УДК 632.4/937; 632.911.2; 581.2

4.1.3. Агрохимия, агропочвоведение, защита и карантин растений (биологические науки, сельскохозяйственные науки)

ПОВЫШЕНИЕ БИОЛОГИЧЕСКОЙ И ЭКОНОМИЧЕСКОЙ ЭФФЕКТИВНОСТИ ЭФИРНЫХ МАСЕЛ ПУТЕМ НАНОИНКАПСУЛИРОВАНИЯ ДЛЯ БОРЬБЫ С ГРИБНЫМИ ЗАБОЛЕВАНИЯМИ КАРТОФЕЛЯ

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Целью данного исследования является синтез новой рецептуры эфирного масла лаванды (LEO) и наночастиц хитозана с помощью процесса ионного гелеобразования и ее применение для защиты клубней картофеля от послеуборочного гниения, вызванного *Fusarium* sp. и *Alternaria* sp., а также для повышения биологической и экономической эффективности масла. Различные виды *Alternaria* были идентифицированы на молекулярном уровне с участком RPB2. Были получены нанокапсулы LEO размером от 200 до 600 нм. Эффективность инкапсуляции достигла своего наивысшего значения - 86,9% при соотношении хитозан-масло 1,0:0,5, а положительный дзета-потенциал составил +38,1%. Влияние MIC инкапсулированного масла (Lav-ChNP) на рост мицелия было получено при концентрации 1,0 г/л против *Fusarium* sp. и *Alternaria* sp., в то время как MIC свободного LOE достигал 10 г/л. При естественном заражении и длительном хранении до 8 месяцев при температуре 4°C Lav-ChNP в дозе 10 г/т

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4.1.3. Agrochemistry, agro-soil science, plant protection and quarantine (biological sciences, agricultural sciences)

ENHANCING OF BIOLOGICAL AND ECONOMIC EFFICIENCY OF ESSENTIAL OILS BY NANOENCAPSULATION TO CONTROL POTATO FUNGAL DISEASES

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The objective of this research is to synthesis a novel formulation of lavender essential oil (LEO) and chitosan nanoparticles through the process of ionic gelation and apply it to safeguard potato tubers from post-harvest decay caused by *Fusarium* sp. and *Alternaria* sp. and to enhance the biological and economic efficacy of the oil. Different *Alternaria* species were molecularly identified with region of RPB2. Nano-capsules of LEO were obtained with a size range of 200 to 600 nm. Encapsulation efficiency reached its highest value, 86.9% with the chitosan-oil ratio of 1.0:0.5, and showed a positive zeta potential of +38.1%. MIC of encapsulated oil (Lav-ChNP) on mycelial growth obtained with concentration of 1.0 g/l against *Fusarium* sp. and *Alternaria* sp., while MIC of free LOE reached 10 g/l. Under natural infection conditions and long periods of storage up to 8 months at 4°C, Lav-ChNP at 10 g/t was the most effective treatment and significantly reduced the disease incidence by 75.1% and disease severity by 91.4%. On the other hand, when tubers were

был наиболее эффективным средством лечения и значительно снизил частоту заболевания на 75,1% и тяжесть заболевания на 91,4%. С другой стороны, когда клубни были обработаны лавандовым маслом в количестве 40 г/т, частота появления гнили снизилась только на 43,3%, а степень выраженности гнили снизилась на 40,0%, что свидетельствует о превосходной эффективности инкапсулированного масла. Уровень рентабельности (как показатель экономической эффективности) применения Lav-ChNP был выше по сравнению с другими методами обработки и достиг 472,75%. Эти результаты свидетельствуют о превосходной эффективности Lav-ChNP в защите клубней картофеля от грибных поражений при хранении

treated with free lavender oil at a rate of 40 g/t, the rot incidence decreased only by 43.3%, and the rot severity decreased by 40.0%, which indicates the superior efficacy of encapsulated oil. The level of profitability (as a parameter of economic efficiency) of using Lav-ChNP was higher compared to other treatments and reached 472.75%. These results show superior efficacy of Lav-ChNP in protecting potato tubers from fungal rots during storage.

