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# **PRELIMINARY STUDIES TOWARDS THE PRODUCTION OF THE INSECTICIDE ROTENONE USING CELL CULTURE**

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## **ABSTRACT**

Detection and quantification of rotenone in the different organs of the plant Pachyrhizus erosus (l) Urban, was achieved by High Pressure Liquid Chromatography (HPLC) using a reverse-phase column. The seeds were found to contain 0.15% rotenone on a dry weight basis, while the tubers, leaves, and the pod shells contained negligible amounts. In attempting to induce high rotenone production in vitro, leaf discs and cotyledon sections of P. erosus were used as explant and these placed on a supplement Murashige and Skoog medium. Cultures are being grown under varying conditions of light requirement, sugar source, and hormone requirement in order to determine the optimum conditions for rotenone production.

## **INTRODUCTION**

The high residual levels of pesticides found in our food and drinking water present severe problems. It is necessary to reduce the amount of chemicals used, but it is also worthwhile to look at a non-persistent insecticide such as rotenone.

Rotenone is a naturally occurring substance, which for centuries was used as a fish poison. In recent years it has been found to be an effective insecticide as well. The traditional method of obtaining this compound was to extract it from field-grown plants. This is an expensive method which makes rotenone uncompetitive with synthetic insecticides.

Two alternatives to producing rotenone are chemical syntehsis and production of rotenone via cell culture. In the first case Jackson (1983) synthesized rotenone, but with great difficulty and only in small amounts. It is the second method which will be attempted here.

In attempting to produce rotenone using cell culture the following was required:

- select an available plant which is known to produce rotenone.
- analyze the different organs of the plant to quantify the amount of rotenone present in each.
- determine the medium composition which will stimulate rotenone production in cell culture.
- attempt to maximize rotenone production by using precursors in the medium.

## MATERIALS AND METHODS

The concentration of rotenone in the different organs of Pachyrhizus erosus was determined. This was done by drying, grinding, extracting with acetone, and partitioning between petroleum-ether and methanol-water. The methanol-water was further partitioned to obtain a chloroform extract, which was then analyzed using High Pressure Liquid Chromatography (HPLC). Separation of the components of the chloroform fraction was achieved with the HPLC using a methanol-water (58:42) solvent, at 2 ml/min, and using a wavelength of 280nm. Quantification of the rotenone content was determined using a standard solution of pure rotenone.

Tissue culture experiments were set up using different media compositions. All explants were placed on supplemented Murashige and Skoog media with the following variations:

- Sugar source : glucose  
sucrose  
fructose
- Type of auxin : 2,4-D  
NAA  
IAA
- Plant tissue used as explant: leaf  
cotyledon
- Light requirement:  
callus grown in dark  
callus grown in 16 hr light/8 hr dark regime  
callus initiated in the dark then placed in 16 hr  
light/8 hr dark regime

This initial study is aimed at determining the optimal conditions for high rotenone production in cell culture.

The resulting calli then dried and extracted as was done with the plant organs, and the rotenone content of each callus found using HPLC.

## RESULTS AND DISCUSSION

As shown in table 1 the rotenone concentrations in the plant organs are low compared to those reported by Duke (1981). Because no reference was given, the methods used in determination of these values are not known. However, Schroeder (1968) stated the concentration of rotenone usually ranged from 0.12 to 0.43 percent of the seed, and the value determined here is in agreement with his findings.

Table 1. Comparison of rotenone concentration of the different organs of P. erosus as reported by Duke (1981) and that evaluated in this study.

Organ	% Rotenone on dry wt basis	
	Reported	Evaluated
Seed	0.66	0.15
Leaf	0.11	0
Pod Shell	0.02	0
Tuber	0	0
Stem	0.03	(not yet determined)

The calli from the cell culture experiment are still being analyzed. However, the presence of rooting in some of the calli is an indication of organogenesis. It is promising since the literature indicates that differentiation usually implies the production of secondary metabolites.