

การสังเคราะห์ไพรีทรินจากเซลล์เพาะเลี้ยงของไพรีทรัม
(*Chrysanthemum cinerariaefolium* Bocc.)



นางสาวอภิตา เวชประสิทธิ์

ศูนย์วิทยทรัพยากร

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SYNTHESIS OF PYRETHRINS IN TISSUE CULTURE OF PYRETHRUM
(*CHRYSANTHEMUM CINERARIAEFOLIUM* BOCC.)



Miss Apitar Vesprasit

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อภิธานศัพท์ : การสังเคราะห์ไพเรทรินจากเซลล์เพาะเลี้ยงของไพเรทรัม

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สารไพเรทรินซึ่งสกัดได้จากพืชไพเรทรัม (Chrysanthemum cinerariaefolium Bocc.) พบว่ามีคุณสมบัติในการฆ่าแมลง ดังนั้นจึงได้มีการศึกษาการสังเคราะห์สารไพเรทรินจากพืชไพเรทรัมที่ได้จากการเพาะเลี้ยงเนื้อเยื่อโดยนำชิ้นส่วนต่าง ๆ (ก้านใบ ใบ และส่วนลำต้น) ของพืชมาเพาะเลี้ยงภายใต้สภาวะแวดล้อมและอาหารสูตรต่าง ๆ ซึ่งมีผลต่อการเจริญเติบโตและการผลิตสารไพเรทริน นอกจากนี้ได้พัฒนาวิธีสกัดและวิเคราะห์สารไพเรทรินที่เหมาะสม เพื่อให้ได้ผลผลิตสารไพเรทรินในปริมาณมากที่สุด และพบว่าเนื้อเยื่อใบให้ผลตอบสนองต่อภาวะของการเพาะเลี้ยงเนื้อเยื่อสูงสุด และการชักนำให้เกิดแคลลัสของพืชไพเรทรัมสูงสุดเมื่อเพาะเลี้ยงชิ้นส่วนของพืชในอาหารสูตร MS เต็มสูตรมาตรฐานเสริมด้วย 2,4-D และ BA (ความเข้มข้น 1.0 และ 3.0 มิลลิกรัม/ลิตร ตามลำดับ) ที่ความเข้มแสงฟลูออเรสเซนต์ 2,000 ลักซ์ และอุณหภูมิ 25 ± 2 °C อย่างไรก็ตามจากการศึกษาผลของสารควบคุมการเจริญเติบโต ต่อการเจริญเซลล์พบว่า IAA และ BA (ความเข้มข้น 1.0 และ 3.0 มิลลิกรัม/ลิตร ตามลำดับ) ให้ผลผลิตสารไพเรทรินจากเซลล์เพาะเลี้ยงมากกว่าที่เลี้ยงในอาหารที่เสริมด้วย 2,4-D และ BA

การสกัดสารไพเรทรินใช้วิธี Soxhlet ที่อุณหภูมิ 60 °C โดยใช้ปิโตรเลียมอีเทอร์เป็นตัวสกัด ซึ่งเป็นวิธีที่สามารถสกัดสารไพเรทรินได้สูงสุด และการศึกษาทางคุณภาพวิเคราะห์ใช้วิธี Thin-layer chromatography โดยใช้ n-hexane:n-heptane:ethylacetate ในอัตราส่วน 40:48:12 เป็นตัวพา สามารถแยกองค์ประกอบทั้ง 6 ชนิดของสารไพเรทรินออกจากกันได้ดีที่สุด การศึกษาปริมาณและองค์ประกอบของสารไพเรทรินโดยละเอียดใช้วิธี gas chromatography และ high performance liquid chromatography โดยใช้ methylstearate เป็นสารมาตรฐาน

จากการศึกษานี้สรุปได้ว่า หากใช้อาหาร และสภาพเพาะเลี้ยงที่เหมาะสมแล้ว ทำให้สามารถเจริญเซลล์และสังเคราะห์สารไพเรทรินจากไพเรทรัมที่ได้จากการเพาะเลี้ยงเนื้อเยื่อได้อย่างมีประสิทธิภาพ

ภาควิชาเทคโนโลยีชีวภาพ.....

สาขาวิชาเทคโนโลยีชีวภาพ.....