Ключевые слова: ЭФИРНЫЕ МАСЛА, НАНОТЕХНОЛОГИИ, ХИТОЗАН, ГРИБЫ, ИНКАПСУЛЯЦИЯ

Keywords: ESSENTIAL OILS, NANOTECHNOLOGY, CHITOSAN, FUNGI, ENCAPSULATION

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Introduction:

Essential oils (EOs) are volatile and abundant sources of various bioactive compounds, including terpenoids and terpenes [1]. Lavender *Lavandula angustifolia* essential oil (LEO) is a potent source of antimicrobial agents [2]. Nevertheless, EOs are unstable substances that can quickly degrade due to various environmental factors, including oxygen, light, and heat. This can result in a reduction in their effectiveness when employed for the purpose of safeguarding agricultural products against fungal infections. Furthermore, for certain purposes, controlled release may be required [3]. To improve the longevity of essential oils and protect them from deterioration, a novel approach to encapsulation has been developed at the nanoscale. The development of nano-encapsulation techniques has provided a novel approach to safeguarding the bioactive components of essential oils from degradation. Additionally, this technology has enabled the creation of innovative materials at the nanoscale, which exhibit enhanced properties compared to conventional materials.

Chitosan, a natural polymer and an encapsulating agent is a cost-effective carrier that is frequently used in the agricultural sectors due to its biocompatibility and biodegradability. Chitosan is widely used to encapsulate biologically active compounds such as, essential oils. [3].

Dry rot of potato tubers caused by *Fusarium* sp. and *Alternaria* sp. is a major concern in the cycle of potato production [4]. The damages caused by the dry rot in the tubers have been described as both numerical and descriptive [5].

The objective of this research is to synthesis a novel formulation of lavender EO and chitosan nanoparticles through the process of ionic gelation and apply it to safeguard potato tubers from post-harvest decay caused by *Fusarium* sp. and *Alternaria* sp. and to enhance the biological and economic efficacy of the oil.

Materials and Methods:

Lavender oil of *Lavandula angustifolia* was purchased from NRC (Cairo, Egypt). The oil main components were Linalyl acetate (32.05%), β-Linalool (24.02%), and Trans-β-ocimene (8.15%). A highly virulence *Fusarium sambucinum* strain SSD-MREZ (OR144017) was used. Rivera Potato variety was used. Alternaria strains were isolated from natural infected plants (tubers and leaves) (Fig. 1). 150 kDa molecular weight and 85% deacetylation degree chitosan was used. Other chemicals were used in technical grades.

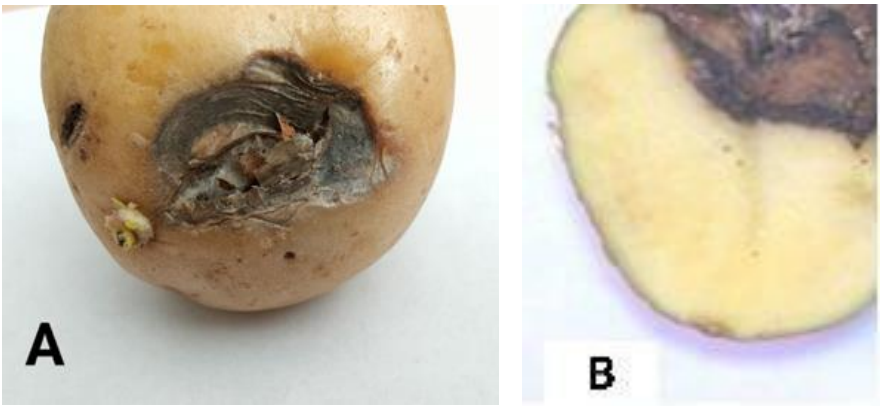

Figure 1. Typical symptoms of Alternaria rot (early blight) on tubers (A and B), and leaves (C and D). (photo by the authors).

Morphological characterization of the pathogens:

Morphological and cultural characteristics of pathogens were studied on PDA. The following parameters were taken for study: color, shape and margins of colony; color, shape, and septa of mycelium; color, size, shape, and segmentation of conidia [6].

Molecular identification:

DNA extraction

Genomic DNA extraction was performed from 7-days fresh cultures using Genomic DNA Mini kit (Geneaid) according to manufacturer's protocol.

