Preparation and Characterizations of Chitosan/Citral Nanoemulsions and their Antimicrobial Activity

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Abstract

Background and Objective: The antimicrobial activity of essential oils has been long recognized, however, they easily evaporate and/or decompose during preparation, owing to direct exposure to heat, pressure and light. The current study deals with the formulation and characterization of bio-based oil in water nanoemulsions and their antimicrobial activity against plant pathogens.

Material and Methods: Citral oil and low molecular weight chitosan were used for preparation of nanoemulsions in the presence of sodium tripolyphosphate. Nanoemulsions were prepared by adding dropwise citral at different ratios into an aqueous solution containing chitosan, sodium tripolyphosphate and surfactant with continuous stirring and then ultrasonication. The success of formulation was confirmed by dynamic light scattering and scanning electron microscopy techniques. Physical stability and viscosity were investigated in details. The antimicrobial activity was evaluated against *Erwinia carotovora*, *Aspergillus niger* and *Rhizopus stolonifer*.

Results and Conclusion: The nanoemulsions had a polydispersity index ranged from 0.508 to 0.614 and particle size from 27 to 1283 nm. The highest antimicrobial activity was observed with F1 formulation (EC_{50} = 23, 278 and 221 mg l^{-1}, against *E. carotovora*, *A. niger* and *R. stolonifer*,respectively). Based on the antimicrobial activity, the prepared chitosan/citral nanoemulsions can be a cost-effective way to protect crops from microbial pathogens. Because such formulations contain bioactive products, the development of resistant pathogens can be delayed.

Conflict of interest: The authors declare no conflict of interest.

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1. Introduction

Research in the development of biologically active formulations in the form of nanoscale has become increasingly popular over the last decades [1,2]. This mainly overcomes the sensitivity and improves the stability of biologically active compounds, especially those that have high volatilization and decomposition such as aromatic and volatile aromatic plant essential oils (EOs). To date, there are a number of potential challenges associated with integrating these products into appropriate combinations [3]. Encapsulation of functional EOs within nanoparticles has been investigated as a potential strategy for improving their utilization, stability, and efficacy [4-6]. Nanoemulsions are currently utilized for the delivery of bioactive components and have been reported to be

especially applicable for the utilization in crop protection [7].

Small particle size may increase interactions between active compounds with biological membranes, as well as being able to transfer through. Furthermore, nanoemulsions can be designed to have good kinetic stability and low turbidity, which is suitable for a wide range of commercial applications [8]. Many techniques have been applied to the production of nanoemulsions, including various low-energy and high-energy methods. Ultrasonic emulsification is a high-energy method that is fast and efficient, capable of preparing nanoemulsions with diameters of small droplets and distribution of narrow size[9,10]. EO nanoemulsions have been reported

previously as effective antibacterial treatments [6,11,12], however, knowledge of their mode of action against microorganisms is currently limited. Citral (R, β-unsaturated aldehyde) is present in the oils of several plants, and has strong antimicrobial activity against bacteria and fungi [13]. Nanoemulsion consists of a phase of oil dispersed in continuous water, with each drop of oil surrounded by a thin layer of surface molecules, which helps to stabilize the nanomolecule system to a more stable formula [14]. Nanoemulsions are stable and transparent in appearance. Recently, nanoemulsion combinations using external and non-ionic surfactants with chitosan have been used as natural antimicrobial formulas in the agriculture sector[15]. In addition, naturally occurring polymers such as chitosan are widely used in agricultural fields in various forms such as nanoparticles, capsules, and emulsions. Chitosan has attractive properties due to its biodegradability, compatibility with life, and the non-toxic nature. Ionic gelatin technology is based on electrical interaction between positively charged primary amino groups of chitosan and negatively charged groups of polyanion, such as sodium tripolyphosphate (TPP) [16].

Therefore, the aim of this article was to prepare and characterize nanoemulsions containing citral and chitosan in order to enhance their antimicrobial effects against some plant pathogens. The antimicrobial effects of the nanoemulsions were compared with technical citral and chitosan against the most important threatening plant pathogens, including *Erwinia (E.)carotovora* (the causal agent of the soft rot disease), *Aspergillus (A.) niger* (the causal agent of the black mold disease)and *Rhizopus (R.) stolonifer* (the causal agent of black bread mold). The novelty and feasibility of this study are to examine the physicochemical characteristics of new nanoemulsions containing natural antimicrobial agents and recommend their application in the protection of plant and food products from microbial spoilage. The best new nanoemulsion will be chosen as an effective delivery system of citral and/or other essential oils to the specific target of microorganisms.

