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Short note

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Nanosponges and CPPU: a scoping review and a pre-test to assess the potentiality for shelf-life prolongation of cut carnations

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Abstract: Nanosponges can favour the gradual release of molecules over a prolonged time, increasing the bioavailability and action of preservatives and phytoregulators, reducing the concentrations usually adopted. In floriculture, they have previously been proposed for the delivery of anti-ethylene compounds to improve the shelf-life of cut flowers. However, the potential of nanosponges is not only limited to these compounds. The present scoping review evaluated the effects of β-cyclodextrin-based nanosponges and growth regulators on the post-harvest longevity of cut flowers of ornamental species. One novelty was the use of Forchlorfenuron (CPPU), a growth regulator belonging to the group of cytokinins predominantly used in fruit cultivation, to evaluate its potential to increase the shelf-life of cut carnations (*Dianthus caryophyllus L*). In particular, an in-depth analysis of a pre-test involving the use of nanosponges and CPPU is proposed. Specifically, as far as post-harvest longevity is concerned, the treatments involved the use of: deionised water; nanosponges and deionised water; nanosponges loaded with CPPU; nanosponges loaded with a classic solution for cut flowers, composed of sucrose, aluminium sulphate and 8-hydroxyquinoline sulphate. Preliminary results show that the nanosponge and deionised water complex and the nanosponge and classical solution complex prolonged the longevity of the cut flower by up to 20 days, compared to the control (17 days). In contrast, the CPPU-nanosponge complex showed similar results to the control. Replication of the research is necessary to validate the results.

1. Introduction

Cyclodextrin Nanosponges (CDNSs) are cage-like network polymers prepared by connecting cyclodextrin molecules with various kinds of bi- or polyfunctional linking agents. The term “nanosponge” was first introduced by Ma and Li, in a paper focusing on the sequestering capacity of polyurethane crosslinked CD polymers, to highlight the nanometric porosity of this class of materials (Ma and Li, 1999). In addition to the central lipophilic cavity of CD molecules, CDNSs exhibit a second type of pores, which are the empty spaces among CD molecules, whose polarity and size can be modulated by varying the chemical structure of the linking agent and its concentration. Such features make CDNSs highly versatile materials with potential applications as nanostructured containers and delivery systems for a broad set of active principles. Furthermore, CDNSs are low-cost, easy to prepare, safe and sustainable materials, as they are mainly composed of starch derivatives and, in some cases, even the linker comes from renewable sources (e.g., citric acid) (Caldera *et al.*, 2017).

The application of CDNSs in the pharmacological field has been widely explored. Several classes of drugs have been successfully encapsulated, stabilized and released with controlled kinetics, including anticancer, anti-inflammatory, antiviral, antibacterial, antifungal drugs and many others. Recently, the functionalization of CDNSs with specific moieties has led to the development of a new generation of stimuli-responsive CDNSs, capable of releasing drugs on-demand and highly selective CDNSs for targeted drug delivery.

In the environmental field, CDNSs have been used as absorbing agents for the removal of organic pollutants as well as heavy metal cations for water decontamination (Krabicova *et al.*, 2020).

Although CDNSs have been extensively studied for biomedical and environmental applications, their full potential in the horticultural field remains largely unknown. To date, only a few studies have explored the use of CDNSs for the encapsulation and release of the herbicide ailanthone and some anti-ethylene molecules (Demasi *et al.*, 2021).

Forchlorfenuron [N-(2-chloro-4-pyridyl)-N'-phenylurea], whose acronym is CPPU, is a plant growth regulator (synthetic cytokinin) that promotes the periclinal cell division and is often used in agriculture to increase the berry size, the quality and the yield of grapes and kiwifruit (Peppi and Fidelibus, 2008; Cruz-Castillo *et al.*, 2014).

Although several studies have evaluated the efficacy of CPPU in postharvest in fruits (Zhang *et al.*, 2017; Chang, 2021), few studies have focused on cut foliage and flowers, in contrast to a similar molecule, Thidiazuron, which has been used for years (Ferrante *et al.*, 2002; Chamani *et al.*, 2006).

The aim of this scoping review is to highlight the application potential of nanosponges in the postharvest context. In addition, an in-depth study is proposed on the production and testing of nanosponges and CPPU in order to assess their effectiveness in prolonging the *shelf-life* of cut carnations. More specifically, we intend to describe the pre-test phase of the two products listed above, which is also useful from an economic point of view in order to produce a quantity of nanosponges in the laboratory suitable for performing an experimental test with replications, as well as to proceed with the purchase of CPPU in larger quantities.

2. Materials and Methods

In the coming sections, the following will be reported: a scoping review on the use of nanosponges in floriculture; a first trial of the use of CPPU and nanosponges on cut flower carnation.

