clove oil concentrations, nanoemulsions with corn oil in oil phase showed stronger antifungal activity than that containing MCT across all tested *Fusarium graminearum* isolates (**Fig. 5**). For instance, when *Fusarium graminearum* isolate F8-1 was treated with nanoemulsions containing same concentration of clove oil (i.e., 2.5 mg/g), the MGI rate was 34.63 % and 26.32 % for ripening inhibitor corn oil and MCT, respectively, proving our hypothesis that different Ostwald ripening inhibitor had varying effects on clove oil antifungal activity (**Fig. 5a**). As the total concentration of clove oil in nanoemulsions was increased from 7.5 to 12.5 mg/g, ripening inhibitor (corn oil and MCT) had no significant influence on the antifungal activity of clove oil nanoemulsions. Similar trend was also found in *Fusarium graminearum* isolate 10-124-1 (**Fig. 5b**).

Fig. 5 inserted here

EC values of the two tested *Fusarium graminearum* isolates were calculated by the cubic regression model to establish the relationship between MGI rate and clove oil concentration in nanoemulsions (**Table 1**). The results showed that increasing the level of ripening inhibitor in oil phase reduced the antifungal activity of clove oil nanoemulsions. For instance, clove oil nanoemulsions with 50 wt% MCT in oil phase (50M) received the smallest EC values (e.g., EC_{50}= 3.569 and 4.140 mg/g in F8-1 and 10-2124-1, respectively) due to the highest net clove oil concentration (25.000 mg/g nanoemulsion) compared with the rest two systems with only 12.500 mg/g nanoemulsion of net clove oil existed in oil phase when the isolates was treated by the same volume (i.e., 500 μl) of nanoemulsions. For this reason, the concentration of clove oil in the nanoemulsion delivery systems had essential impact on the antifungal activity. When same concentration of clove oil was loaded in oil phase (75C and 75M), nanoemulsions using

corn oil as ripening inhibitor displayed stronger antifungal activity than the ones using MCT in oil phase as reflected by both EC_{50} and EC_{70} against two isolates ($p<0.05$). This study demonstrated that net clove concentration and ripening inhibitor type had a profound influence on the antifungal activity of clove oil nanoemulsions against *Fusarium graminearum*. This can be explained by the higher oil-water partition coefficient of clove oil when mixing with MCT than with corn oil. Accordingly, higher amount of clove oil will be dissolved and physically trapped in MCT than in corn oil under same initial clove oil concentration. As a result, the incorporation of MCT in oil phase of nanoemulsions renders a relatively lower antifungal activity of clove oil by attenuating the efficient amount of clove oil to be delivered to the site at which it acts as antifungal agent. Similar results had also been reported that MCT decreased the antimicrobial ability of thyme oil nanoemulsions larger than that of corn oil against an acid-resistant spoilage yeast. [17] In contrast, there was no distinctive differences between 75C and 75M in EC_{90} value, which reveals that the limited impact of oil-water partition coefficient of clove oil in oil phase is no longer an important factor to influence the antifungal activity of nanoemulsions at such higher clove oil concentration. From the result described above, the antifungal activity of clove oil nanoemulsions was determined not only by the concentration of active compounds in oil phase, but also by the location of active compounds in the system being determined by the type Ostwald ripening inhibitor.

Table 1 inserted here

In order to better understand the mechanism by which clove oil nanoemulsions inhibit fungal growth, the morphology of *Fusarium graminearum* isolate F 8-1 treated with clove oil nanoemulsions was examined by phase contrast light microscope (**Fig. 6**). In the control group,

396 prevailing germ tubes from spores were observed, some of which became branched hyphae,
397 representing the fast growth of fungal (**Fig. 6a**). Such morphology was in consistent with
398 normal spore germination and hyphae growth in *Fusarium graminearum.*[29] Conversely, No
399 spore germination and hypha growth were observed in clove oil nanoemulsions treated
400 *Fusarium graminearum,* indicating that clove oil nanoemulsions had remarkable effect on
401 retardation of *Fusarium graminearum* growth (**Fig. 6b & c**). Based on light microscopy studies
402 along with agar dilution method, clove oil nanoemulsions could inhibit *Fusarium graminearum*
403 growth by retarding the mycelial growth.

404 Fig. 6 inserted here

405 **3.5 Influence of Oil Phase Composition on inhibition of mycotoxin production**

406 In terms of food safety, the main issue that needs to be addressed is the consumption of
407 mycotoxin contaminated food. The inhibitory activity of clove oil nanoemulsions to *Fusarium*
408 *graminearum* growth cannot be extrapolated to the inhibition of mycotoxins production because
409 antifungal agents might trigger the production of secondary metabolisms and mycotoxins as a
410 response to environmental stress.[2] Thus it is crucial to evaluate the effect of clove oil
411 nanoemulsions on the production of mycotoxin in *Fusarium graminearum* isolates. The effect
412 of clove oil nanoemulsions on mycotoxins production by two chemotypes of *fusarium*
413 *graminearum* isolates (10-124-1 and F8-1) in rice culture was studied upon incubation at 25 □.
414 The isolate10-124-1 of *Fusarium graminearum* used in this study produces deoxynivalenol
415 (DON, **Fig. 7a**) and 3-acetyldeoxynivalenol (3-ADON, **Fig. 7b**), whereas isolate F8-1 produces
416 deoxynivalenol (DON, **Fig. 7c**) and 15-acetyldeoxynivalenol (15-ADON, **Fig. 7d**).

417 Fig. 7 inserted here

In general, all clove oil nanoemulsions showed an inhibition on mycotoxins production over incubation times. The oil phase composition (i.e., ripening inhibitor type and clove oil concentration) had an appreciable influence on the mycotoxin inhibition of *Fusarium graminearum* isolates. The inhibitory activity of clove oil nanoemulsions increased with increasing clove oil concentrations (50M > 75M). At the same concentration of clove oil in oil phase (75C and 75M), clove oil nanoemulsions with corn oil as ripening inhibitor in oil phase performed stronger inhibitory activity on mycotoxins production than those with MCT in oil phase for both fungal isolates studied (**Fig. 7**). The possible reason for this phenomenon was again due to the higher amount of clove oil being physically trapped in MCT than in corn oil at the same initial clove oil concentration. As a result, the existence of MCT in oil phase causes a bigger reduction in the mycotoxin inhibitory effect of clove oil than that of corn oil. Furthermore, mycotoxin inhibition of bulk clove oil (700 μg clove oil/g rice) was significant lower than that of nanoemulsions with equivalent amount of clove oil. For example, a complete inhibition (100 %) of all three mycotoxins were achieved in the two isolates across the entire incubation time with the addition of 50M nanoemulsions bearing700 μg clove oil/g rice; however, DON was only reduced by ~ 80% in *Fusarium graminearum* isolate F8-1 upon the addition of 700 μg bulk clove oil /g rice after 9 days incubation (**Fig. 7c**). Interesting, it is also noticed that bulk clove oil had shown some inhibition of DON production in the first 5 days of incubation, after which it promoted the production of DON in *Fusarium graminearum* isolate 10-124-1 (**Fig. 7a**). Presumably, the nanoemulsion systems would be useful to increase the stability and solubility of clove oil in the rice culture medium to further control the release of bioactive components in clove oil during incubation, resulting in the extending of mycotoxin inhibition effect.