2. Materials and Methods

2.1. Chemicals and reagents

Citral oil, 3,7-dimethyl-2,6-octadienal (a mixture of geranial (Trans, 55-70%) and neral (Cis, 35-45%), low molecular weight chitosan (made from coarse ground crab, 89% degree of deacetylation), tween 80, dimethyl sulphoxide(DMSO), sodium tripolyphosphate (TPP)and 2,3,5,-triphenyltetrazolium chloride (TTC)were purchased from Sigma-Aldrich (St. Louis, MO, USA). Potato dextrose agar (PDA), Nutrient broth (NB), and Nutrient agar (NA) media were purchased from Oxoid Ltd. (Basingstoke, Hampshire, UK). All other commercially available solvents and reagents were used without further purification.

2.2. Bacterial and fungal strains

Plant pathogenic bacterium *E. carotovora* ATCC 39048 was obtained from Microbiology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Egypt. The culture was maintained on NA medium at 37°C. Two plant pathogenic fungi *A. niger* ATCC 16620and *R. stolonifer* ATCC 6227B were provided by Microbiology Laboratory, Department of Plant Pathology, Faculty of Agriculture, Alexandria University, Alexandria, Egypt, and kept during the experiments on PDA medium at 27±2°C.

2.3. Preparation of chitosan/citral nanoemulsions

A biopolymer chitosan was dissolved in aqueous acetic acid solution (1% v v^{-1}) to prepare a solution of 1% (w v^{-1}). Tween 80 as a surfactant was added to chitosan solution and stirred at 45°C for 2 h, to obtain a homogeneous mixture. The citral (0.04, 0.08, 0.16, and 0.32 g) was dissolved individually in DMSO (4 ml). This oil phase was gradually added dropwise to the chitosan solution (40 ml) with homogenization at 15100 g for 10 min under ice-bath conditions, for obtaining an oil-in-water emulsion. A solution of the TPP (0.4% w v^{-1}) was then added dropwise into the agitated emulsion and stirred constantly for 40 min. Finally, ultrasonication was achieved by a Sonicator (Ultrasonic Homogenizers HD 2070 with HF generator (GM 2070), ultrasonic converter UW2070, booster horn SH 213 G and probe microtip MS 73, Ø 3 mm) as shown in Figure 1. The tip of the horn was symmetrically placed in the coarse emulsion, and the process was carried out at 10 min, power 50 kHz and pulses or cycles 5 cycle/sec controlled by the software of the device to produce the nanoemulsions. Different ratios including 0.1:1, 0.2:1, 0.4:1, and 0.8:1 of citral: chitosan were examined respectively.

