ENHANCED PRODUCTION OF SECONDARY METABOLITES BY METHYL JASMONATE AND SILVER NANOPARTICLES ELICITATION IN TISSUE CULTURE OF *CATHARANTHUS ROSEUS* (APOCYNACEAE)

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ABSTRACT

Methyl jasmonate (MeJA) and silver nanoparticles (SNPs) are one of the elicitors that can induce the production of secondary metabolites of *Catharanthus roseus* L., the two of which incite defense responses.

Methyl jasmonate (MeJA) treatments with different concentrations (0, 10, 50, and 100 µM) were used to enhance high yielding of alkaloid content in *Catharanthus. roseus*. The content of vincristine and vinblastine in the callus of *Catharanthus. roseus* were determined by HPLC.

High content of vinblastine was seen in those explants treated with 75 mg/L SNPs (1.4 µg/g Fresh Weight). The most estimation amount of vinblastine and vincristine were seen in plants treated with 50µM MeJA (1.23 and 0.9 µg/g FW).

Keywords:

Catharanthus roseus, Silver nanoparticles, Methyl jasmonate, Secondary metabolites.

Introduction

Catharanthus roseus (*C. roseus*) (Apocynaceae) is a medicinal plant that has traditionally been used. It is planted as a herbaceous plant or under a shrub of latex. It reaches a height of 1 m in the subtropical zone. It was native to the Island of Madagascar, and is now growing wildly in most warm regions of the world especially in Egypt (**Dobelis, 1989 and Heywood, 1993**). The plant produces many pharmaceutically important alkaloids of which the bisindole alkaloids vinblastine and vincristine. They are antineoplastic medicines and the monoindole alkaloids ajmalicine and serpentine are antihypertension drugs (**Zhao and Verpoorte, 2007 ; Auriola et al., 1990**).

The term "nanotechnology" can be defined as design, synthesis, manipulation and use of atomic or molecular aggregates with a size between 1 and 100 nm (**Bleeker et al., 2013)**. The technical method and processing that produce metallic nanoparticles (NPs) change their physical and chemical properties, as well as their reactivity, due to their small size and high volume ratio **(Boverhof et al., 2015)**. This new nanomaterial technology has been applied to modern and sustainable agricultural practices such as innovative synthetic pesticides and potential fertilizers in the new green revolution. Silver nanoparticles (SNPs) have long been known to exhibit strong anti-microbial

activity (**Rai et al., 2009)**. Secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles (**Annamalai and Nallamuthu, 2016**)

Methyl jasmonate (MeJA) plays a significant regulatory role in the coordination of plant growth and defense (**Wasternack, 2007**). Exogenous application of MeJA has been shown to elicit responses in plants that are naturally induced by insect herbivore and fungal infestation **(Zeneli et al., 2006).**

Jasmonates are endogenous phytohormones of the plants. They are powerful elicitors or signal generators that influence a wide range of physiological and biochemical processes. Several studies have shown that jasmonates cause accumulation of secondary metabolites, such as alkaloids and anthocyanins, in plants. Increases of paclitaxel in Taxus, rosemarinic acid in *Culeus blumei* and anthocyanins in strawberries and vines have been reported after the addition of jasmonate to their suspension cultures **(Akula and Ravishankar, 2011)**.

In vitro, the propagation of plants has enormous potential for the production of high-quality plant medicines **(Murch et al., 2000**). In an in vitro culture a tissue is wound and the induced callus is further subcultured on nutrient media (**Ikeuchi et al., 2013**). Auxin and cytokinins are used almost in all plant tissue culture systems because of their effects on the induction of callus and induce differentiation in vegetative parts and roots.

However, as far as we know, the effect of SNPs on secondary metabolites in plants still need more work to be done. In this study we investigated the effects of SNPs and MeJA on secondary metabolites of *Catharanthus roseus*.

Material and Methods

Catharanthus roseus L. shoots were obtained from the cultivated sample in the *Genetic Engineering and Biotechnology Research Institute* Farm at Sadat city, Egypt, in March 2017, and authenticated by the morphological comparison against authentic herbarium specimens kept at the herbarium of Orman Botanical Garden, Giza, Egypt.

