

## ACKNOWLEDGMENTS

**Investigations on the *in vitro* Production of Rotenone using  
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This study investigated *in vivo* and *in vitro* production of rotenone (% dry matter) using *Pachyrhizus erosus*. Quantitation of rotenone was achieved using high pressure liquid chromatography (HPLC). Analysis of the organs of *P. erosus* showed that seed contained the highest rotenone content (0.184%). The cotyledon of the seed contained 97% of the total quantity of rotenone while the testa contained the remaining 3%. Leaf (0.029%) and podshell (0.003%) contained less rotenone while no rotenone was detected in tuber and stem. Rotenone content of seeds of *Pachyrhizus* species were also determined and ranged from 0.019% in *P. tuberosus* to 0.426% in EC104 (a variety of *P. erosus*).

Plant material from *P. erosus* was used for attempting *in vitro* production of rotenone. Four parameters were varied- sources of explant, auxin and carbon as well as light regime to determine the effect on callus and rotenone production. Maximum callus production (939 mg dry matter) was obtained with cotyledonary-derived callus grown in 2,4-D and glucose containing medium. The explant was initiated in the dark and then placed in a 16 hour photoperiod. Maximum rotenone content (0.02%) was obtained from cotyledonary-derived calli grown in IAA-containing medium. Carbon source and light regime were not significant in stimulating rotenone production.

The results of this study showed that rotenone content in the seed of the plant was much higher than that produced under *in vitro* conditions. To enhance rotenone production *in vitro*, it is necessary to optimize the conditions under which the highest rotenone content was found. The establishment of high rotenone-producing cell lines may further enhance the *in vitro* production of rotenone.