PCR amplification

The amplification of the RPB2 region was done with RPB2–5F2 [7] and RPB2–7cR [8]. The PCRs were performed in a MyCyclerTM Thermal Cycler in a total volume of 15 μL. The PCR mixtures consisted of 1x HotMaster Taq Buffer with Mg2+, 0.2 mM dNTP Mix, 1 U HotMaster Taq DNA Polymerase, 0,3 uM of each primer and 50-100 ng of DNA template. Conditions for PCR amplification consisted of an initial denaturation step of 2 min at 95°C followed by 35 cycles of

30 s at 94°C, 30 s, annealing for 30 s, 72 °C for 50 s and a final elongation step of 7 min at 72°C. Annealing temperatures 57°C for gpd gene region. The partial RPB2 gene was obtained using a touchdown PCR protocol of 5 cycles of 45 s at 94 °C, 45 s at 60 °C and 2 min at 72 °C, followed by 5 cycles with a 58°C annealing temperature and 30 cycles with a 54°C annealing temperature. Amplifications were evaluated by electrophoresis of the PCR products in 1.5% agarose gel for 50 min at 80 V in Tris-Borate-EDTA (TBE) buffer.

DNA sequencing

PCR products were sequenced in both directions using the PCR primers and the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's recommendations, and analyzed with an ABI Prism 3730XL Sequencer (Applied Biosystems) according to the manufacturer's instructions.

Phylogenetic analysis

RPB2 analysis

Sequence alignment search was done using the (blastn) tool of GenBank database (www.ncbi.nlm.nih.gov/blastn). Indistinctly ends were eliminated from all analyses and gaps were treated as fifth character in the parsimony analysis. The evolutionary history was conducted in MEGA7 [9], using the Maximum Likelihood method based on the Tamura-Nei model Tamura and Nei (1993) [10]. Bootstrap value was set to 1000 replications to assess the robustness of the phylogeny. The percentage of trees on which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Bayesian analyses were performed with MrBayes v. 3.1.1 [11] using the Markov Chain Monte Carlo

(MCMC) algorithm to generate trees with Bayesian probability values. Four chains were run simultaneously from a random tree topology and ended at 200,000 generations, and trees were saved every 100th generation and 75% of trees were discarded in the burn-in phase. The burn-in value was graphically estimated from the likelihood scores and the consensus tree was constructed from the remaining trees.

Assessment of the resistance of various varieties potato tubers to *Fusarium* sp. and *Alternaria* sp.: To study the resistance of potato tubers to the pathogen of each of the *Fusarium* and *Alternaria* strains, isolates belonging to the group of highly pathogenic (highly aggressive) were selected. A mixture of *Fusarium* and *Alternaria* fungal strains was used for research. The resistance of varieties was determined by the method of artificial infection according to the lesion index according to [12] (Table 1). The following varieties were used in the study: Riviera, Gala, Red Scarlet, Arizona.

Table 1 - Potato assessment scale for resistance to *Fusarium* and *Alternaria*

Degree of resistance of the variety	Lesion index, % (disease severity, %)
Resistant	1–25
Medium Resistant	26–50
Susceptible	51–75
Very susceptible	76–100

Synthesisof Lav-ChNP:

Lav-ChNP were prepared as described by Yoksan et al. (2010) [13] method with some modifications: 1- chitosan solution 1.0% was used; 2- Using of various contents of LEO (0.25, 0.50, 0.75, 1.0 and 1.25 g); 3- Centrifugation at 4,000 rpm for 30 min at room temperature to collected the nanoparticles.

Characterization of Lav-ChNP:

Particles shape and size of both CSNP and Lav-ChNP were analyzed by atomic force (AFM) microscope in contact mode (SOLVER NEXT, Limerick, Ireland). It was also analyzed using the Advanced Watershed software platform. The loaded content (Encapsulation efficiency, EE) of LEO in ChNP was determined by UV spectrophotometry by the formula: EE%= (weight of loaded LEO) / (weight of initial LEO) × 100. The surface charge (zeta-potential, ZP) of nanoparticles were measured using a Malvern nano-series Zeta-sizer (UK) at 25°C.

Antifungal assay was performed as described by Askarne et al. (2012) [14].