The young shoot tip explants were trimmed and washed under running tap water for 5 min followed by a rinse with 70% ethanol (v/v) for 1 min and 0.1% $HgCl_2$ for 5 min, the following explants were washed with sterile distilled water two to four times each, axillary buds were cultured on sterile MS medium (**Murashige and Skoog, 1962)**.

The culture medium consisted of MS media gelled with 0.2% (w/v) gelrite. The growth regulator 2, 4-dichlorophenoxyacetic acid (2,4-D) at 2µM were used for culturing the explants then transferred to 8% sucrose medium as described by **Knobloch and Berlin, 1980**

A seven- days old callus of *C. roseus* was treated for 24 h with 0, 50, and 100 µM of MeJA (Sigma-Aldrich, St. Louis, MO, USA), and (25, 50, 75 and 100 mg /l) of SNPs (US Research Nanomaterial Inc., USA). Stock solutions of MeJA in MeOH 50 % and of SNPs in water were prepared and sterilized by filtration (0.2µm, Millipore). After treatment, the plants were harvested and washed. Aliquots were snap-frozen in liquid nitrogen prior to storage at -80 °C until used in biochemical measurements. All tests were carried out in triplicate using a destructive sampling method, which consisted

of treating the entire sample at each harvest time. Methanol solution 50% (v / v) was used as a control in these experiments.

Extraction and quantification of vincristine and vinblastine by HPLC analysis

The extraction of indole alkaloids and quantification of vincristine and vinblastine using HPLC were carried out as described by **Schripsema and Verpoorte (1992)** and **Ramani and Jayabaskaran (2008)**. Briefly, freeze-dried cells (~50 mg) were extracted twice with 5 ml of dichloromethane, and the extraction solutions were combined and concentrated under vacuum. The residues were dissolved in 0.5 ml of HPLC mobile phase [50 mM sodium phosphate pH 3.9: acetonitrile: 2 methoxyethanol (80:15:5 v/v)]. (**Aerts et al., 1994).**

Statistical analysis

All experiments were conducted in triplicate at least three independent times. All data are expressed as the mean values ± standard error (SE). Differences between treatment outcomes were calculated using Student's t-test. Differences were considered statistically significant at $p \leq 0.05$.

Results and Discussion

***SNPs uptake by Catharanthus roseus* L.**

The SNPs size in the plant extract was approximately 200 nm which, in comparison to their original size (40 nm), suggests that the SNPs aggregated within the plant matrix.

Effect of SNPs and MeJA on secondary metabolite production

The result of the effects of SNPs and MeJA on secondary metabolite content in *C. roseus* is shown in Fig 1 and2.

High concentration of vinblastine was seen in those explants treated with 75 mg/L SNPs (1.4 µg/g FW) and with 50 mg/L that give 1.22 µg/g FW. The most estimation of vinblastine and vincristine were seen in plants treated with 50µm MeJA (1.23 and 0.9 µg/g FW respectively). On the other hand, the lowest concentration of vincristine and vinblastine were noticed on untreated explants (control) (0.31 and 0.5 µg/g FW respectively).

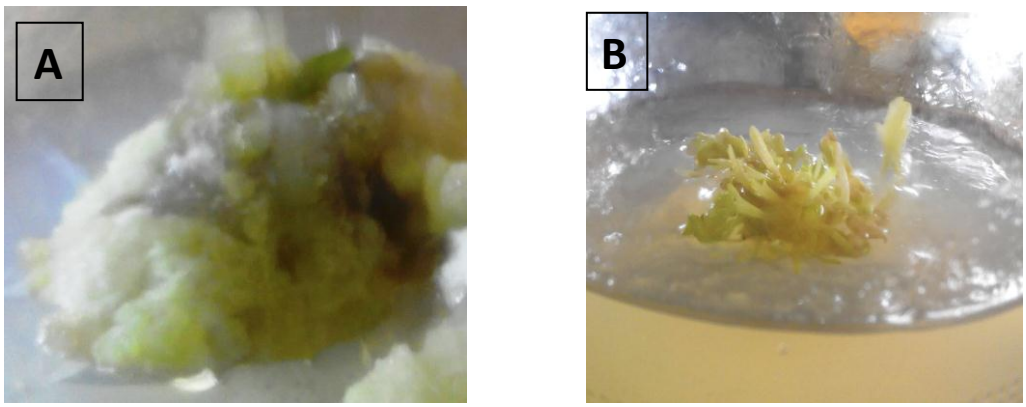

Fig 1. (A) Effect of exogenous MeJA treatment on callus of *C. roseus*). (B) Effect of exogenous SN treatment on callus culture of *C. roseus*)