2.4. Characterization of chitosan/citral nanoemulsions

2.4.1. Scanning electron microscopy (SEM)

SEM analysis was done by using a JEOL JSM-5410 (Japan) electron microscope with a W-source and operating at 25 kV. Sample was prepared on a glass slide (1×1 cm) after washing it with ethanol. A small drop of nanoemulsion has spread evenly over the glass slide and allowed to air dry. In order to make it conductive, gold coating with Jeol Quick Auto Coater was performed (JFC-1500). The slides were then subjected to SEM analysis under ambient conditions.
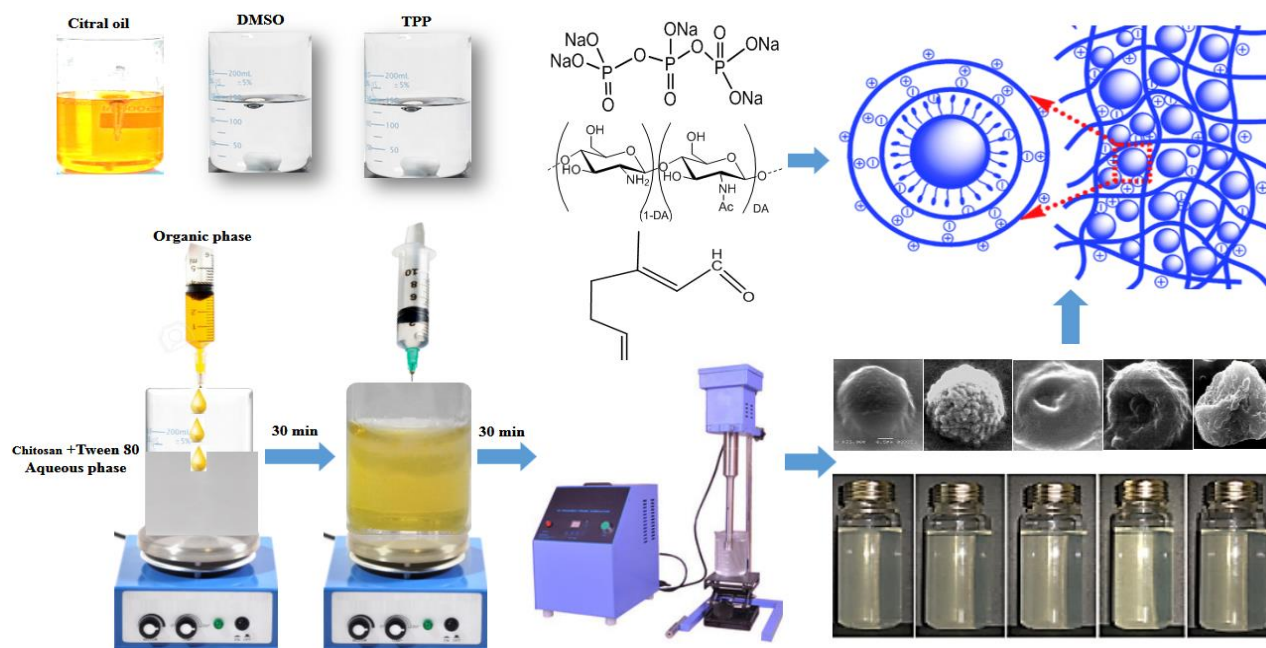
Figure 1. Schematic representation of preparation of chitosan/citralnanoemulsions. The oil phase containing citral at different amounts (0.04, 0.08, 0.16, and 0.32 g) and DMSO was added dropwise to the aqueous chitosan solution (at different ratios) with stirring. A solution of the TPP was then added dropwise into the agitated emulsion and stirring and finally the nanoemulsions were performed by ultrasonication.

2.4.2. Droplet size and poly dispersity index (PDI)

The droplet size distribution (analysis by volume) and PDI of citral/chitosan nanoemulsions were performed by a dynamic light scattering (DLS) method using Zetasizer Nano ZS (Malvern Instruments, UK) at room temperature. The samples were diluted before measurements to 10% with deionized water to avoid multiple scattering effects. Emulsion droplet size was estimated by the average of three measurements and presented as mean diameter in nm. The higher the PDI value refers to the lower uniformity of globules size of nanoemulsion [17].

2.4.3. Viscosity

The dynamic (absolute) viscosity (μ)of the nanoemulsions was measured by a Rotary Myr VR 3000 digital viscometer with L4 spindle at 200 rpm at 25°C without further dilution. Each reading was taken after the equilibrium of the sample for two min. The samples were repeated three times and the data expressed in mPa.s.

2.4.4. Stability at centrifugation

The formulated nanoemulsions were centrifuged for 30 min at 300 ×g and observed for phase separation, creaming and cracking. The nanoemulsions should have maximum stability, which is not a phase separation. Successful formulations exposed to other thermodynamic stability tests [18]. The measurements were performed in triplicate.

2.4.5. Stability at freeze thaw cycle

This test was carried out for the determination of the accelerated stability of the nanoemulsions. This type of test, puts the formulation through a series of extreme, rapid temperature changes that may encounter during the normal handling processes without any significant changes in physical properties. The formulations were stored at -21°C for 24 h and then at 21°C until melt for also 24 h [18]. Separation or creaming layer was examined. The measurements were performed in triplicate.

2.4.6. Stability at room temperature

About 25 ml of freshly prepared nanoemulsions were transferred to a glass tube. The transition from steady state to creaming and coalescence was examined during the storage period of 4 weeksat temperature of 25°C.

2.5. Antimicrobial assay

2.5.1. Antibacterial activity

Nutrient Broth (NB) medium was used to grow the bacterial strains to a final inoculum size of 5×10^5CFU ml. Nanoemulsion formulations were added to wells of a sterile 96 well microtitre plate, followed by the addition of NB medium and then 20 μl of bacterial suspension. The final volume in each well was 200 μl and the concentrations of 0.0, 56.25, 62.5, 112.5, 125, 225, 250, 450, 500, 900, and 1000 mg l were tested for all products including the nanoemulsions and technical chitosan and citral. Control wells were prepared with culture medium, bacterial suspension only, and solvent. The contents of each well were mixed on a microplate shaker at 200 rpm for 1 min prior to incubation for 24 h at 37°C. To indicate respiratory activity the presence of color was determined after adding 25 μl\well of TTC dissolved in water