Among the two *Fusarium graminearum* isolates, isolate 10-124-1 was more sensitive to the action of three types of clove oil nanoemulsions in comparison with isolate F8-1 towards the inhibition of mycotoxins production. For *Fusarium graminearum* isolate 10-124-1, both DON and 15-ADON level gradually increased during the first 6 days of incubation, and subsequently increased to high levels in control group. With addition of clove oil either in bulk oil form (700 μg clove oil/g rice) or in nanoemulsion forms (50M, 75C and 75M), a strong inhibition of both DON and 15-ADON production were observed (**Fig. 7c & d**). For example, only 0.55±0.24 μg/g level of DON produced in *Fusarium graminearum* isolate 10-124-1 by adding 75C nanoemulsions after 9 days of incubation (**Fig. 7c**). In contrast, DON was completed suppressed by 50M nanoemulsions containing 50 wt % MCT (700 μg clove oil/g rice) in oil phase, while for 75C nanoemulsions containing 75 wt % corn oil (350 μg clove oil/g rice) in oil phase, over 140 μg/g level of DON could be detected after 9 days of incubation for *Fusarium graminearum* F8-1 (**Fig. 7a**). Similar trend was observed in the inhibition of 3-ADON (**Fig. 7b**). On the basis of the results described above, it is clear that clove oil nanoemulsions exerted higher inhibitory activity on mycotoxins production of *Fusarium graminearum* isolates 10-124-1 and F8-1 than bulk clove oil under the same concentration.

The effects of EOs on fungal growth and mycotoxin production of some toxigenic fungal genera like *Fusarium* spp., *Aspergillus* spp., and *Penicillium* spp. had been reported in literature.[9, 29-31] Some of possible mechanisms of the action have been proposed. For instance, it is suggested that the fungi respond by limiting secondary metabolite mycotoxins production with respect to the stress induced by certain active compounds from EOs such as phenolic compounds. [2] Recent study have also indicated that EOs components decreased the mRNA levels encoding proteins in

463 fungi, which is prerequisite for DON biosynthesis.[33] The mechanism of bulk clove oil to inhibit
464 the growth and mycotoxins production of *Fusarium graminearum* isolates 10-124-1 and F8-1
465 may be largely due to the abovementioned reasons because bulk clove oil contained bioactive
466 phenolics eugenols. The striking enhancement of antifungal and detoxification activity of clove
467 oil when incorporated in nanoemulsions could be attributed to the increased solubility and
468 controlled release of clove oil.

469 **4. Conclusions**

470 In summary, physical stable clove oil nanoemulsions could be fabricated by mixing clove oil
471 with either ≥ 50 wt% of MCT or ≥ 75 wt% of corn oil. The particle size of clove oil
472 nanoemulsions containing appropriate amount of ripening inhibitors such as MCT or corn oil
473 remained stable during 30 days storage under 4 and 25 □. The ripening inhibitor type and
474 concentration had a remarkable influence on antifungal activity and mycotoxins inhibitory
475 activity of the clove oil nanoemulsions. In general, the incorporation of corn oil in oil phase
476 enhanced antifungal activity and mycotoxins inhibitory activity of clove oil nanoemulsions
477 compared to MCT. The antifungal activity and mycotoxins inhibitory effect decreased with
478 increasing ripening inhibitor levels in oil phase. Our study has also shown that nanoemulsions
479 based delivery system substantially increase the mycotoxins inhibitory activity of the clove oil:
480 nanoemulsions containing 350 μg clove oil/g rice was required for a complete inhibition of
481 mycotoxins production from *Fusarium graminearum*, whereas a double concentration (i.e., >
482 700 μg clove oil/g rice) of bulk clove oil was needed to achieve the same efficacy. This effect
483 may be attributed to: (i) the nanoemulsion based delivery systems could significantly increase
484 the stability and solubility of the essential oil in medium; (ii) the controlled release of essential

485 oil bioactive constituents in rice culture medium, thus extending the mycotoxin inhibitory
486 activity. The results reported in this study have important implications for the design and
487 utilization of nanoemulsions as effective antifungal and efficient detoxification delivery systems
488 in food or other industries. For example, essential oils often have a strong flavor profile. The
489 addition of low concentrations of the nanoemulsions encapsulated essential oils might be able to
490 completely inactivate fungi and mycotoxins production, while minimizing the impact on the
491 organoleptic properties of the foods.

492 ## Conflicts of interest

493 There are no conflicts to declare.

494 ## Acknowledges

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496 and USDA National Institute of Food and Agriculture Multistate Research Project (NC1183).

497 ## REFERENCES

498 1 B. Kabak, *J. Sci. Food Agric.*, 2009, **89**, 549–554.
499 2 L. da Cruz Cabral, V. Fernández Pinto and A. Patriarca, *Int. J. Food Microbiol.*, 2013,
500 **166**, 1–14.
501 3 C. M. Placinta, J. P. F. D'Mello and A. M. C. MacDonald, *Anim. Feed Sci. Technol.*, 1999,
502 **78**, 21–37.
503 4 L. B. Bullerman and A. Bianchini, *Int. J. Food Microbiol.*, 2007, **119**, 140–146.
504 5 L. Escrivá, G. Font and L. Manyes, *Food Chem. Toxicol.*, 2015, **78**, 185–206.
505 6 R. Gyawali and S. A. Ibrahim, *Food Control*, 2014, **46**, 412–429.