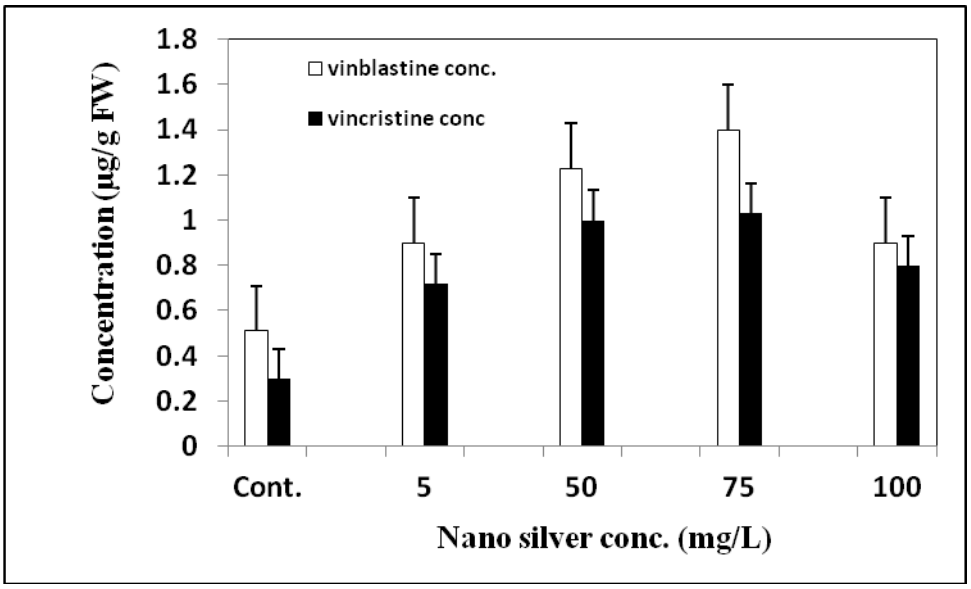

Fig. 2. Effects of silver nano particles on alkaloid contents (vinblastine and vincristine conc.) in callus cultures of *C. roseus.* Vertical bars represent standard error.

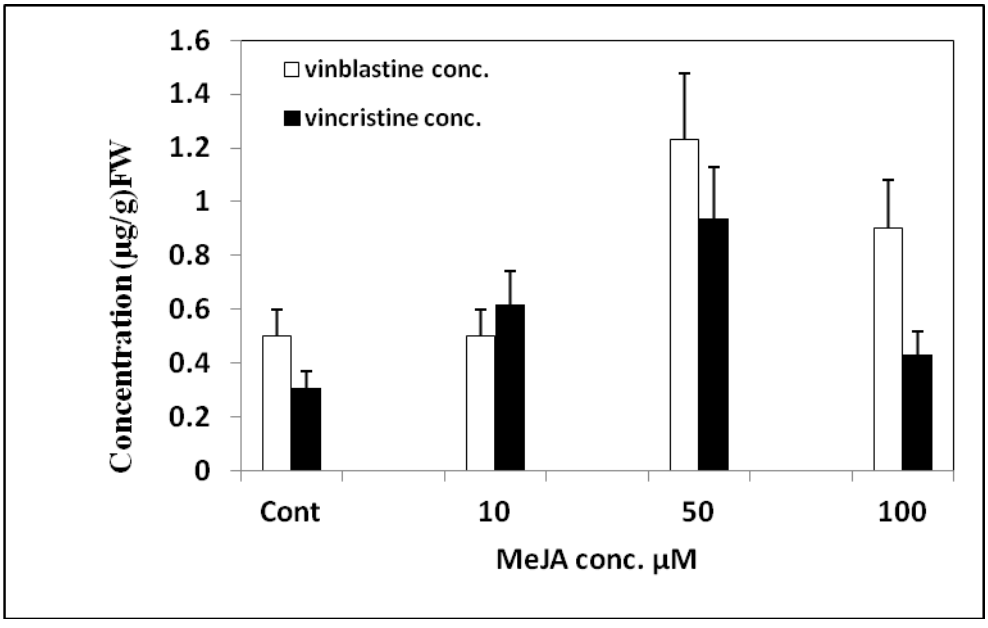

Fig. 3 Effects of Methyle jasmonate on alkaloid contents (vinblastine and vincristine conc.) in callus cultures of *C. roseus.* Vertical bars represent standard error.