(0.01%, w v^{-1}) as a chromogenic marker and incubated under appropriate cultivation conditions for 30 min in the dark [19]. The absorbance was measured at 492 nm in an Ultra Microplate Reader (Robonik, PVT, LTD). Negative controls were wells with the growth medium, concentrations of each product was tested (nanoemulsions, chitosan and citral) and the TTCreagent. Positive controls were wells containing a growth medium, bacterial suspension and TTC reagent. The minimum inhibitory concentration (MIC) was determined as the lowest concentration with no viability was observed after 24 h based on metabolic activity.

2.5.2. Antifungal activity

The antifungal activity was tested using mycelia radial growth technique [20]. Different concentrations of each product ranging from 50 to 3000 mg l^{-1} were tested. The aliquots of the stock solutions were added to the PDA medium and then transferred to Petri dishes. After solidification, the mixtures were inoculated with a 5 mm in diameter mycelium fungi at the center of Petri dishes and these were incubated in the dark at 27 ± 2ºC. Fungal growth was measured when the control had grown to the edge of the plate. The inhibition of fungal growth was calculated as the percentage of inhibition of radial growth compared to the control. The effective concentration that inhibits 50% of mycelial growth (EC$_{50}$) for each compound was estimated by probit analysis [21] using SPSS 21.0 software.

2.6. Statistical analysis

Experimental data are presented as mean ± standard error (SE). Statistical analysis was performed using SPSS ver. 21.0. The log dose-response curves were used for determination of the EC$_{50}$ values for the fungal bioassay according to the probit analysis [21].The 95% confidence limits for the range of EC$_{50}$ values were determined by the least-square regression analysis of the relative growth rate (% control) against the logarithm of the compound concentration.

3. Results and Discussion

3.1. Characterization of chitosan/citral nanoemulsions

Chitosan-citral oil based nanoemulsions were formulated in two phases as shown in Figure 1. First, coarse emulsion was prepared by adding chitosan to the oil phase containing citral with different ratios (0.1:1, 0.2:1, 0.4:1 and 0.8:1, respectively) and then subjected to the ultrasonic emulsification. SEM studied the morphology and shape of the citral-loaded nanoemulsions and the data are presented in Figure 2. The shape of droplets was found to be spherical for F1-F4.However, the droplet of F5 was uniform shape and size. The different ratios between chitosan and citral significantly affected the final droplet size and form. The results are in agreement with those of

Lu and others who reported that the shape of the citral droplet was spherical [22].

Tween 80 was favored as an effective surface-active agent due to its high hydrophilic-lipophilic balance (HLB=15), which is satisfactory for the formulation of nanoemulsion oil in water. In addition, a small molecule is more efficient at minimizing droplet diameter. Surface active agents act as emulsifiers and serve the process by reducing the free energy needed to prepare nanoemulsions by reducing the tension between the faces in the oil/water interface[14].
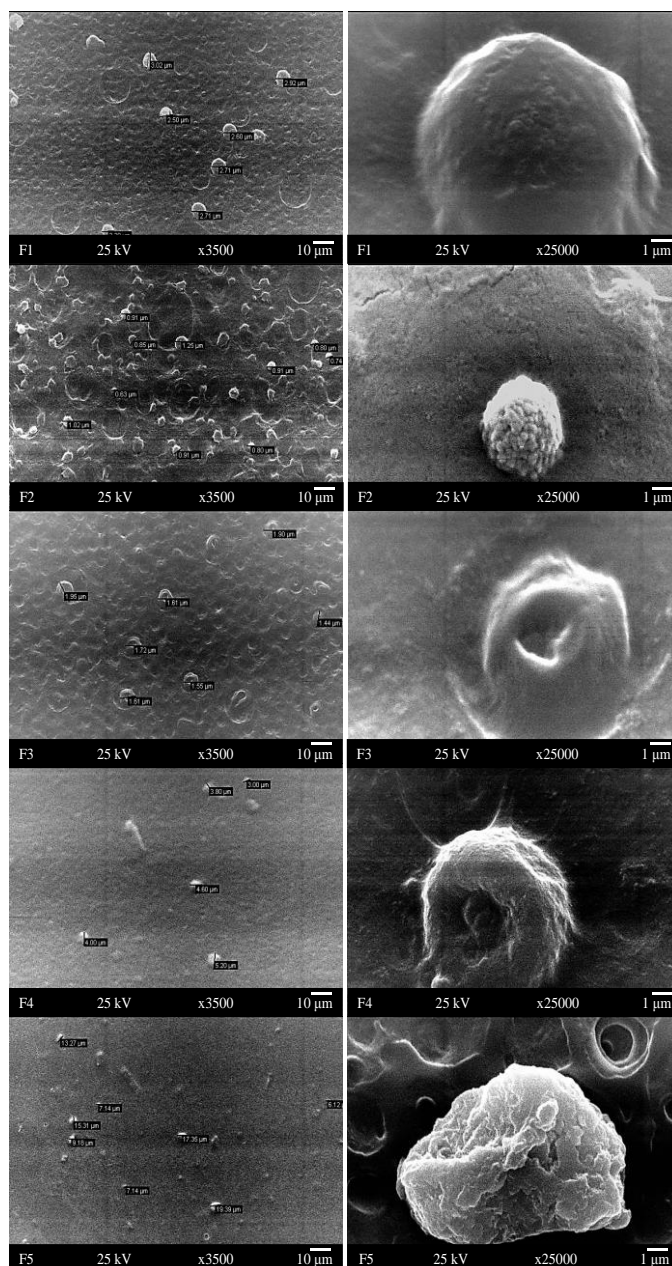