7 V. K. Juneja, H. P. Dwivedi and X. Yan, *Annu. Rev. Food Sci. Technol.*, 2012, **3**, 381–403.

8 J. Gutierrez, C. Barry-Ryan and P. Bourke, *Int. J. Food Microbiol.*, 2008, **124**, 91–97.

9 A. Velluti, V. Sanchis, A. J. Ramos, J. Egido and S. Marín, *Int. J. Food Microbiol.*, 2003, **89**, 145–154.

10 S. Burt, *Int. J. Food Microbiol.*, 2004, **94**, 223–253.

11 M. E. Guynot, A. J. Ramos, L. Setó, P. Purroy, V. Sanchis and S. Marín, *J. Appl. Microbiol.*, 2003, **94**, 893–899.

12 A. Amiri, R. Dugas, A. L. Pichot and G. Bompeix, *Int. J. Food Microbiol.*, 2008, **126**, 13–19.

13 F. Donsì, M. Annunziata, M. Sessa and G. Ferrari, *LWT - Food Sci. Technol.*, 2011, **44**, 1908–1914.

14 J. Rao, E. A. Decker, H. Xiao and D. J. Mcclements, *J. Sci. Food Agric.*, 2013, **93**, 3175–3183.

15 A. H. Saberi, Y. Fang and D. J. McClements, *J. Colloid Interface Sci.*, 2013, **391**, 95–102.

16 D. J. McClements and J. Rao, *Crit. Rev. Food Sci. Nutr.*, 2011, **51**, 285–330.

17 Y. Chang, L. Mclandsborough and D. J. Mcclements, *J. Agric. Food Chem.*, 2012, **60**, 12056–12063.

18 R. Liang, S. Xu, C. F. Shoemaker, Y. Li, F. Zhong and Q. Huang, *J. Agric. Food Chem.*, 2012, **60**, 7548–7555.

19 F. Donsì, M. Annunziata, M. Vincensi and G. Ferrari, *J. Biotechnol.*, 2012, **159**, 342–350.

20 L. Salvia-Trujillo, A. Rojas-Graü, R. Soliva-Fortuny and O. Martín-Belloso, *Food Hydrocoll.*, 2015, **43**, 547–556.

21 K. Ziani, Y. Chang, L. McLandsborough and D. J. McClements, *J. Agric. Food Chem.*,

2011, **59**, 6247–6255.

22 M. R. Zahi, H. Liang and Q. Yuan, *Food Control*, 2015, **50**, 554–559.

23 K. D. Puri and S. Zhong, *Phytopathology*, 2010, **100**, 1007–1014.

24 H. Patzke, S. Zimdars, N. Schulze-Kaysers and A. Schieber, *Food Res. Int.*, 2017, **99**, 821–827.

25 R. R. Burlakoti, S. Ali, G. A. Secor, S. M. Neate, M. P. McMullen and T. B. Adhikari, *Appl. Environ. Microbiol.*, 2008, **74**, 6513–6520.

26 Z. Jin, B. Zhou, J. Gillespie, T. Gross, J. Barr, S. Simsek, R. Brueggeman and P. Schwarz, *Food Control*, 2018, **85**, 6–10.

27 S. Abbaszadeh, A. Sharifzadeh, H. Shokri, A. R. R. Khosravi and A. Abbaszadeh, *J. Mycol. Med.*, 2014, **24**, e51–e56.

28 L. M. VictorRyu, David J.McClements, Maria G.Corradini, *Food Chem.*, 2018, **245**, 104–111.

29 K. Naveen Kumar, M. Venkataramana, J. A. Allen, S. Chandranayaka, H. S. Murali and H. V. Batra, *LWT - Food Sci. Technol.*, 2016, **69**, 522–528.

30 R. V. Bluma and M. G. Etcheverry, *Food Microbiol.*, 2008, **25**, 324–334.

31 J. Nguefack, V. Leth, P. H. Amvam Zollo and S. B. Mathur, *Int. J. Food Microbiol.*, 2004, **94**, 329–334.

32 A. Kumar, R. Shukla, P. Singh and N. K. Dubey, *Food Chem. Toxicol.*, 2010, **48**, 539–543.

33 A. Yaguchi, T. Yoshinari, R. Tsuyuki, H. Takahashi, T. Nakajima, Y. Sugita-Konishi, H. Nagasawa and S. Sakuda, *J. Agric. Food Chem.*, 2009, **57**, 846–851.

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553

554 **Table 1.** Antifungal activity against *Fusarium graminearum* isolates on PDA media. Effective
555 concentration (EC) was expressed by the concentration of clove oil in nanoemulsions. For
556 example, EC_{50} was calculated when isolate mycelia growth was observed in half inhibition. For
557 each EC value against a certain isolate, means with different letters are significantly different
558 ($p<0.05$).

F. graminearum isolates	Nanoemulsions	EC_{50} (mg/g)	EC_{70} (mg/g)	EC_{90} (mg/g)
F8-1	75C	4.674^{b}	7.731^{b}	11.174^{a}
	75M	5.814^{c}	9.013^{c}	11.785^{a}
	50M	4.140^{a}	6.764^{a}	10.760^{a}
10-124-1	75C	4.150^{B}	7.260^{B}	11.249^{B}
	75M	5.300^{C}	7.866^{C}	11.103^{B}
	50M	3.569^{A}	6.125^{A}	10.339^{A}

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570 ## Figure Captions

571 **Figure 1.** Time-dependence of (**a**) mean particle diameter; (**b**) particle size distribution of 5
572 wt % bulk clove oil-in-water emulsions stored at 25 ^{o}C (0.5 wt% Tween 80, 94.5 wt% of 10mM
573 phosphate buffer, pH 7; the inserted pictures were the visual observation of emulsions)
574
575 **Figure 2.** Dependence of oil phase Ostwald ripening inhibitor (a) Medium Chain
576 Triacylglycerol (MCT); (b) corn oil on mean particle diameter of 5 wt% clove oil-in-water
577 emulsions after 24 h storage at 25 ^{o}C (0.5 wt% Tween 80, 10mM phosphate buffer, pH 7; the
578 inserted picture was the visual observation of oiling off in emulsion)
579
580 **Figure 3.** Evolution of mean particle diameter of 5 wt% oil-in-water nanoemulsions with oil
581 phase containing (a) 75 wt% corn oil (75C); (b) 75 wt% MCT (75M); (c) 50 wt% MCT (50M)
582 upon 30 days storage at 4 and 25 □
583
584 **Figure 4**. Particle size distribution of 5 wt% oil-in-water nanoemulsions with oil phase
585 containing (a) 75 wt% corn oil (75C); (b) 75 wt% MCT (75M); (c) 50 wt% MCT (50M) upon
586 30 days storage (the inserted pictures were visual appearance of nanoemulsions during storage
587 at 25 □)
588
589 **Figure 5**. Influence of clove oil concentrations and Ostwald ripening inhibitor type on mycelia
590 growth inhibition rate (MGI) in (a) *Fusarium graminearum* isolate F8-1; (b) *Fusarium*
591 *graminearum* isolate F 10-124-1 after 4 days of incubation. The mycelial growth inhibition
592 (MGI) rate was calculated as MGI rate (%) =100× (mycelial colony's diameter of control –

