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Research Article

SALICYLIC ACID ELICITATION ON PRODUCTION OF SECONDARY METABOLITE BY CELL CULTURES OF *JATROPHA CURCAS* L.

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ABSTRACT

Objective: *Jatropha curcas* (Euphorbiaceae) is native of Latin America origin and widely distributed throughout tropical and sub tropical regions. The present work was carried out to identify the chemical composition in the presence of various concentration of salicyclic acid by GC-MS analysis.

Methods: *In vitro* callus was treated with 200, 300, 400, 500 µM of salicyclic acid in Murashige and Skoog (MS) medium under *in vitro* condition. Ethyl acetate extract of control and treated callus was analysed for their chemical constituents using Gas Chromatography-Mass Spectroscopy (GC-MS).

Results: The major constituents obtained from control and salicylic acid treatments are 1-docosene, 1-octadecene, 1-hexadecene, (E)-3-eicosene, (E)-5-eicosene, 1-hexacene, nonahexacontaoic acid and methyl ester-3-oxocyclohexane carboxylic acid. Salicylic acid treatment altered the chemical content and increased percentage of compounds in all treatments, and also resulted in the production of higher percentage of alkanes and fatty acid.

Conclusion: This study highlights production of secondary metabolites using salicylic acid elicitation. Hence, production of higher level of alkanes and fatty acids may be utilized for pharmaceutical uses.

Keywords: *Jatropha curcas*, Salicyclic acid, Elicitors, 1-docosene, Alkanes, Fatty acids.

INTRODUCTION

Jatropha curcas commonly known as physic nut belongs to Euphorbiaceae family and grows in tropical and subtropical regions in Central and South America, Africa, India and South East Asia [1]. Seeds of *Jatropha curcas* is a potential source of oil for the production of biofuel [2]. This species have been reported to possess various biological properties that include antioxidant, anticancer, anti-inflammatory, antimicrobial [3-5], anti-tumuor, cytotoxicity [6,7], anti-HIV, antimalaria [8].

Production of secondary metabolites through *in vitro* methods is useful for large-scale exploitation in agrochemical and pharmaceutical industries. Saw et al. [9] reported that plant cell suspension culture is being utilized for higher yield and increased quality products than whole plants. Elicitation can be applied to increase the yields of secondary metabolites in plant cell cultures [10,11] and increase the accumulation of umbelliferone [12]. Salicylic acid resulted in two fold increase in Trigonelline production in *Trigonella foenum* [13]. Salicylic acid treatments resulted in hyoscyamine and scopolamine in *Datura metel* root culture increased 3.5 and 4 times more than control [14], capsaicin content increased to 1.5 fold in the cells compared to control [15] and enhanced the azadirachtin content up to 6-9 folds as compared to control cultures [16].

The present study attempted *in vitro* production of secondary metabolites using various concentration of salicylic acid elicitation from *Jatropha curcas*.

MATERIALS AND METHODS

Preparation of explants

Seed material was collected from a single tree (MSSRF-0019) at *Jatropha* Genetic Garden in Kudankulam, Tirunelveli District. Seeds were soaked in double distilled water (DDH_2O) over night and treated with *Tricoderma viride* before sowing in plots. Plots were irrigated at two days intervals up to 45 days. Petiole explants were obtained from 45 days old seedling and kept under running water for 20 min and 2 drops of Tween-20 added to it and soaked for 10 min. Explants were washed with DDH_2O for four times, followed by washing sodium hypochlorite (0.1%) for 5 min and once again with DDH_2O for two times. Later the explants were washed with 70% alcohol for 3 min and three times with DDH_2O, rinsed with mercuric chloride (0.1%) for 3 min and again washed five times with DDH_2O.

Inoculation and culture condition

Surface sterilized petiole were cut into small pieces and inoculated in MS medium [17] containing NAA (0.5 mg/l) and KN (0.1 mg/l) and the culture kept under dark condition at 27±1°C for 60 days. Callus induction obtained from petiole after 15 days were transferred to MS medium supplemented with NAA (0.5 mg/l) and KN (0.1 mg/l). The developed callus was transferred to a flask in MS medium supplemented NAA (0.5 mg/l) and Kn (0.1 mg/l) with various concentration of salicylic acid (200, 300, 400 and 500 µM). Cell suspension culture was kept in a rotary shaker at 100 rpm for 15 days under dark condition maintained at 27±1°C. The control and treated callus were removed from culture and dried under laminar flow without light. Dried callus were grounded and transferred to a soxhlet apparatus ethyl acetate added to it and boiled at 65°C for 3 h. The ethyl acetate containing each extract were separately transferred to a rotary flask and boiled at 65°C. Each extract was stored in glass screw amber cap bottle and stored at 4°C refrigerator until further use.