Biological efficacy on potato tubers: The effectiveness of free and nano-form of LEO against *Alternaria* and *Fusarium* rot was evaluated during long storage period (at 4 °C) on natural infected Riviera variety (2020-2022 years), each treatment had 10 kg tubers and four replications at the following application rate: LEO 100 g/t; ChNP 20 g/t; Lav-ChNP 10 g/t.

Economic efficacy during potato storage: The economic efficiency of production is characterized by the ratio of the economic effect to the costs that caused this effect. The calculation of the economic efficiency of using experimental treatments is based on processing costs and net income (from the sale of saved products).

Results and Discussion.

Identification of the pathogens:

Different isolates of Alternaria were obtained from the diseased tissue and classified according to morphological parameters: 1- isolate number (AA1) showed olivaceous-black colony; abundant, branched, septate, brownish mycelium; dark brown conidia with short beaks, several vertical and transverse septa; 2- isolate number (AT 10) showed brown with a cottony texture, a rough upper surface colony; pale olive gray, branched, septate mycelium; light, golden-brown conidia, grow individually or in short chains of 2-5 units, have a median transverse septum (Table 2).

Table 2 - Morphological characterization of different *Alternaria* isolates recovered from potato plants and tubers

№	Colony morphology	Conidia
AA1		
AT10		

The outputs of molecular analysis: Amplicons of approx. 570, 400 bp were generated for gpd and rpb2, respectively. The data set of RPB2 consisted of 31 taxa including the out-group taxa composed of 689 characters. The tree with the highest log likelihood (-249.3205) is shown in (Fig.2). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 200.0000)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 13.6210% sites). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 31 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were

eliminated. There was a total of 39 positions in the final dataset. The isolated strains were defined as *A. alternata* strain 1, and *A. tenuissima* strain 10