Figure 2. Scanning electron micrograph of prepared chitosan-citral nanoemulsions, F1 to F5. The SEM was performed on a JEOL JSM-1200EX II scanning electron microscope operating at an acceleration voltage of 25.0 kV. Scale bar 10 µm with low magnification (left) and scale bar 1.0 µm with high magnification (right) for observation of their surface morphologies.

The PDI values (Table 1) were measured with a range from 0.508 to 0.614, indicating that all nanoemulsions had a relatively narrow range of size distribution. PDI decreased significantly from 0.614 to 0.508 as the mass ratio of citral/chitosan increased from 0.1:1 to 0.8:1, respectively. In addition, the droplet size distribution is presented in Figure 3.The nanoemulsion appeared to be transparent and the particle size is concentration dependent where the low concentrations of citral have the lowest particle size (27.0 and 28.5 nm for F1 and F2, respectively) as compared to the other formulations (F3 to F5).

The droplet size increased with the increase in concentration of oil phase in the formulations (Table 1). The droplet sizes of the nanoemulsions is a function of oil-surfactant weight ratio [23,24]. Increased ratio causes separation of the excess oil phase, resulting in higher droplets sizes. The concentration structure changes with the increases in ratio, increasing the volume of oil fields in the bilateral phase, leading to an increase in the volume of droplets.

The viscosity of nanoemulsions was found in the range of 5.1 to 8.0 mPa.s. F5 had the lowest viscosity (3.1 mPa.s.) compared to the other formulations (5.1-8.0 mPa.s.) (Table 1). The difference in viscosity of the F5 was significant from other formulations but it was observed that the viscosity of all formulations was very low, which is expected as one of the characteristics of the nanoemulsion [25]. It can also be observed that viscosity is concentration dependent. F1 with the lowest proportion of citral: chitosan (0.1:1.0, respectively) had the lowest viscosity. There are several factors affecting viscosity such as disperse phase volume fraction, rheology of component phases, droplet size, colloidal interactions, or droplet charge [15,26,27]. Pal stated that the high volume droplets and low emulsion viscosity, in the same spread phase difference and shear rate [27].
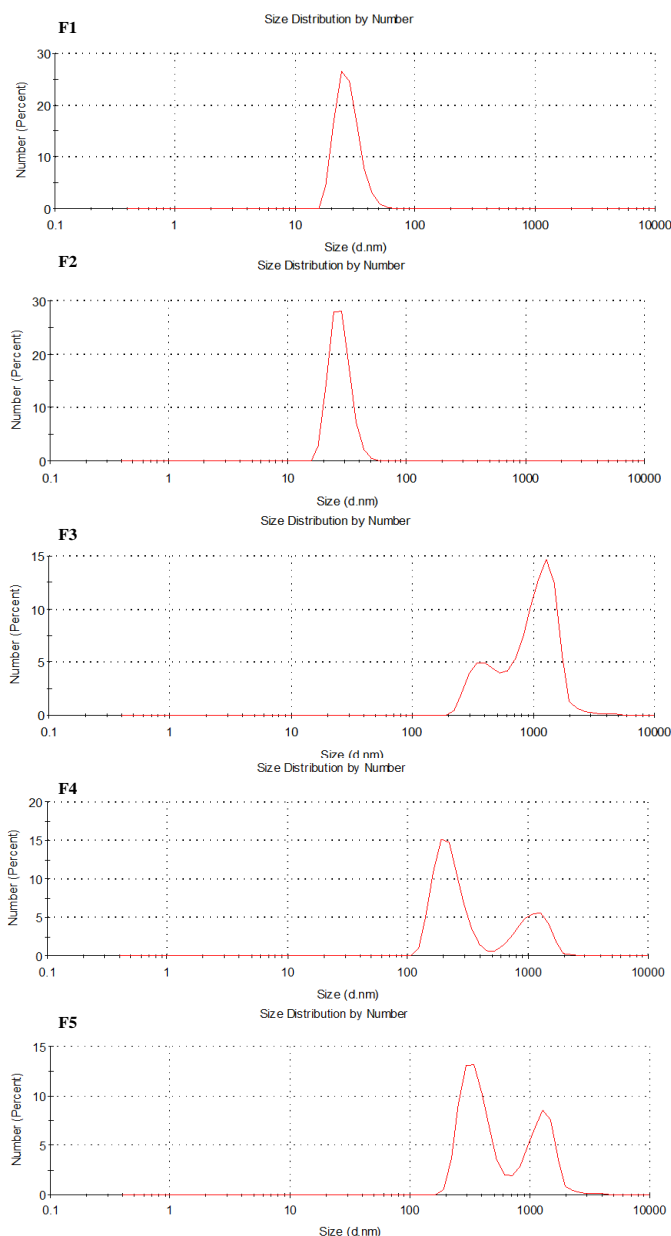

