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Research Article,

Effect of Growth Regulators on Callus Formation and Alkaloids Production on Vinca Rosea Plant (Catharanthus Roseus L.)

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Abstract:
Vinca alkaloids are a subset of drugs obtained from the periwinkle plant they are naturally extracted from the pink periwinkle plant (Catharanthus roseus), and they have been used to treat diabetes, high blood pressure and have been used as disinfectant. The vinca alkaloids are also important for being Cancer resistant there are four major vinca alkaloids in clinical use: Vinblastine (VBL), vinorelbine (VRL), vincristine (VCR) and vindesine (VDS). Vinca alkaloids are the second most used class of cancer drugs and will stay among the original cancer therapies. Catharanthus roseus is still source used for the powerful antitumor drugs. Callus culture had been done on MS-medium containing Different Concentrations from BA – 2,4-D – NAA – IAA. Where the different effects of growth regulators were studied when different parts of the plant have been cultured from leaves and stems this is to induce callus formation and encourage growth and the concentrations that were prepared: BA (0,1 – 0,3 – 1 – 2 mg/l) - NAA (0,1 – 1 mg/l) - IAA (0,1 mg/l) - 2,4-D (0,75 – 1 mg/l). And after culture the explants we transferred it to the growth room with a temperature ranging from 27 to 29 ° C relative humidity around 80% And in complete darkness. The results from HPLC analysis for the dry weight of callus were as follows ajmalicine (0.18 mg/g DW), catharanthine (0.07 mg/g DW), serpentine (0.37mg/g DW), vindoline (0.11 mg/g DW).

Keywords: Alkaloids, Catharanthus roseus, growth regulators High Performance Liquid Chromatography (HPLC), Fluorescence, UV

Introduction:

It was found that the Vinca rosea plant contains more than 50 alkaloids thathave a rapid medicinal effect for the treatment of some serious diseases. The aqueousextracts of the Vinca rosea are used as a drink for dentin for gum infections, dental pain and decay, as well as for the dentin of gastrointestinal ulcers, and recently it was possible to know the vital effect of some alkaloid substances in Chronic constipation dentin, More than 40 years ago, other substances have been shown to have an effective effect in the dentin of many types of cancerous diseases, including: vincristine, vinblastine, Ajmalicine and Serpentine, in addition to the substance Hypertension, which plays a role in the dentin of hypertension. These compounds are important for the plant as they are defensive materials against Pathogens, in addition to their use as pharmaceuticals, food flavors, dyes, perfumes, or pesticides, and plants are considered the continuous source in the work to produce important secondary metabolites, Plant tissue culture is the science of growing plant cells, tissues or organs isolated from mother plant on artificial media [1]. It includes techniques and methods appropriate to research into many botanical disciplines and several practical objectives. Both organized and unorganized growth is possible in vitro [2]. For most of the world's population, medicinal plants are the only source of life-saving medicines. They still serve as an important therapeutic aid in the treatment of human diseases. Catharanthus roseus produces more than 100 monoterpenoids indole alkaloids

(TIA) in different organs. Leaves and stems are sources of dark alkaloids, Vincristine and vinblastine are indispensable cancers medicines, while the roots have antihypertensive, ajmalicine and serpentine. [4]. Catharanthus roseus (L.) G. Don. (Habit Known as the family Periwinkle Apocynaceae) it has gained commercial importance due to its alkaline contents in various parts of the plant. Some are used in the pharmaceutical industry to treat childhood leukemia, Hodgkin's disease, testicular cancer and carcinoid tumors [3]. Alkalis are a class of organic compounds consisting of Carbon, hydrogen, nitrogen, and usually oxygen, which are often derived from plants such as C. alkaloids It has physiological effects that make the vinca plant of medicinal value.

Materials and Methods:

The experiments and Cultivation process and chemical analysiswere carried out in the Laboratory of Biotechnology Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt.

Plant material:

The main plant part used in the cultivation process the parts that were used incultivation were a stalk 2 cm long [2] and a part of the leaf 1 cm long and 0.5 cm wide free of middle sweat [5] was obtained for Vinca Rosa plant (one years-old produced by explant) cultivated in the farm of Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt.

Explant preparation:

Surface sterilization of explants The branch of a plant has been collected fromgarden and it cut into small parts and Separate the leaves from the stem and it cut the stems the plant to small part it is 2 cm long [2] and these parts were put in a bigjar, and wash with industrial detergent and they were washed with running water 4 times [5], and It's surface was sterilized with ethanol at 97% concentration for 2 minutes , Then we washed 3: 5 times with sterile distilled water in a laminar flow hood [2], Now the parts are ready to be cultured.