593 mycelial colony's diameter of treatment)/mycelial colony's diameter of control (the inserted
594 images were the appearance of mycelia growth inhibition zone)
595
596 **Figure 6.** Light microscope images (400 × magnification) of spores in *Fusarium graminearum*
597 isolate F8-1 grown on MBA after 11 days of incubation (a) in control group; (b) treated with
598 4.674 mg clove oil/g nanoemulsions (EC_{50}) of 75C; (c) treated with 5.814 mg clove oil/g
599 nanoemulsions (EC_{50}) of 75M. Spore germination was only observed in control group. Scale bar
600 indicates 10 μm
601
602 **Figure 7.** Mycotoxins production behavior of *Fusarium graminearum* isolates in rice culture
603 during 9 days of incubation after treatment with different clove oil nanoemulsions. (a) DON
604 produced from isolate F8-1; (b) 3-ADON produced from isolate F8-1; (c) DON produced from
605 isolate 10-124-1; (d) 15-ADON produced from isolate 10-124-1
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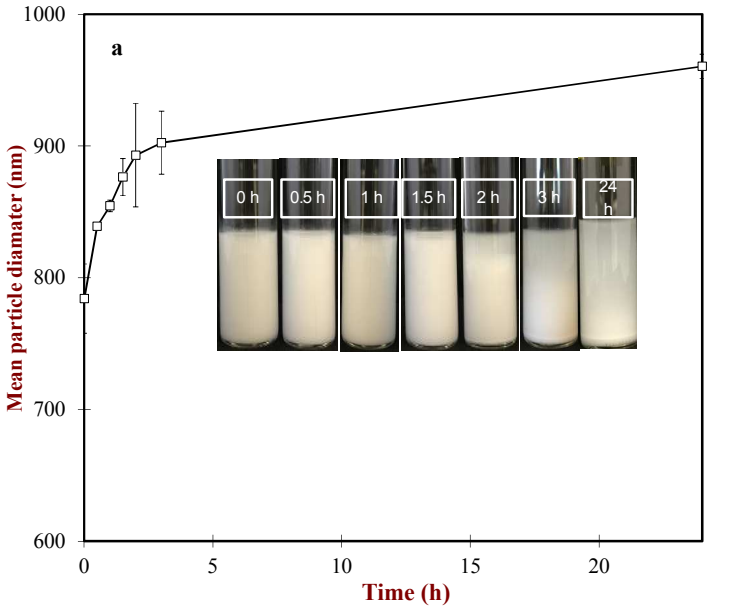
616 **Fig. 1**



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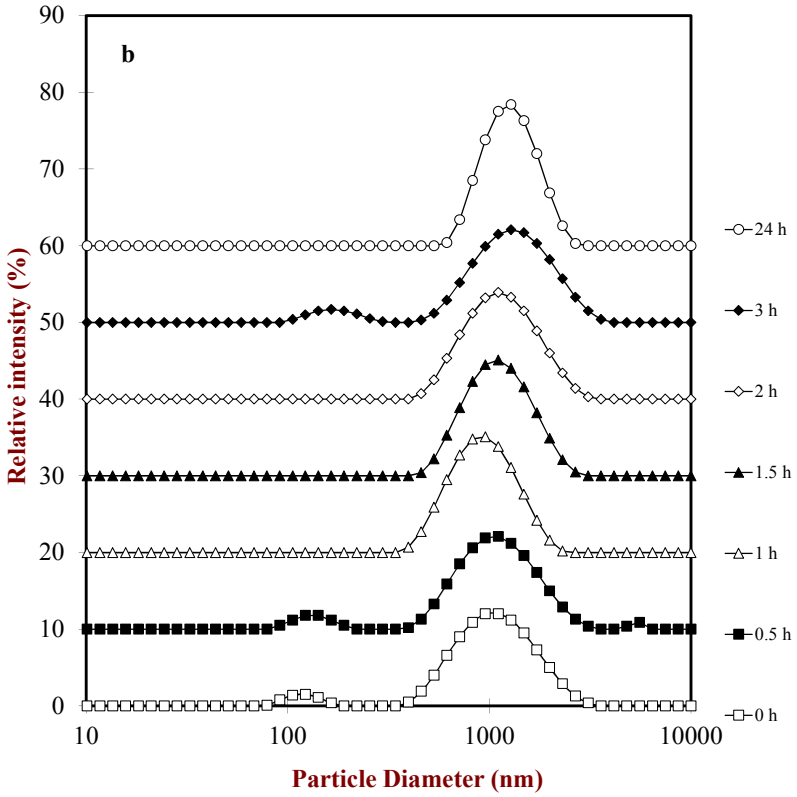

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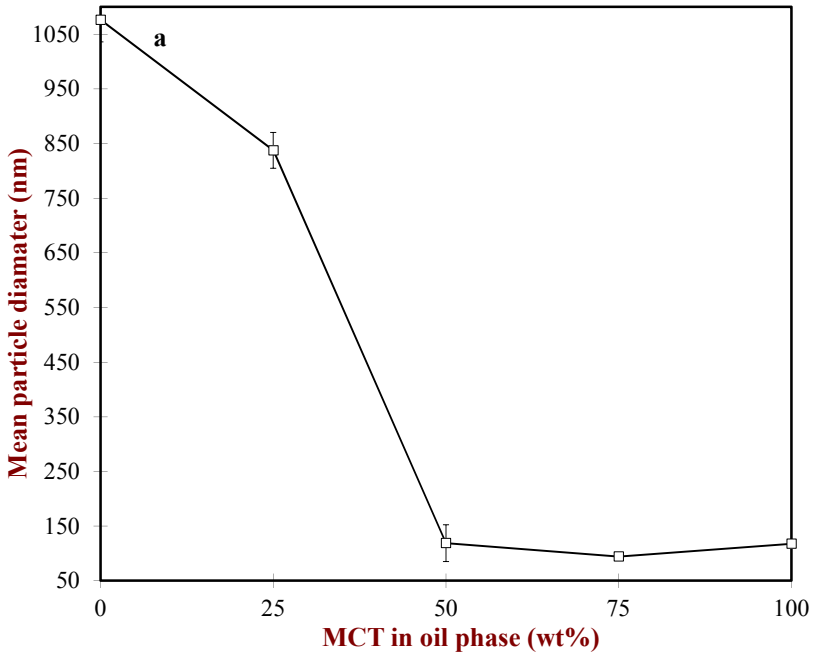
621 **Fig. 2**