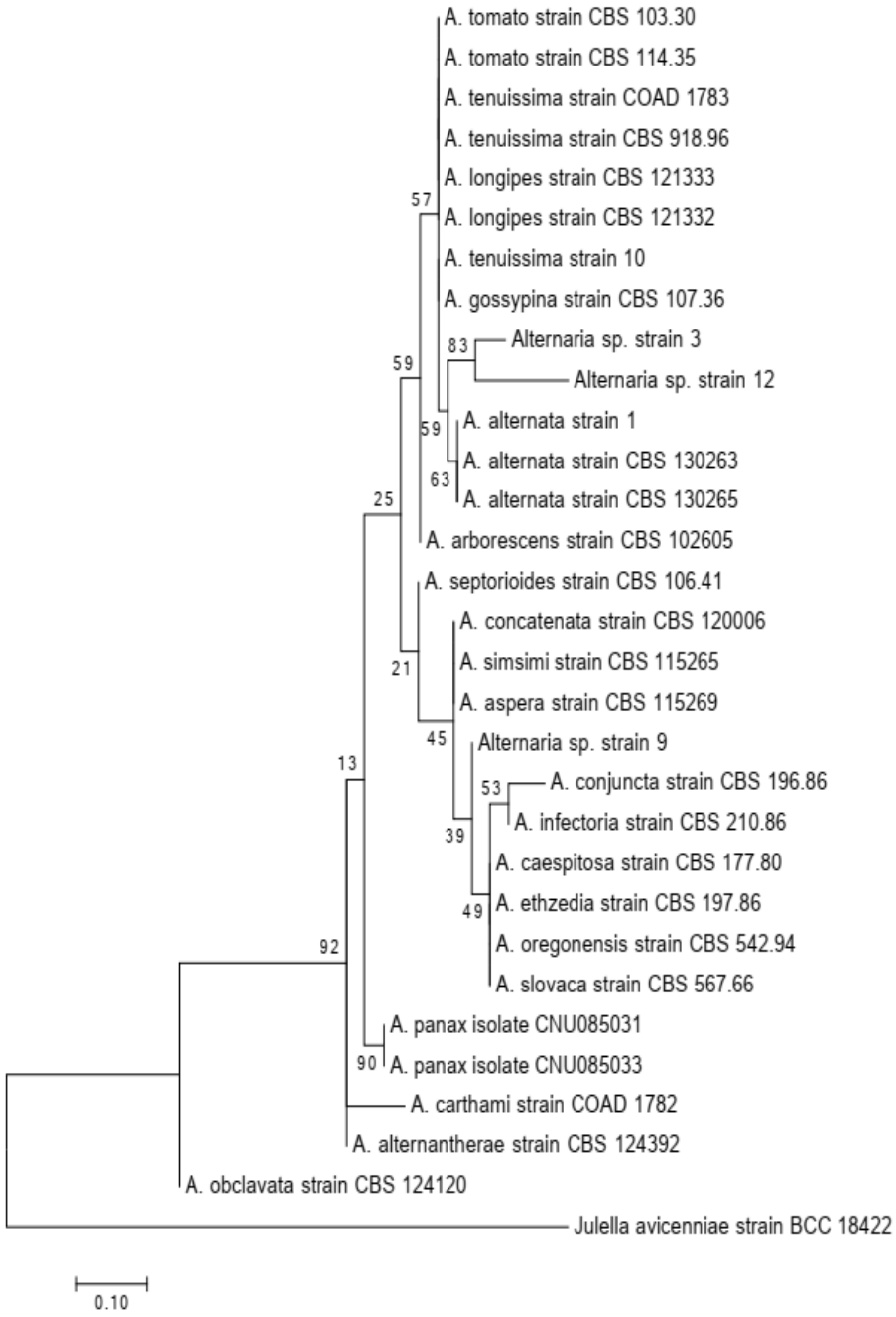

Figure 2 - Maximum Likelihood analysis tree based on rpb2 gene region of 31 Alternaria species using Maximum Composite Likelihood (MCL) approach. The bootstrap support values are given at the nodes. The tree was rooted to *Julella avicenniae* (BCC 18422).

The development of dry rot of *fusarium* and *Alternaria* on tubers under conditions of artificial infection has shown that there are no varieties resistant to the pathogens of the Astrakhan pathogenic population. Regarding *Fusarium*, the Arizona and Red Scarlet varieties have shown to be susceptible, Gala and The Riviera are very susceptible (Table 3). Regarding *Alternaria* rot, variety Arizona proved to be moderately resistant, while Riviera, Red Scarlet, and Gala proved to be susceptible (Table 3).

Table 3 - Resistance of potato tubers of various varieties to *Fusarium* and *Alternaria*

Resistance group	*Fusarium*		*Alternaria*	
	Variety	Disease index %	Variety	Disease index %
Resistant (index 1–25%)	-	-	-	-
Medium Resistant (index 26–50%)	-	-	Arizona	38.6
Susceptible (index 51–75%)	Arizona Red Scarlet	72.2 69.1	Riviera Red Scarlet Gala	57.5 62.7 54.1
Very susceptible (index 76–100%)	Gala Riviera	81.7 89.5	-	-

Synthesis and characterization of Lav-ChNP: The AFM images of the ChNP and Lav-ChNP demonstrate spherical shape (Fig. 3(A) and (D)). ChNP size ranged from 20 to 90 nm as analyzed by Advanced Watershed software platform (Fig. 3(B) and (C)), whereas Lav-ChNP exhibited a broader size range from 200 to 600 nm.