Synthesis of a carbonate cyclodextrin nanosponge

A carbonate cyclodextrin nanosponge was synthesized by heating β-cyclodextrin (β-CD, 6.500 g, 5.73 mmol) and 1,1'-carbonyldiimidazole (CDI, 3.715 g, 22.92 mmol) in 39 mL of N,N-dimethylformamide at 90°C for 4 h. In the end, the solid gel formed during the crosslinking reaction was crushed and washed with approximately 2 L of deionized water via Buchner filtration. The nanosponge was further purified in a Soxhlet extractor with ethanol for 20 h and, once dry, finely ground in a planetary ball mill.

CPPU loading in the nanosponge

The loading of CPPU into the nanosponges was performed subsequent to the synthesis and purification of the nanosponge. Briefly, 450 mg of nanosponge was added to a solution of 50 mg of CPPU dissolved in 25 mL ethanol. Then, the dispersion was stirred at room temperature for 24 h. Finally, the CPPU-loaded nanosponge was collected by removing the entire volume of ethanol in a rotary evaporator at 50°C under vacuum. The complex was stored in a desiccator at room temperature until use.

Pre-test setting

The pre-test was conducted in March and April 2022, in the laboratories of the Department of Chemistry and Department of Agricultural, Forest and Food Sciences, University of Turin.

The carnations were collected, packaged and purchased at the flower market in Sanremo (Italy) on 27 March at 6 p.m. and were transported to Turin and kept in the transported condition until 3 p.m. on 28 March, when the pre-test began. The four packages, purchased from 'Cooperativa Tre Ponti' (intermediary), contained 20 flowers each.

The first phase consisted of eliminating flowers that visually showed diseases, lesions and flowers at a different stage of flowering from the others. A total of 70 flowers were selected and the stems were cut into the water leaving a length of 30 cm.

The pre-test comprised seven different treatments (solutions with deionized water), each applied to 10 flowers. Each flower was placed in a glass tube containing 50 ml of solution. On day 8 and 16, 10 ml of the specific solution was added to all tubes. Measurements were conducted daily, and data were acquired for the following parameters: cut carnation weight (g) and flower diameter (cm). The pre-test lasted 20 days, with the following environmental conditions: 12 hours of light and 12 hours of darkness; temperature 19±2°C. The treatments are shown in Table 1.

3. Results and Discussions

Pre-test results

Preliminary results (Fig. 1) show that the treatment A and the treatment C prolonged the longevity of the cut flower by up to 20 days, compared to the Control (17 days). In contrast, the treatment E complex showed similar results to the control. There was no inhomogeneity in the diameters of the flowers within the various treatments; however, the largest diameters (9.5 cm) were more frequently reached by the treatment F. Lateral shoot development was a common side effect of CPPU and commercial solution treatments.

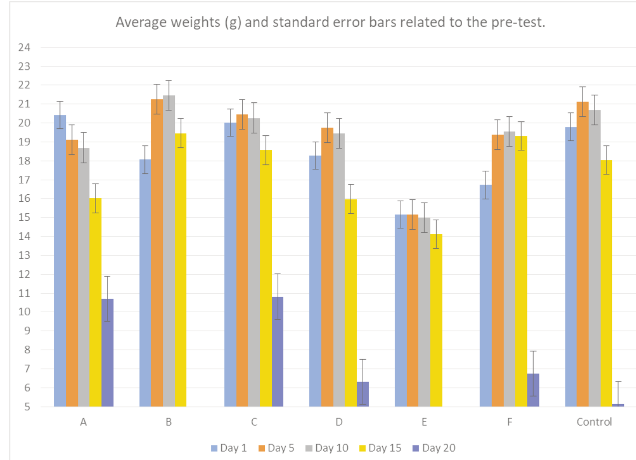

Fig. 1 - Average values of weights (g) and standard error bars of cut carnations during the pre-test.

The pre-test yielded interesting results that allow planning the production of nanosponges and the purchase of CPPU in order to set up a scientific trial. Of particular interest could be the application in solution and spray of nanosponges and CPPU on cut foliage.

Final discussions and next steps

The application of CDNSs in floriculture, and more specifically post-harvest preservation, is an emerging line of research still widely unexplored. To date, only few scientific papers focusing on the encapsulation of anti-ethylene compounds in CDNSs have appeared in the literature. Of these ethylene inhibitors, 1-methylcyclopropene (1-MCP) is one of the most studied, especially in combination with CDs and CD-derivatives.