Discussion

The biosynthesis of medicinally important alkaloids in Catharanthus has been studied (**Aerts et al., 1994; De Luca et al., 1986 and 1988**). Alkaloids in Catharanthus

are derived from tryptophan, which is first decarboxylated into tryptamine by the enzyme tryptophan decarboxylase (TDC)**; (Knobloch et al., 1981**). In this experiment, the induction of plant defense mechanisms, for instance by wounding or pathogens, is mediated by methyl jasmonate (MeJA). The jasmonates arise from linolenic acid that is liberated from plasma membranes. Wounding- or elicitor-induced increases in endogenous jasmonate levels have been observed (**Creelman et. al., 1992; Gundlach et. al., 1992**). Besides regulating defense responses, jasmonates may play a central role in regulating plant growth and development as well (**Farmer and Ryan, 1992**). Exogenously applied jasmonates have been shown to influence diverse physiological processes in plants and plant cells (**Farmer and Ryan, 1992**). A role of jasmonates in the initiation of secondary metabolite synthesis in cell suspension cultures has also been shown (**Dittrich et al., 1992; Gundlach et al., 1992**). Exogenous application of MeJA to cultures of undifferentiated cells of several plant species triggered the synthesis of various secondary compounds, such as flavonoids and alkaloids. Untreated cultures, on the other hand, showed only marginal accumulation of these compounds (**Gundlach et al.,1992**). In contrast to cell cultures, the accumulation of alkaloids in intact plants occurs as part of the developmental program of plant tissues (**Aerts et al., 1991; Frischknecht et al., 1986 and 1987; Robinson, 1974**). Our data show that MeJa can markedly enhance alkaloid production during explant growth.

Silver nanoparticles (SNPs) have long been known to exhibit strong anti-microbial activity **(Savithramma et al,. 2011)**. Secondary metabolites present in plant systems may be responsible for the reduction of silver and synthesis of nanoparticles **(Savithramma et al., 2011)**. However, to the best of our knowledge, the effect of SNPs on secondary metabolites in plants has not been well studied. In this study, we investigated the effects of SNPs and MeJA on secondary metabolites of *C. roseus*.

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تعزيز إنتاج الأيضات الثانوية بواسطة جاسمونات الميثيل والفضيحات النانوية الفضة في زراعة الأنسجة من نبات الوينكا (العائلة الدفلية)

للسيدة الدكتورة

هبه شاهين

مـــــن

مدرس بقسم بايوتكنولوجيا النباتية معهد الهندسة الوراثية والتكنولوجيا الحيوية بجامعة مدينة السادات

ميثيل جاسمونات (MeJA) والجسيمات النانوية الفضية (SNP) هي واحدة من المحفزات التي يمكن أن تحفز إنتاج المستقلبات الثانوية من نبات الوينكا ،يعتبر كلا المركبين من المركبات التى تحفز استجابات الدفاع.

استخدمت معاملات ميثيل جاسمونيت (MeJA) مع تركيزات مختلفة (٠ ، ١٠ ، ٥٠ ، و ١٠٠ ميكرومتر) لتحسين إنتاجية عالية من محتوى قلويدى في الوينكا. تم تحديد محتوى كلا من الفينكريستين و الفينبلاستين في الكالس من نبات الوينكا بواسطة HPLC.

شوهد مادة عالية من فينبلاستين في تلك المستكشفات بعد المعاملة مع 75 ملغ / لترمن نلنو الفضة (١.٤ ميكروغرام / غرام الوزن الطازج). شوهدت معظم تقديرات vinblastine و vincristine في النباتات بعد المعاملة ب MeJA بتركيز 50µm 1.23 و ٠.٩ ميكروغرام / غرام من الوزن الطازج).