Figure 3. A typical particle size distribution by a dynamic light scattering of the formulated chitosan-citralnanoemulsions, F1 to F5.

Table 1. Mean droplet size, poly dispersing index, and dynamic viscosity of chitosan/citralnanoemulsions

Formulation	Weight ratios (chitosan: citral)	Droplet size (nm)	Poly dispersing index (PDI)	Dynamic viscosity (mPa.s.) ± SE
F1	1.0 : 0.1	27.0	0.614	5.1±0.05
F2	1.0 : 0.2	28.5	0.594	6.0±0.05
F3	1.0 : 0.4	387	0.536	7.0±0.04
F4	1.0 : 0.8	1115	0.508	8.0±0.05
F5	1.0 : 0.0	1283	0.571	3.1±0.04

3.2. Stability tests of chitosan/citral nanoemulsions

The nanoemulsions were stable at centrifugation of 300 ×g, heating cycle, and freeze-thaw cycle for 4 weeks (Table 2). No creaming or phase separation was observed on these formulations. Nanoemulsions are thermodynamically stable systems and formed at a particular composition of oil, surfactant and water, with no phase separation, creaming, cracking, or coalescence. Centrifugation can accelerate the rate of sedimentation or incineration, demonstrating that degradation of an emulsion may be related to the action of gravitational force. O/W emulsion often appears rather than deposition of precipitation due to low oil droplet density as compared to aquatic medium. If nanoemulsions are to be used as antimicrobial delivery systems, it is important to have good physical storage during long-term storage. Thus, selected citral nanoemulsions were selected to various thermo-couple tests using centrifugation, cooling and heating cycle, and freezing stress tests. The steric effect of non-ionic surfactant plays a vital role in the stability of nanoemulsions [14].

Table 2. The *in vitro* antibacterial activity of chitosan/citralnanoemulsion formulations against *E. carotovora* by ELISA technique

Formulations	MIC (mg l^{-1})
F1	23
F2	40
F3	113
F4	238
F5	520
Chitosan	635
Citral	>700

MIC is the minimum inhibitory concentration.