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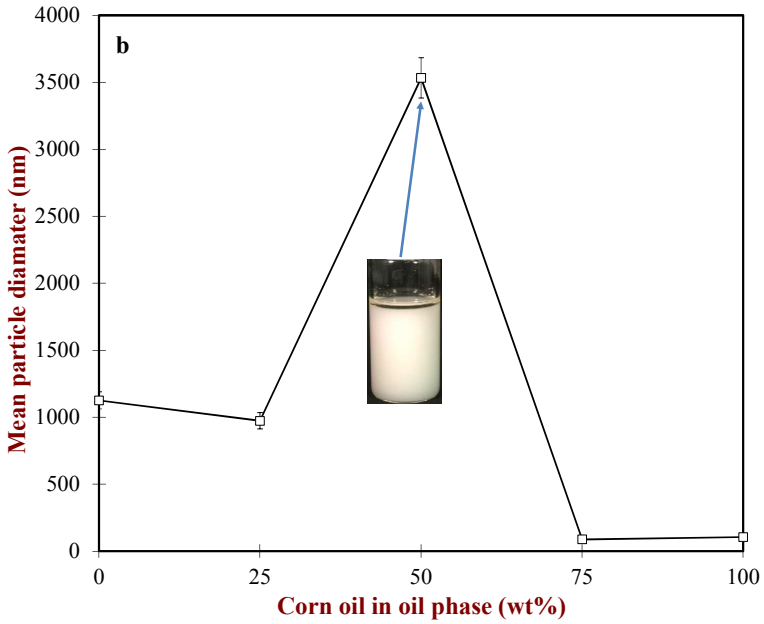

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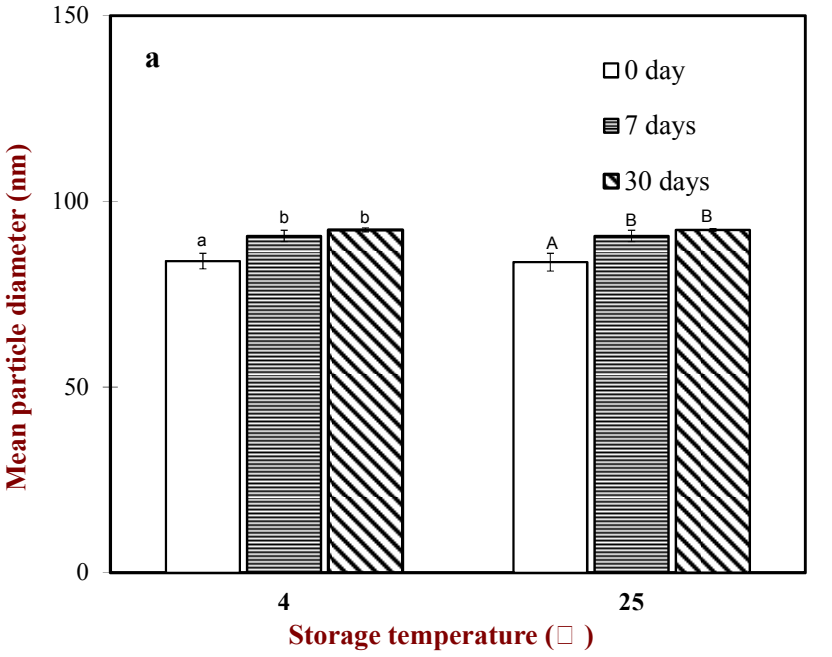
627 **Fig. 3**



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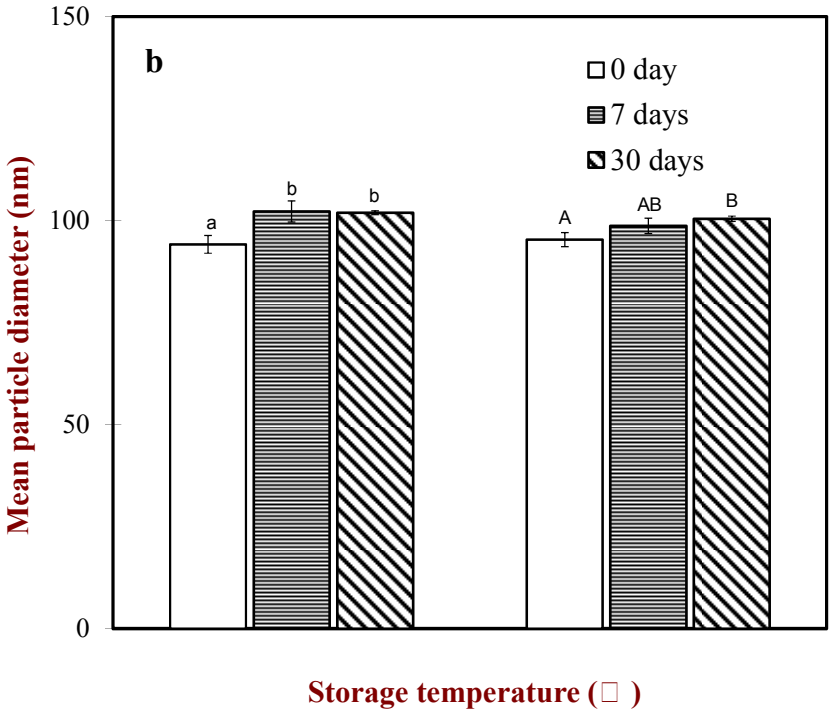

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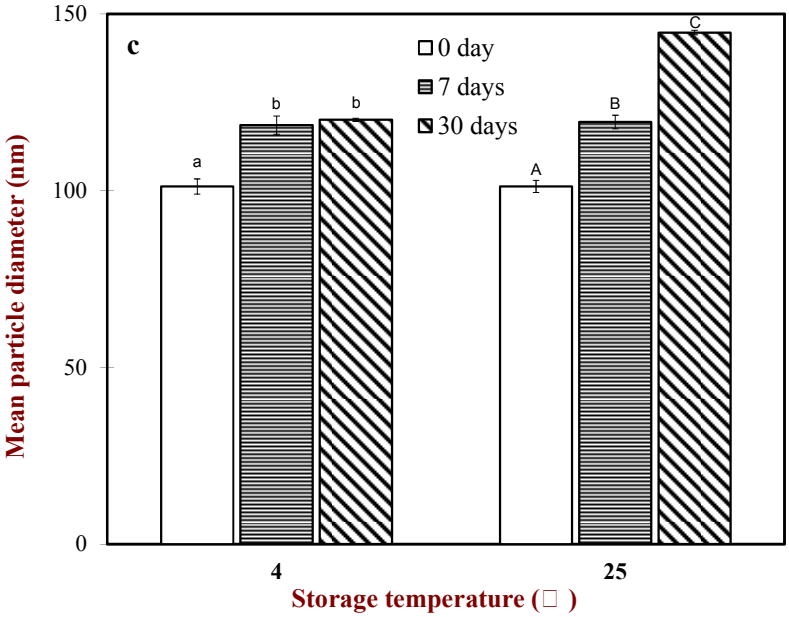

