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SHORT COMMUNICATION

Chemical composition and potentiation of insecticidal and fungicidal activities of *Citrus trifoliata* L. fruits essential oil against *Spodoptera littoralis, Fusarium oxysporum* and *Fusarium solani via* nano-cubosomes

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ABSTRACT
Development of natural nano-based plant-protection formulations represents an emerging phenomenon that has been widely improved for crops protection and for enhancing the efficiency and safety of pesticides. In the present study we isolated the essential oil from the fruits of *Citrus trifoliata* L. and investigated it using gas chromatography-mass spectrometry analysis. Limonene (78.46%) was the major component followed by β-Myrcene (7.94%) and Caryophyllene (4.20%). *Citrus trifoliata* essential oil (CTEO) loaded nano-cubosomes were successfully prepared by the emulsification technique. The insecticidal and fungicidal activities of formulated CTEO nano-cubosomes and unformulated CTEO were tested. While both of them exhibited substantial activities, CTEO nano-cubosomes were more effective than unformulated oil. It is the first time to formulate CTEO in nano-cubosomes and examine their insecticidal and fungicidal activities. In light of the current study, CTEO as it is or as nano-cubosomes is recommended as a promising candidate for pest and fungal pathogens control.

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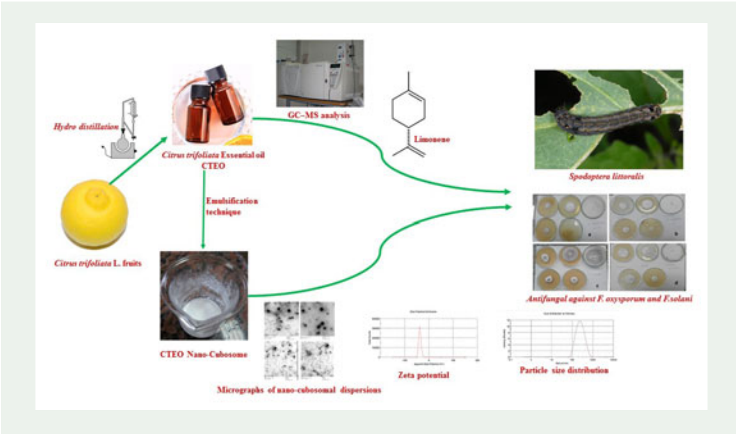

1. Introduction

The Egyptian cotton leaf worm *Spodoptera littoralis* is considered one of the most dangerous pests that attack cotton, vegetables and more than 29 economically important crops in many countries around the world (El-Din and El-Gengaihi 2000). Genus *Fusarium* is one of the most important soil-borne pathogens that cause fungal diseases in a wide variety of crops, produce mycotoxins in stored grains (Maheshwar et al. 2009) and has the ability to survive for very long periods in soil without a host (Larena et al. 2003). *Fusarium oxysporum* causes a destructive disease of cotton which leads to crop losses in the most cotton-producing countries (Abd-Elsalam et al. 2014). On the other hand, the soil-borne fungus *Fusarium solani* is involved in sudden death syndrome of soybean and resulting in reduced quality and quantity of soybean products (Nelson and Toussoun 1989). Some species can survive in the soil even under unfavourable conditions so, there is no total elimination of this disease (Rupe and Gbur 1995).

Use of synthetic pesticides and fungicides not only resulted in several negative effects in the environment (Sitaramaraju et al. 2014) but also led to the appearance of pesticide and fungicide-resistant pathogens. The focus is, therefore, shifted to search potent pesticides and fungicides from natural origin. Plant essential oils are known to possess remarkable fungicidal and insecticidal activities (Siddiqui et al. 2017). Citrus fruits essential oils were used as food preservatives (Javed et al. 2014) and their use as insecticides and antifungals has increased in food and packaging industries. Limonene is the major component of citrus EOs; ranging from 32 to 98% (Svoboda and Greenaway 2003). It has reported insecticidal activity *via* fumigant effect, contact or ingestion (Prates et al. 1998).

Development of environment-safe pesticidal and antifungal agents-based nano materials is urgently needed to improve the current crop management protocols (Kah et al. 2013). Recently, nanotechnology offers the opportunity to facilitate formulation and delivery of pesticide active ingredients and ensure the efficient and safe utilization of pesticides and fungicides without any pesticidal drift or environmental contamination (Gogoi et al. 2009).

The current study aimed to enhance the insecticidal and fungicidal activities of *Citrus trifoliata* L. fruits essential oil (CTEO) through the formulation of nano-cubosomes (self-assembled cubic liquid crystalline nanoparticles) incorporating the essential oil. To the best of our knowledge, formulation of CTEO in nano-cubosomes and examine its insecticidal and fungicidal activities compared to the unformulated EO is offered for the first time by this study.

2. Results and discussion

2.1. Chemical composition of CTEO

The separated oil was colourless with mandarin like odour. The yield was 3.6% (w/w) and the refractive index was 1.476. Twenty-four components were identified in CTEO at different percentages (Table S1). The GC-MS analysis of the oil revealed that most of the compounds were monoterpenes. Limonene (78.46%) is the major component in agreement with those reported by (Geraci et al. 2017; Yang et al. 2017) followed by β-Myrcene (7.94%) and Caryophyllene (4.20%).