3.3. Antibacterial activity

The MICs of pure and emulsified citral showed significantly different inhibitory effects and all formulations showed higher inhibition (MIC ranged from 23 to 520 mg l^{-1}) than citral (MIC >700 mg l^{-1}) against the tested bacterium (Table 3). Increased inhibition with the reduction of the citral concentration and most effect was observed with F1. Several studies have reported enhancements in the physical properties and antimicrobials of EO-loaded nanoemulsions compared to conventional emulsions [28-31]. Comparison of antibacterial activity with pure EOs and emulsification showed that the nanoemulsion was much more effective. This is presumably due to the fact that small fat particles within the nanostructures are able to bring primary oil to the surface of the cell membrane, while pure oil (low water solubility) cannot easily interact with cell membranes. The results obtained are in agreement with other recent studies, which have shown that flavor conversion or EOs in nanoemulsions have greatly improved antibacterial activity, D-limonene[12] and oregano oil [32]. Lambert and co-authors reported that the EOs containing carvacrol and thymol as monoterpenes, such as thyme oil have a strong bactericidal action [33]. However, eugenol and citral, the main components of clovs, lemon or rosewood EO, were found to disrupt against a wide spectrum of microorganisms [34]. Other aromatic compounds such as linalool, pinene, geraniol and borneol, which can be found in plant EOs, showed less inhibition effect against bacteria[35]. Hamouda and Baker reported that the soybean oil based nanoemulsion had a good bactericidal activity against gram-positive bacteria [36].

Table 3. The *in vitro* antifungal activity of chitosan/citralnanoemulsion formulations against *A. niger* and *R. stolonifer* by mycelia radial growth technique

Formulations	EC$_{50}$[a] (mg l^{-1})	95% confidence limits		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
		Lower	Upper			
A. niger						
F1	278	150	514	3.45±0.33	-8.44±0.82	7.22
F2	396	337	449	2.54±0.31	-6.60±0.84	1.92
F3	710	626	827	2.40±0.32	-6.85±0.88	2.09
F4	1887	1293	4587	1.57±0.35	-5.16±0.97	1.75
F5	1568	1146	3068	1.66±0.34	-5.31±0.95	0.85
Chitosan	3016	2363	4157	1.19±0.25	-4.13±0.84	0.99
Citral	474	410	538	2.38±0.30	-6.37±0.83	0.62
R. stolonifer						
F1	221	162	309	6.16±0.49	-14.45±1.15	6.72
F2	340	128	514	5.42±0.47	-13.75±1.24	9.10
F3	708	610	856	1.98±0.30	-5.65±0.84	1.92
F4	1074	861	1600	1.70±0.32	-5.15±0.87	0.86
F5	1433	1045	2903	1.47±0.32	-4.63±0.89	1.08
Chitosan	2649	1222	7494	2.10±0.26	-7.05±0.89	4.73
Citral	437	72	785	4.67±0.39	-12.35±1.04	13.04

[a] The concentration causing 50% mycelialgrowth inhibition.
[b] Slope of the concentration-inhibition regression line ± standard error.
[c] Intercept of the regression line ± standard error.
[d] Chi square value.

3.4. Antifungal activity

The antifungal activity of chitosan/citralnanoemulsions against *A. niger* and *R. stolonifer* is presented in Table 3. F1 exerted significantly potent antifungal activity (EC_{50}=278 and 221 mg l^{-1} against *A. niger* and *R. stolonifer*, respectively). However, F4 and F5 were the lowest active (EC_{50} = 1887, 1568, 1074 and 1433 mg lagainst *A. niger* and *R. stolonifer*, respectively). Citral only showed good antifungal activity (EC_{50} = 474 and 437 mg l^{-1}, respectively) compared to chitosan (EC_{50} = 3016 and 2649 mg l^{-1}, respectively). This result is in agreement with those of Caccioni and co-authorswho reported that citral exerted antifungal activity against *P. italicum* and *P. digitatum, Magnaporthe grisea* and *Botrytis cinerea*[37]. When the viability of microorganisms are considered, another point deserves attention. It can be suggested that *R. stonolifer* was more susceptible than *A. niger* for all the formulas (Table 3). In previous literature, a number of studies have been published investigating antimicrobial activity using nanoemulsions against fungi and yeast [6,38]. Saddiq and Khayyat reported the growth of *P. italicum* and *R. stolonifer* on the solid media, and reported reductionin the presence of citral and its epoxide [39]. Low concentrations (12.50 - 200 mg l^{-1}) of citral exhibited strong inhibition of *M. grisea* and *B. cinerea* with inhibition values from 22.84% to 91.34% and 9.76% to 92.12%, respectively [33]. Moreover, citral produced low inhibition rate of mycelium growth of *Rhizoctonia solani* with EC_{50} of 193 mg l^{-1}. Luo and others reported irreversibly damages plasma membrane and DNA with consequent spore loss germination were found after citral penetrating cell wall of *A. flavus*[40]. Also in *A. niger*, citral work appears to be the central location of the cell membrane. Moreover, citral was able to form charge transfer complexes with tryptophan, which is a good electron donor. Apparently, the antifungal action of aldehydes, as the citral, is due to their ability to form charge transfer complexes with electron donors in addition to their interaction with SH-groups. The use of nano-delivery systems may increase cellular absorption mechanisms, reducing resistance to mass transfer and increasing antimicrobial activity [4]. In general, the mechanism of action of EOs against microorganisms includes the interaction of phenolic compounds with proteins in the cytoplasmic membrane, which can precipitate and lead to leakage of ions and other cell contents causing cell breakdown[41].