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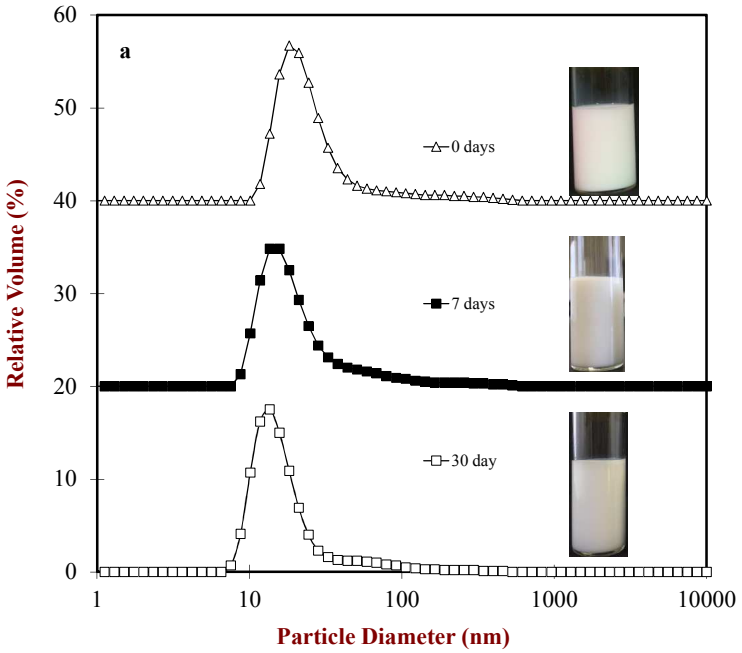
645 **Fig. 4**



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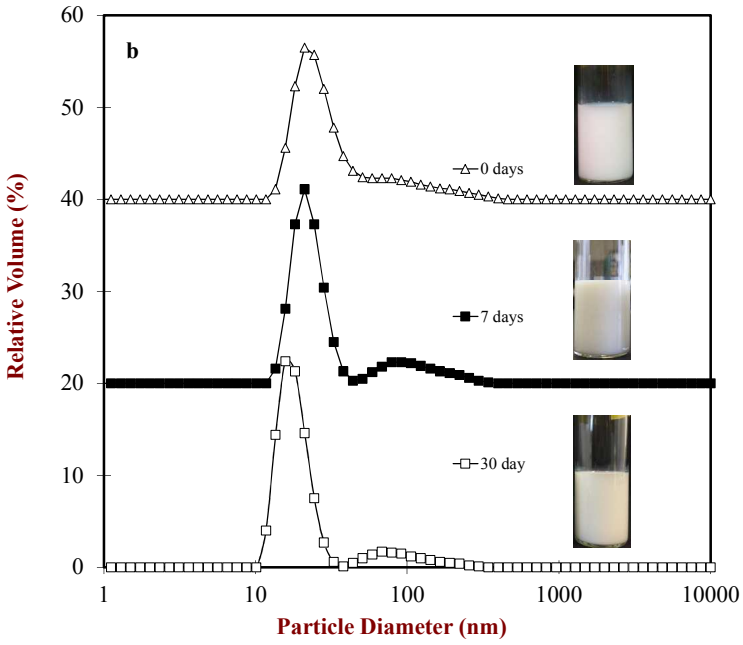

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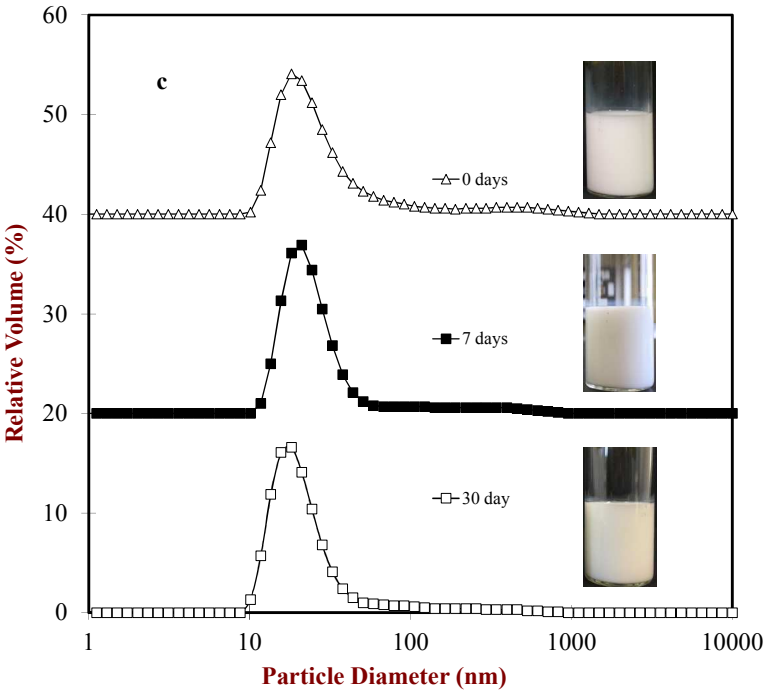

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663 **Fig. 5**

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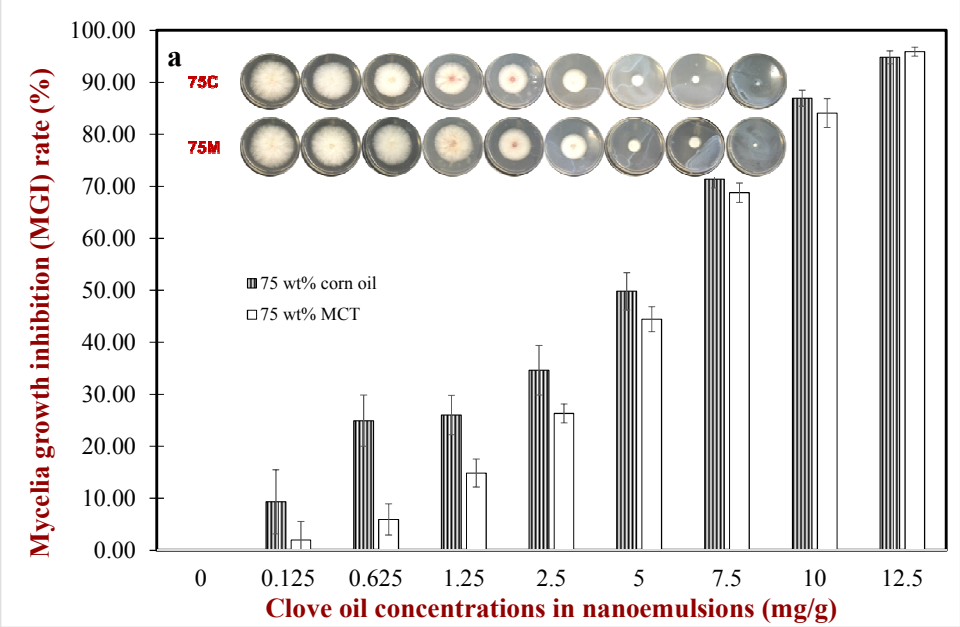