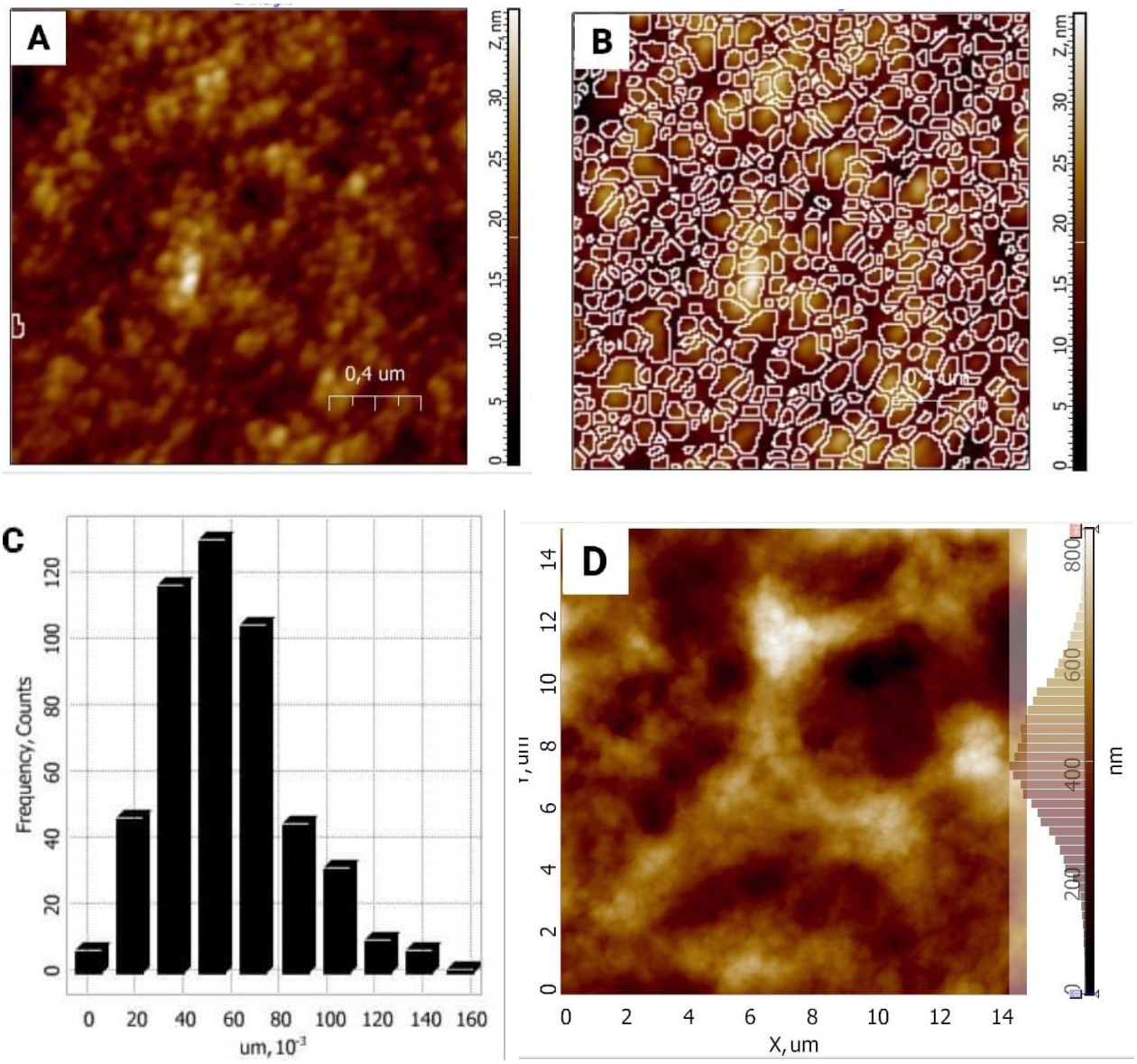

Figure 3 – AFM image of ChNP (A), Nanocapsule sizing of ChNP using the Advanced Watershed software platform (B), nanocapsule size distribution of ChNP (C), AFM image of Lav-ChNP.

The encapsulation efficiency of LEO in Lav-ShNP was shown in (Table 4), the EE% ranged from 42.46% to 86.9% with the highest value of 86.9% achieved at a chitosan-to-oil ration of 1.0:0.5 (Table 4). The nanoparticles size increasing is due to loading of lavender oil in chitosan nano-capsules, which is concurrent to the findings of [15], where *Zataria Multiflora* essential oil was successfully

encapsulated into ChNP. As presented in (Table 4) ChNP showed a ZP of +51.9±0.6 mV, also Lav-ChNP showed ZP ranged between +28.1±0.6 to +41.1±0.7 mV. Decreasing of ZP value was observed as the initial lavender oil content increased. Similarly, [16]reported that the ZP of ChNP was reduced with increasing initial content of L-ascorbic acid.