4. Conclusion

Based on this study, the conversion of citral to nanoemulsion has significantly enhanced its antimicrobial activity against important plant pathogenicbacteria(*E. carotovora*) and fungi (*A. niger* and *R. stolonifer*). Nanoemulsion may particularly be an effective delivery systems for EOs and their components because of their ability to facilitate the application of antimicrobials and increase the effectiveness of antimicrobials. However, as these tests were conducted in vitro, the next step may be in vivo research on plants.

5. Acknowledgements

6. Conflict of Interest

The authors confirm that this article content has no conflict of interest.

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تهیه نانوامولسیون‌های[1] کیتوزان/سیترال و تعیین ویژگی‌ها و فعالیت ضدمیکروبی آنها

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چکیده

سابقه و هدف: فعالیت ضدمیکروبی اسانس‌های روغنی به‌خوبی شناخته شده است، اگرچه به‌آسانی تبخیـر و/یـا در حین آماده سازی هنگام تماس مستقیم با حرارت، فشار و نور تجزیه می‌شوند. مطالعه حاضر به فرموله کردن و تعیین ویژگی‌های نانوامولسیون‌های روغن در آب و برپایه روغن و فعالیت ضدمیکروبی آنها دربرابر عوامل بیمـاریزای گیـاهی می‌پردازد.

مواد و روش‌ها: برای تهیه نانوامولسیون‌ها از روغن سیترال و کیتوزان با وزن مولکولی پایین در حضور سدیم تـری پلی فسفات استفاده شد. سیترال قطره قطره به محلول آبی حاوی کیتوزان، سدیم تری فسفات و عامل سطح فعـال [2]، در نسبت‌های گوناگون، و در حال همزدن مداوم اضافه شد و سپس با استفاده از تکنیک فراصوت نانوامولسیون‌ها تهیه شدند. موفقیت تشکیل نانوامولسیون با میکروسکوپ الکترونی روبشی[3] و روش پراکنـدگی دینامیـکی نـور یـا تفـرق پویای نور[4] تایید شد. پایداری فیزیکی و گرانروی با جزئیات مورد بررسی قرار گرفتند. فعالیـت ضـدمیکروبی در برابـر *Erwinia carotovora*، *Aspergillus niger* و *Rhizopus stolonifer* ارزیابی شد.

یافته‌ها و نتیجه‌گیری: نانوامولسیون‌ها شاخص بس‌پراکندگی[5] بین ۰/۵۰۸ تا ۰/۶۱۴و اندازه ذرات ۲۷ تا ۱۲۸۳ نانومتر داشتند. بیشترین فعالیت ضدمیکروبی در فرمولF1 مشاهده شد ($^{-1}$221mg L و۲۳،۲۷۸= EC_{50} به ترتیب در برابر *Erwinia carotovora*، *Aspergillus niger* و *Rhizopus stolonifer*). براساس فعالیت ضدمیکروبی، نانوامولسیون‌های کیتوزان/سیترال می‌توانند روشی مقرون به‌صرفه برای حفاظت محصولات کشاورزی در برابر عوامل بیماریزای میکروبی باشند. از آنجا که چنین فرمول‌هایی حاوی ترکیبات زیست فعال می‌باشند، بروز مقاومت عوامل بیماریزا می‌تواند به تاخیر انداخته شود.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ تعارض منافعی وجود ندارد.

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[1] نانوامیزه (nanoemulsion)، نوعی کلوئید مایع‌درمایع که در آن ذره‌های یک مایع در مایع دیگر به حالت تعلیق پراکنده باشد. اندازه این ذرات ۱۰۰-۱ نانومتر می باشد.

[2] Surfactant(هر ماده‌ای که از کشش سطحی یا بین‌سطحی محیطی که در آن حل شده است بکاهد)

[3] Scanning Electron microscopy Technique (SEM)

[4] Dynamic Light Scattering Technique (DLS)

[5] Polydispersity index(پهنای توزیع وزن مولکولی یک بسپار یا پلیمر)