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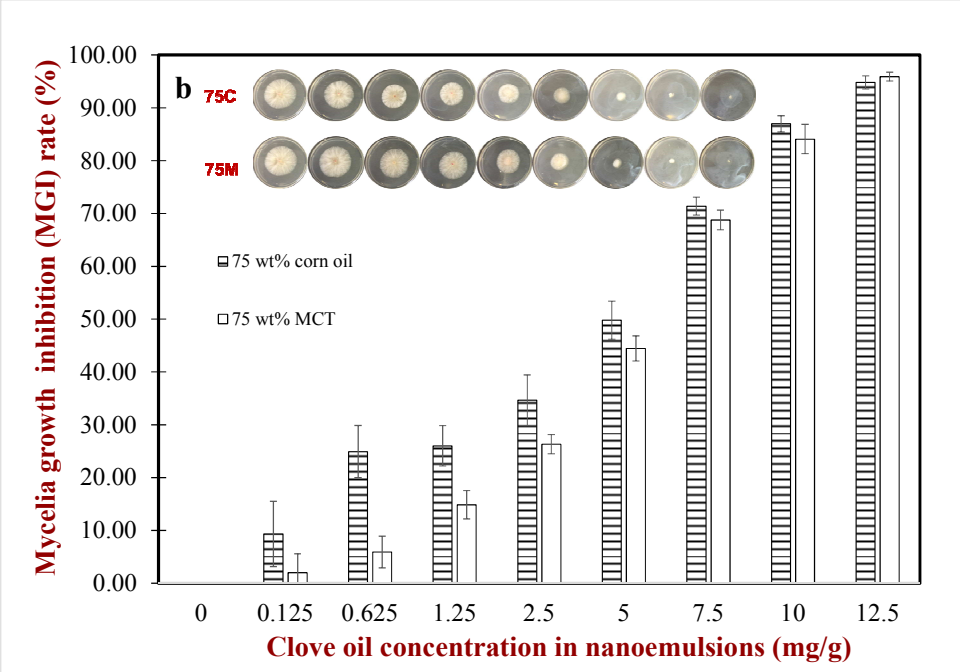

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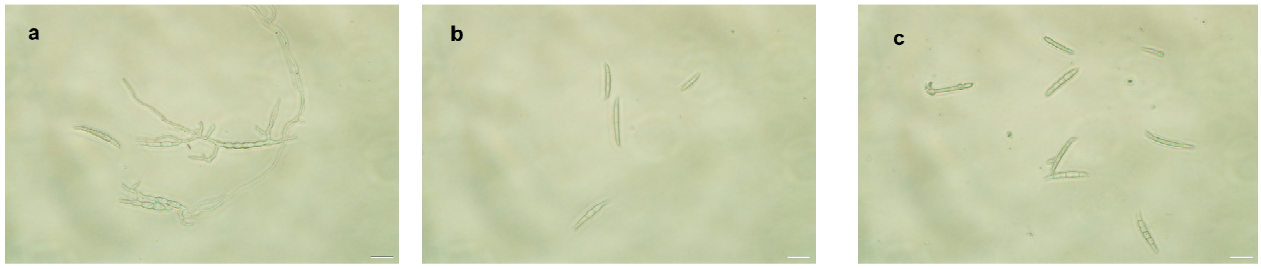
Fig. 6



693 **Fig. 7**

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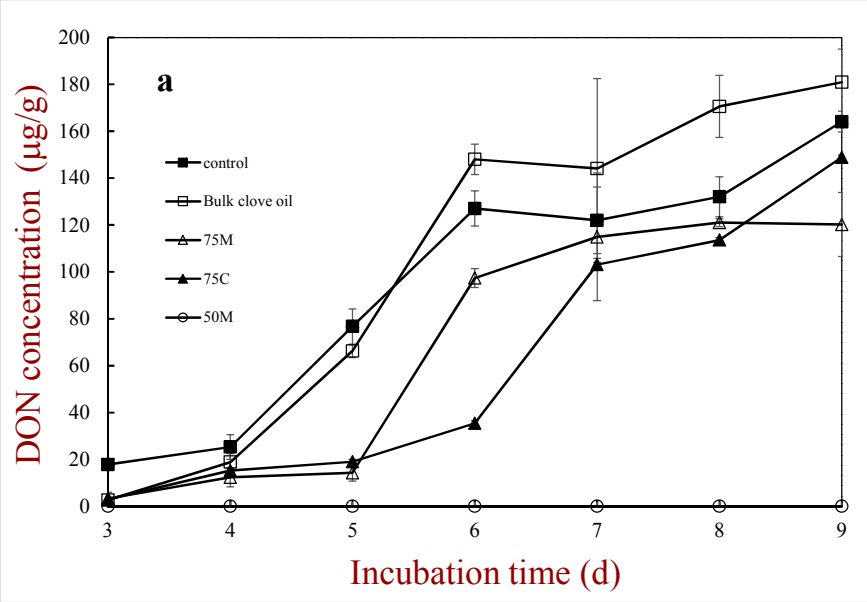

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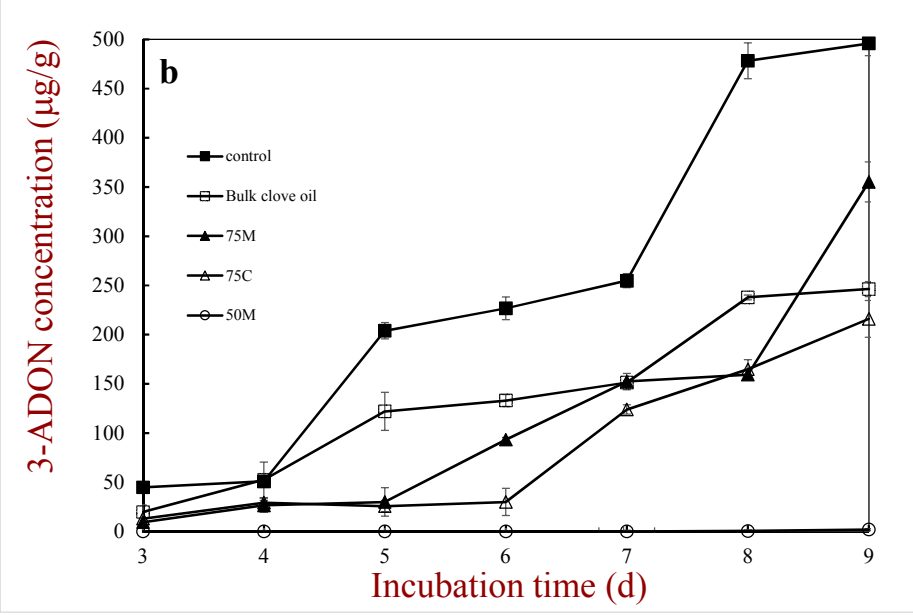