Table 4 – Encapsulation efficiency (EE) and zeta potential of encapsulated lavender oil

Chitosan: essential oil mass ratio (weight/weight)	EE (%) UV-spectrophotometry	zeta potential (ζ) (mV)
1:0.0	-	+51.9±0.6
1:0.25	42.46±2.36	+41.1±0.7
1:0.50	86.99±3.56	+38.1±0.7
1:0.75	61.31±2.11	+33.6±0.5
1:1.00	48.54±1.98	+30.7±0.1
1:1.25	43.62±3.35	+28.1±0.6

Antifungal activity: Antifungal activity of free and encapsulated Lavender oil was tested on the mycelial growth of *A. Alternata* and *F. sambusinum* as shown in (Figure 4 A and B). The minimal inhibitory concentrations (MICs) of free and encapsulated oil were 10.0 g/L and 1.0 g/L, respectively (Figure 4 A and B). These results highlight the augmented antifungal activity of encapsulated oil compared to its free form. These results are in agreement with [15], who found that the MIC was lower in case of encapsulated *Zataria multiflora* essential oil than its free form in relation to *Botrytis cinerea*.

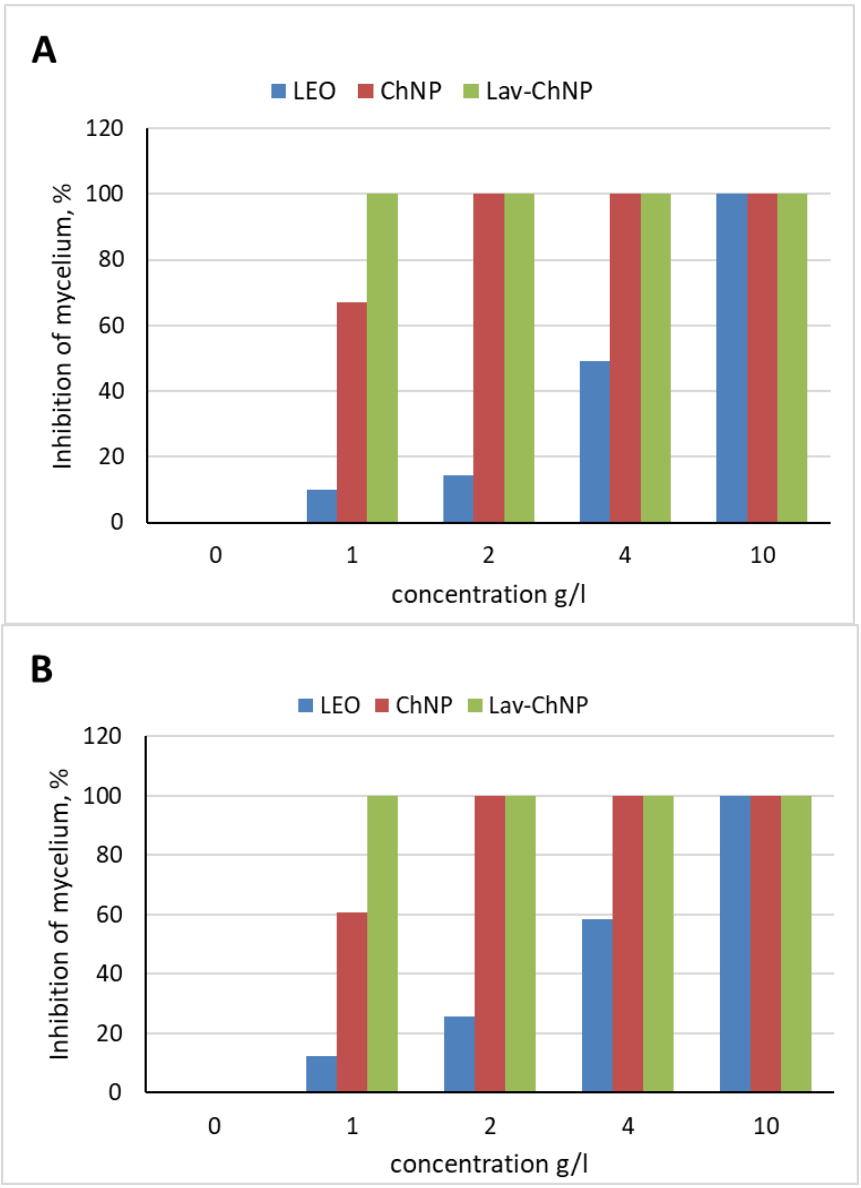

Figure 4 - Comparative antifungal activity of free and encapsulated LEO against *Alternaria* (A) and *Fusarium* (B) mycelial growth in vitro. (LEO) lavender essential oil, (ChNP) chitosan nanoparticles, (Lav-ChNP) lavender oil encapsulated in chitosan nanoparticles.