Effect of growth regulators on callus formation:

Sterile uniform explants i.e. 1.0*0.5 cm for leaf blade is free of middle sweat and placed in the outside of it and main stem are a 2 centimeter long placed in italics in media were aseptically transferred to jars containing 30 mL of Morishige and Skoog (MS) medium 2 liters of media were prepared to contain (8.86g/2L MS + 60 g / 2L sucrose + 14 g agar) then we take 1.75 litters from them and were individually transferred into 250 ml jars and different combinations of 2,4-D (2,4-dichlorophenoxyacetic acid) - BA (Benzyl adenine) - NAA (1-Naphthaleneacetic acid) - IAA (indole acetic acid).

Table 1. our concentration from hormones that used.

	Hormonal Transactions	Concentrations
1	BA + NAA	0.1 mg/l + 1 mg/l
2	BA + NAA	1 mg/l + 0.1 mg/l
3	BA + 2,4-D	0.3 mg/l + 0.75 mg/l
4	BA + 2,4-D	0.1 mg/l + 1 mg/l
5	BA + 2,4-D	2 mg/l + 1 mg/l
6	BA + 2,4-D	2 mg/l + 0.5 mg/l
7	BA + IAA	0.1 mg/l + 1mg/l

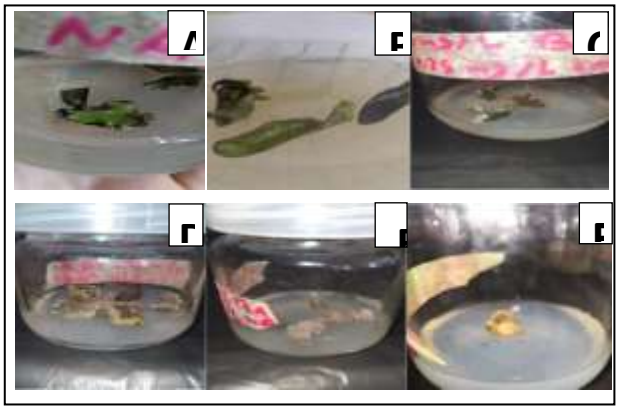

Figure1.Induction of callus. After 45 days from culture it was observed callusinduction with good grade by using: (BA 0.1mg/L + 2.4-D 1 mg/L) , (B A 0.3mg/L+ 2.4-D 0.75mg/L)

Figure 2. Light white to yellowish friable callus produced in vitro cultured leaf explants of Catharanthus roseus at MS medium with BA 0.3 mg/l and 2,4-D 0.75 mg/l hormonal combination.

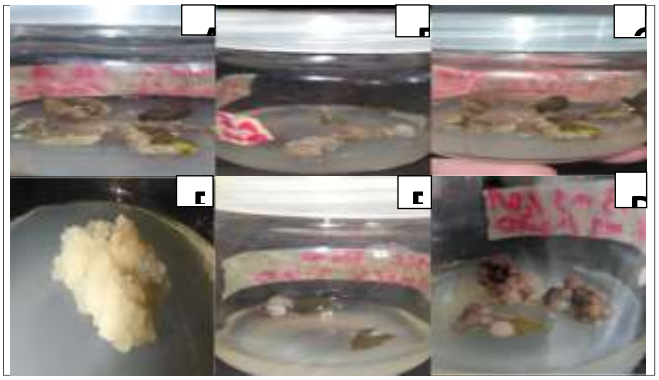

Figure 3. difference of callus formation when used different concentration from hormones.

Table .2 Specific properties of C. roseus' TIAs and precursors for HPLC separation and detection, And the result from HPLC analysis was as follows: Ajmalicine (0.18 mg/g DW), Catharanthine (0.07 mg/g DW), Serpentine (0.37mg/g DW), Vindoline (0.11 mg/g DW) .

Alkaloid	**Fluorescence (max Ex/Em in nm)**	**Absorption UV (nm)**
Tryptophan	**250/330**	**198,231**
Ajmalicine	**250/350**	**217,265**
Serpentine	**310/380**	**272,367**
Vindoline	**201/261**	**201,239**
Strictosidine	**216/289**	**211,236**
Catharanthine	**129/181**	**160,197**

Result and Discussion:

These results show the variation and difference from the effect of different concentrations of hormones on tissue formation, as well as the effect of subcultureson the size and purity of the callus.

Conclusion:

The Vinca rosea plant contain indole alkaloids that have medical applications.High performance liquid chromatography was used with tandem mass spectrometry to determine Tryptophan, Ajmalicine, serpentine, vindoline, catharanthine, and Strictosidine. Overall, vinca alkaloids have the second most-used class of cancer drugs and will stay among the original cancer therapies. Different research and studies for new vinca alkaloid applications will be carried out in this regard. So, weconclude from this research that the different concentrations of growth hormones affect the differentiation and formation of callus Where it was found that the use of hormonal BA and 2,4-D is what gives the highest efficiency and vitality of the resulting callus.

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