ปีการศึกษา 2532

ลายมือชื่อนักศึกษา

ลายมือชื่ออาจารย์ที่ปรึกษา



APITAR VESPRASIT : SYNTHESIS OF PYRETHRINS IN TISSUE CULTURE OF
PYRETHRUM (*CHRYSANTHEMUM CINERARIAEFOLIUM* BOCC.). THESIS
ADVISOR : ASSO. PROF. SANHA PANICHAJAKUL, PH.D. AND ASSO. PROF.
ORADEE SAHAVACHARIN, PH.D., 147 PP. ISBN 974-577-786-2

Pyrethrins extracted from pyrethrum (*Chrysanthemum cinerariaefolium* Bocc.) has been found to possess an active insecticidal property. Through this property an attempt to synthesize pyrethrins in tissue culture of pyrethrum was carried out. Explants of three organ sources (petiole, leaf and stem piece) were cultured under varying influential factors on the growth and production of pyrethrins such as the medium constituents, and the environmental conditions. In addition, to maximize the pyrethrins extracted from pyrethrum grew under those conditions, the suitable extraction and analysis procedures were established. Through this study it was found that leaf tissue gave the highest response to those treated conditions and the maximum pyrethrum callus initiation could be achieved when the explants were grown on full strength MS medium supplemented with 2,4-D, BA (1.0:3.0 mg/ml) under 2,000 lux fluorescent illumination and maintained at the temperature of $25 \pm 2^\circ\text{C}$. However, it was found that the effect of growth regulators, IAA and BA recombined at a ratio of 1.0 to 3.0 mg/ml was more sustained on the pyrethrins production in culture cells than the 2,4-D and BA recombined at the ratio 1.0 to 3.0 mg/ml respectively.

Maximum yield of pyrethrum extract could be established when petroleum ether was employed as an extracting solvent in the Soxhlet apparatus at performing temperature of 60°C for at least 7 hours. The TLC qualitative analysis developed by n-hexane-n-heptane-ethylacetate (40:48:12) comprised the good separation of all six compounds. GC and HPLC analysis of the culture cells revealed to facilitate for more separation of pyrethrins constituents. It can be concluded that with appropriate media constituents and environmental culture conditions, an efficient synthesis of pyrethrins in tissue culture of pyrethrum can be achieved.

ภาควิชา เทคโนโลยีชีวภาพ
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ABBREVIATION



AcOH	=	acetic acid
BA	=	Benzyladenine
p-BAP	=	p-benzylaminopurine
°C	=	degree celcius
ca.	=	circa (approximately)
cm	=	centimeter
2,4-D	=	2,4-dichlorophenoxyacetic acid
EtOAc	=	ethylacetate
EtOH	=	ethanol
et al.	=	et alli (and others)
g	=	gram
Glu	=	glucose
GC	=	gas chromatography
GI	=	growth index
hr	=	hour
hg	=	hugnur (mercury)
HPLC	=	high performance liquid chromatography
H ₂	=	hydrogen gas
IAA	=	indoleacetic acid
kg	=	kilogram
l	=	liter
M	=	molarity
MS medium	=	Murashige and Skoog's medium
MeOH	=	methanol
μl	=	microliter
mg	=	milligram

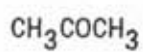
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min.	=	minute
ml	=	milliliter
mm	=	millimeter
m.p.	=	melting point
NAA	=	α -naphthaleneacetic acid
N ₂	=	nitrogen gas
nm	=	nanometer
no.	=	number
P	=	page
ppm	=	parts per million
rpm	=	revolutions per minute
RT medium	=	revised tobacco medium
wt	=	weight
v	=	volume



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CHEMICAL FORMULA



= acetone



= benzene



= chloroform



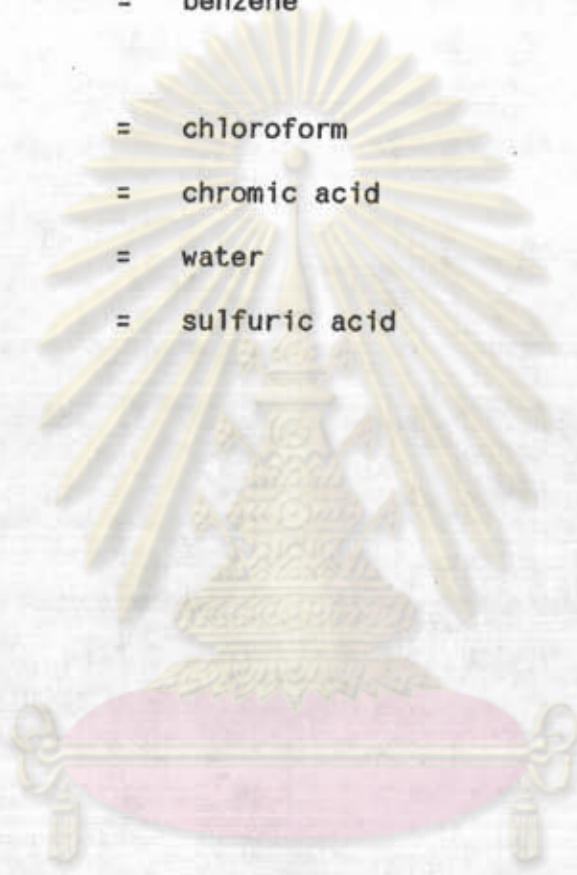
= chromic acid



= water



= sulfuric acid



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