Evaluation of biological and economic efficacy of experimental preparations: After 8 months of potato treatments by the experimental preparations and storage at 4°C, the analysis of disease incidence and severity revealed significant differences between all treatments. Lav-ChNP at a rate of 10 g/t was the most effective treatment and significantly reduced the disease incidence by 75.1% and disease severity by 91.4%. On the other hand, when tubers were treated with free lavender

oil at a rate of 40 g/t, the rot incidence decreased only by 43.3%, and the rot severity decreased by 40.0% compared with the control (Fig. 5). These data show superior efficacy of encapsulated lavender oil in reducing the disease incidence and severity during potato storage.

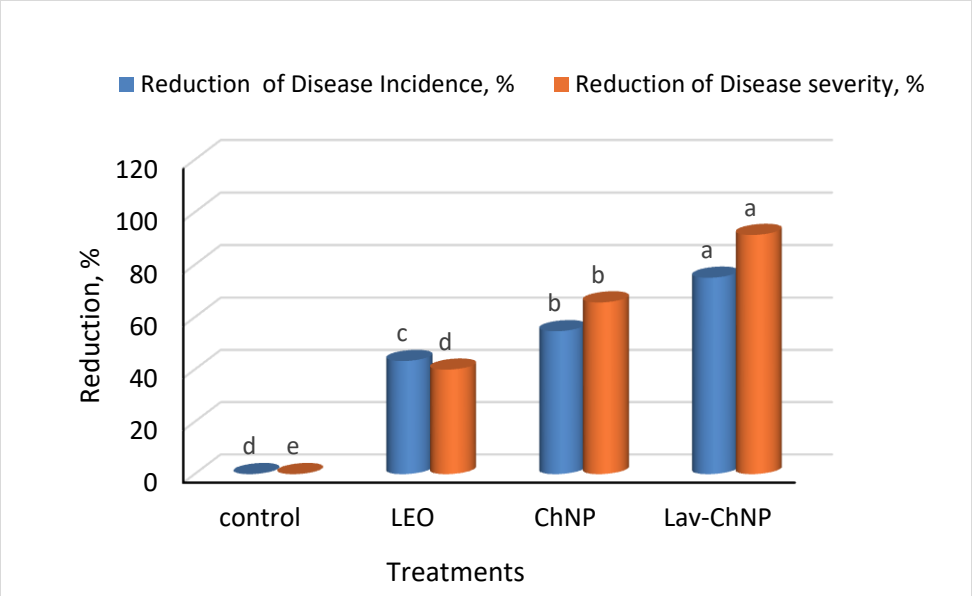

Figure 5 – Biological efficacy of free and encapsulated lav oil against *Fusarium* and *Alternaria* of tubers during storage (average for 2020-2022). Note: values with different letter indexes inside the graph (in each parameter) significantly differed at $p < 0.05$. (LEO) Lavander essential oil 100 g/t, (ChNP)= Chitosan nanoparticles 20 g/t, (Lav-ChNP) Lavender oil encapsulated into chitosan nanoparticles 10 g/t.

Economic efficacy during potato storage: The development prospects of the potato industry largely depend on its economic efficiency. The calculation of the economic efficiency of this treatment method consists in reducing the losses of food potatoes during storage, making a profit from the sale of saved products after storage, and increasing the profitability of food potato production. The level of profitability of using encapsulated lavender oil was higher compared to other treatments and reached 472.75% (Table 5).

Table 5 - Economic efficiency of free and encapsulated lavender oil against *Fusarium* and *Alternaria* rots of tubers during long storage of potato (2020-2022)

Indicators	Treatments			
	Control	Lavender oil, 100 g/t	Chitosan nanoparticles, 20 g/t	Lavender oil encapsulated in Chitosan nanoparticles, 10 g/t
Losses per 1 ton, kg	157	89	71	39
Saved products from 1 ton, kg	-	68	86	118
Total processing costs of 1 ton, USD.	-	6.47	3.09	2.90
The selling price of the saved products, USD.	-	9.57	12.11	16.61
Net income, USD.	-	3.1	9.02	13.71
Profitability level, %	-	47,91	300.66	472.75

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