2.2. Preparation of nano-cubosomal dispersions

The average particle size and poly dispersity index as measured by dynamic light-scattering technique were found to be 220 nm and 0.17, respectively (Figure S1). Figure S2 demonstrated that the prepared nano-formulation exhibited a zeta potential of −37 mV indicating that they have sufficient charges that would inhibit their aggregation (Abdelbary et al. 2016). Transmission electron microscope (TEM) revealed that the prepared cubosomes are in the nano-size, which confirms the results of particle size measurement, and red arrows indicate more or less cubic nano-particles (Figure S3).

2.3. Insecticidal activity of CTEO and its nano-cubosomes against *S. littoralis*

Data of the accumulative mortality against 2nd instar larvae of *S.litteralis* along 24 h exposure to CTEO or its nano-cubosomes (Table S2 and Figure S5) revealed that both were toxic in a dose-dependent manner in agreement with the previous study of insecticidal citrus essential oils (Abdel-Sattar et al. 2010; Oboh et al. 2017). CTEO at the lowest tested dose 25 μl/5g artificial diet caused 40% mortality after 24 h, while a dose of 100 caused 100% mortality after 24 h. Treatment with nano-cubosomes of CTEO showed potent insecticidal activity and caused 100% mortality after 24 h exposure to the highest tested dose of 200 μl. The results (Table S2) demonstrated that LC_{50} and LC_{90} of CTEO were 41.6 and 75 μl, respectively, while LC_{50} and LC_{90} of nano-cubosomes of CTEO were 77.3 and 165.8 μl respectively which are equivalent to 7.73 and 1.65 μl of CTEO as nano-cubosomes contain only 10% CTEO (see the experimental section), so nano-cubosomes have enhanced the insecticidal activity of CTEO at much lower volumes, therefore the nano-cubosome offers a more economical alternative than using the pure oil. Results showed that development of nano-cubosomes of CTEO significantly enhanced insecticidal activity against 2nd instar larvae of *S. litteralis* which could be resulted from improving the chemical stability of the essential oil, reducing rapid evaporation, increasing the contact time with the insect, increasing passive cellular absorption mechanism and diminishing transfer resistance. This finding was evidenced by earlier studies of essential oils nano-formulations (Wang et al. 2018). The possible sites of action of essential oil toxicity are acetylcholinesterase and the octopamingeric system in insects (Jankowska et al. 2018).

2.4. Antifungal activity of CTEO and its nano-cubosomes against F. solani and F. oxysporum

The antifungal activity of CTEO and its nano-cubosomes against *F. solani* and *F. oxysporum* using well agar diffusion assay at different volumes were evaluated, the EC_{50} were determined and compared (Table S3 and S4, and Figure S6).

The percentage inhibition of mycelia growth has increased with an increasing volume of CTEO or its nano-cubosomes. While EC_{50} of *F. solani* and *F. oxysporum* treated with CTEO was 65.5 ± 0.8 and 55.1 ± 0.7, respectively, EC_{50} after treatment with nano-cubosomes have markedly improved to 41.5 ± 0.6 and 35 ± 0.5, respectively (Table S3 and S4) proving that *F. oxysporum* was more sensitive to CTEO and nano-cubosomes inhibitory action. Our results confirm the previously reported antifungal activity of Citrus (Trabelsi et al. 2016) and the efficacy of Citrus essential oils in insect/pest control for stored food products and processed food preservation (Mahato et al. 2019).

On the other hand, treatment with nano-cubosomes of CTEO showed more potent fungicidal activity than CTEO, it had lower EC_{50} values (Table S3 and S4). Moreover, considering that the cubosome contained lower amount of the CTEO is an indication of how greatly the cubosomes have enhanced the activity of the oil. Our study proved that Monoolein-based nano-cubosomes are among the most cost-effective drug-loaded lipid-based systems (Saber et al. 2018) that deliver essential oils in their bioactive form (Sagiri 2014).

Significant fungicidal activity of CTEO could be attributed to the rich monoterpenes content especially limonene with agreement of previous studies (Aggarwal et al. 2002; Chee et al. 2009).

3. Conclusion

The present study gave deep insights into the chemical composition of CTEO, preparation of CTEO loaded nano-cubosomes and verification of their insecticidal and fungicidal activities against *Spodoptera littoralis*, *F. oxysporum* and *F. solani*. Limonene is the major component which is mostly the effector mediator for their insecticidal and fungicidal activities. CTEO is effective enough to be used as a natural biopesticide. Nano-cubosomes of CTEO exhibited more powerful insecticidal and fungicidal activities. Concisely, nano-cubosomes is considered a potential carrier for enhancing the pesticidal activity of CTEO and can be used as natural nano-pesticide in plant disease control. Further field studies will provide more evidence for the potential use of CTEO for pest and fungal pathogens control.

Disclosure statement

The authors have no conflict of interest to declare.

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