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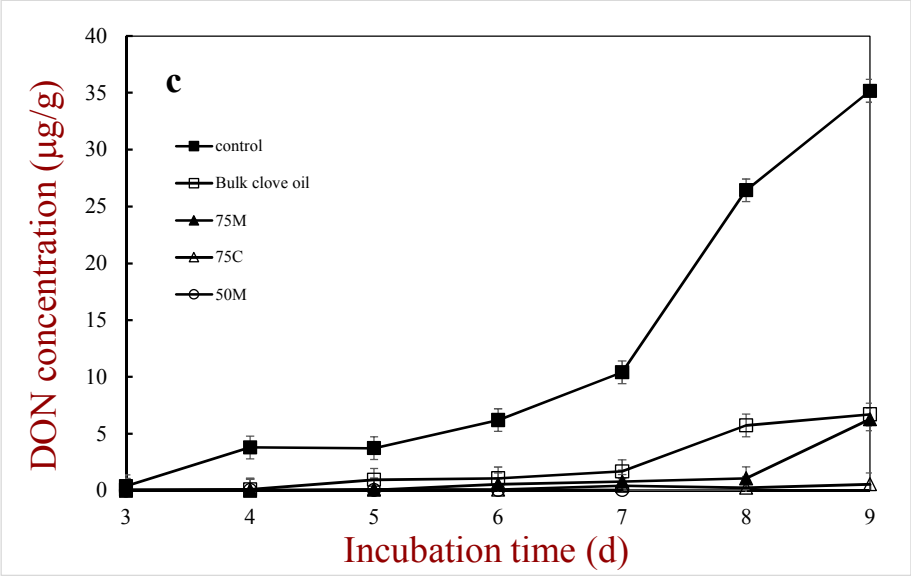

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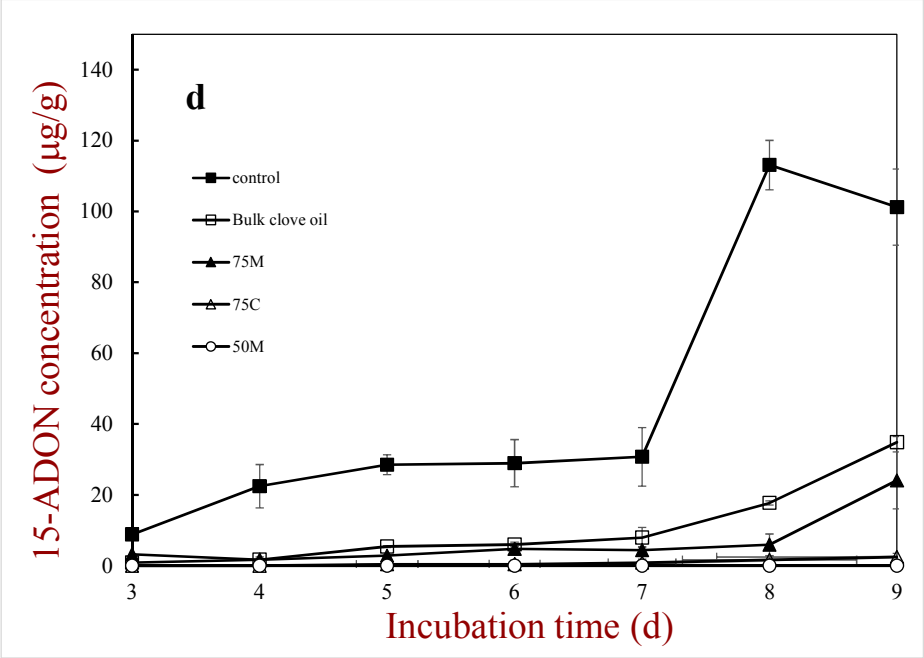

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709 **Graphic abstract**

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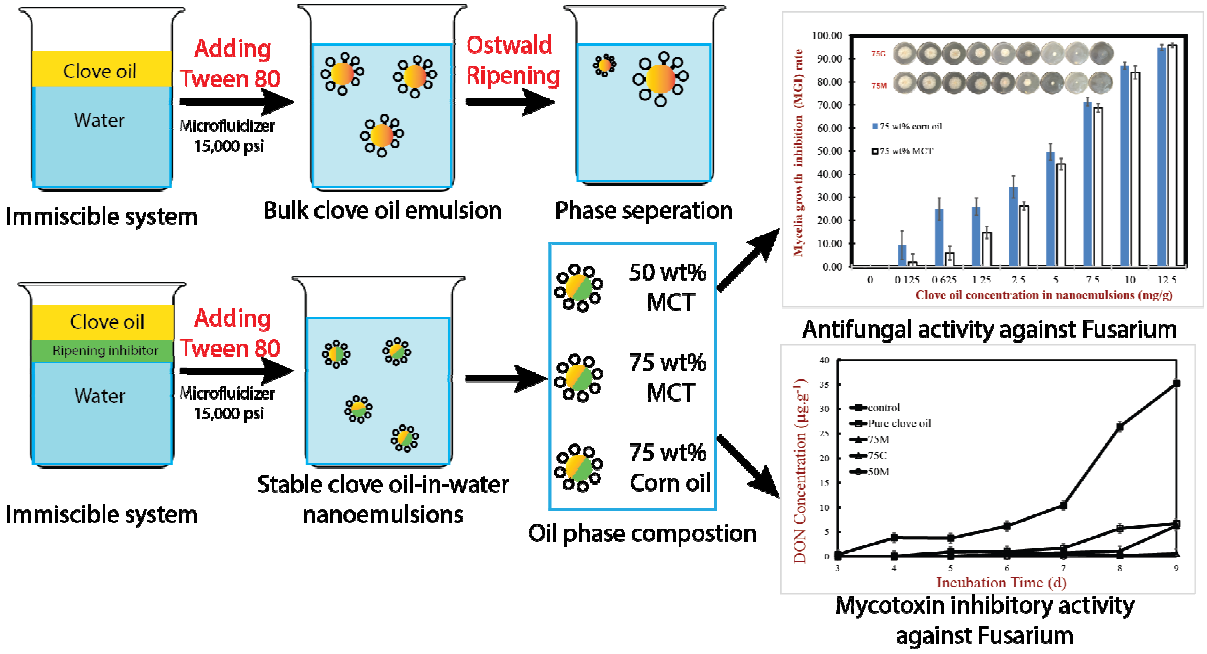

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713 Functional performance, including antifungal and mycotoxins inhibitory activity of clove oil

714 can be enhanced by nanoemulsion based delivery systems