In 1994, Serek *et al.* described for the first time the ability of 1-MCP to prolong the post-harvest shelf-life of plant material (Serek *et al.*, 1994). Since then, 1-MCP has been extensively studied as a non-toxic anti-ethylene agent to inhibit the senescence

Table 1 - Pre-test treatments

Code	Treatments
A	Nanosponges (5 g/l)
B	Sucrose 30 g/l + aluminium sulphate (200 mg/Kg) + 8-hydroxyquinoline sulphate (200 mg/l)
C	Sucrose 30 g/l + aluminium sulphate (200 mg/Kg) + Nanosponges (5g/l)
D	CPPU (200 mg/l)
E	CPPU in Nanosponges (200 mg/l)
F	Commercial solution - Chrisal prof. 2 (10 ml/l)
Control	Deionised water

process of cut flowers, vegetables and fruits at the receptor level (Blankenship *et al.*, 2003; Nasiri *et al.*, 2020). In 2000, 1-MCP appeared on the market in two different formulations commercialized by Floralife, Inc. (Walterboro, SC) and AgroFresh, Inc. (Spring House, PA). The Floralife product is named EthylBloc® and it is meant to be applied on ornamentals only. While, Agrofresh provides a formulation, named SmartFresh®, that can be used with edible crops. However, both products are based on the inclusion complex of 1-MCP gas in α-CD (Gehla *et al.*, 2003).

The efficacy of these formulations is time-limited, as most of 1-MCP is released within 20-30 minutes from application, under normal temperature and pressure conditions (Blankenship *et al.*, 2003). To overcome such limitations, new materials such as CDNSs have been tested for the encapsulation and prolonged release of 1-MCP.

In a first attempt, 1-MCP, 2,5-norbornadiene and silver nitrate were encapsulated in a carbonate CDNS and added to two flower species, specifically *Dianthus caryophyllus* 'Idra di Muraglia' and *Ranunculus asiaticus* 'Elegance'. While the formulation containing 1-MCP allowed to extend the vase life of *Dianthus caryophyllus* up to 23 days, the other formulations did not show significant effects and none of the formulations was able to improve the longevity of ranunculus (Devecchi *et al.*, 2009).

With the aim of understanding the role played by the central cavity of CD molecules in the encapsulation and controlled release of 1-MCP, carbonate NSs based on α-CD (6 glucose units, diameter of the cavity: 4.7-5.3 Å) were used in comparison with the analogous carbonate NSs prepared with β-CD (7 glucose units, diameter of the cavity 6.0-6.5 Å). The tests were performed on *Dianthus caryophyllus* 'Idra di Muraglia'. Results demonstrated that β-CD is remarkably more effective than α-CD in extending the postharvest life of the *Dianthus* flowers, as no evidence of deterioration was observed up to nearly 11 days. Whilst, the 1-MCP-loaded NSs based on α-CD did not show any significant anti-ethylene activity (Sceglie *et al.*, 2011 a).

The next step was to determine the influence of the NS's degree of crosslinking on the encapsulation and slow release of 1-MCP. A series of samples of β-CDNS were synthesized with different carbonate to CD molar ratio (i.e., 2, 4 and 8), resulting in different degree of crosslinking. After loading with 1-MCP, the NSs were used to extend the vase life of carnation cut flowers. All the NS formulations exhibited anti-ethylene activity. However, the NS with the highest degree of crosslinking (i.e., linker/β-CD molar ratio of 8), and therefore the most densely crosslinked polymer structure, showed the highest efficacy for prolonged time, even at low concentration (Sceglie *et al.*, 2011 b).

The protective effect of the 1-MCP-loaded β-CDNS with monomer ratio 1:8 against infection by Botrytis cinerea on *Dianthus caryophyllus* L. 'Idra di Muraglia' was studied as well. After eleven days of treatment, the NS at low dosage reduced the spreading of the grey mould by approximately 60 %. At higher dosage, the NS formulation outperformed the commercial 1-MCP product, used as a reference, reaching a value above 90%, while the commercial formulation stopped at 76% (Sceglie *et al.*, 2012).

As a final step, the effectiveness of the 1-MCP/ β-CDNS(1:8) complex in delaying the senescence process of cut flowers was evaluated in different floral species. Specifically, *Anemone coronaria L. multicolor*, *Paeonia lactiflora L.* 'Sarah Bernhardt', *Helianthus annuus L.* 'Sunrich Orange', *Ranunculus asiaticus L.* 'Minou Abrown', *Papaver nudicaule L. multicolor* and *Rosa hybrida L.* 'Jupiter' were treated with the NS formulation in the presence of exogenous ethylene. Although the mechanism of action was different, depending on the flower species, the CDNS increased the anti-ethylene activity of 1-MCP in all the tested samples (Sceglie *et al.*, 2013).

Despite the positive results described above, the use of 1-MCP has some disadvantages, including its high cost, difficult handling and volatility. Moreover, EthylBloc® and SmartFresh® are not available in some countries. Therefore, the development of new formulations based on easily manageable active principles, such as salicylic acid (Cocetta and Ferrante, 2018), synergistic formulations of cumin essential oil and 8-hydroxyquinoline sulfate (Mirjalili *et al.*, 2018), CPPU and others with prolonged release kinetics and thus long-term efficacy, are of special interest.

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