Gas Chromatography-Mass Spectrometry analysis

GC-MS analysis were conducted using Agilent MSD (5975B-inert XL MSD) apparatus equipped with reference NIST libraries (National Institute of Standards and technology); column DB-5MS (J&W Scientific) cross-linked fused-silica capillary column (30 m × 0.25 mm × 0.25 µm thickness), coated with 5% phenyl-polymethylsiloxane; column temperature, 80°C for 0 min, rising to 150°C at 10°C/min, then 250°C at 5°C/min, then rising to 270°C at 20°C held for 6 min. injector temperature 270°C, injection mode, split; split ratio 1:20; volume injected, 2µl of the oil. Helium was used as a carrier gas; interface temperature 270°C; acquisition mass range, m/z 55-550. The compounds of the extracts were identified by comparing their retention indices with NIST library.

RESULTS AND DISCUSSION

Ethyl acetate extract of salicylic acid treated callus were analyzed using GC-MS, which showed total number of compounds in control (28), 200 µM (23), 300 µM (20), 400 µM (18) and 500 µM (13) salicylic acid treatments and presented in Table 1. The major compounds present in

control are 1-Docosene (18.80%), 1-Hexadecene(13.38%), 1-Octadecene (13.25 %) and other compounds (Figure 1). 200 µM salicylic acid are 1-Octadecene (13.20%), 1-Hexadecene (12.77%), (E)-3-Eicosene (12.45%) and other compounds as shown in GC-MS chromatogram (Figure 2). 300 µM salicylic acid are 1-Docosene (20.97 %), 1-Octadecene (12.37%), 1-Hexadecene (11.99%) and other compounds as shown in Figure 3. 400 µM salicylic acid are 1-Docosene (16.00%), Methyl ester-3-oxocyclohexanecarboxylic acid (13.29%), (E)-3-Eicosene (12.99%) and other compounds obtained from GC-MS chromatogram as shown in Figure 4. 500 µM salicylic acid are 1-Octadecene (16.19 %), (E)-5-Eicosene (15.36 %), 1-Docosene (14.71%) as shown in GC-MS chromatogram (Figure 5).

Table 1: GC-MS analysis of *in vitro Jatropha curcas* callus elicitated with salicylic acid

S. No.	RT▲	Compounds	Control (%)	200 µM (%)	300 µM (%)	400 µM (%)	500 µM (%)
1	17.181	1-Dodecene	1.22	1.75	1.52	1.15	1.74
2	22.808	1-Tetradecene	-	7.47	-	-	-
3	22.818	3- Tetradecene	-	-	6.98	-	-
4	22.839	2-Tetradecene	10.14	-	-	5.02	-
5	22.849	5-Tetradecene	-	-	-	-	10.70
6	26.229	2,4-*bis*(1,1-Dimethylethyl) phenol	1.23	1.62	1.47	1.14	1.89
7	27.312	1-Hexadecene	13.38	12.77	11.99	8.70	13.36
8	27.536	1-Methyl-1-(1-methylethyl)-2-nonyl- cyclopropane	0.84	0.81	0.62	-	-
9	29.569	Nonahexacontanoic acid	-	0.38	-	-	-
10	30.232	1-Octadecene	13.25	13.20	12.37	10.21	16.19
11	30.396	1-Undecene	-	-	5.35	-	-
12	30.406	(E)-9-Octadecene	4.68	-	-	4.88	6.10
13	30.406	3-Tridecyl ester-3-methyl-2-butenoic acid	-	8.40	-	-	-
14	30.927	6,10-Dimethyl-2-undecanone	0.53	-	-	-	-
15	32.346	Dibutyl phthalate	1.10	1.09	0.85	0.62	1.18
16	32.489	(E)-3-Eicosene	12.69	12.45	-	12.99	-
17	32.489	(E)-5-Eicosene	-	-	11.60	-	15.36
18	33.541	Propyl tetradecyl ester oxalic acid	0.47	-	-	-	-
19	34.328	Cyclopentadecane	0.61	-	-	-	-
20	34.430	1-Docosene	18.80	9.89	20.97	16.00	14.71
21	34.563	Methyl ester-3-oxocyclohexanecarboxylic acid	-	-	-	13.29	-
22	35.339	Heptadecane	0.82	-	-	-	-
23	35.400	Z,E-3,13-octadecadien-1-ol	-	0.71	-	-	-
24	35.492	1,2-Diethyl cyclohexadecane	0.78	1.01	0.43	-	3.35
25	35.635	(Z)-9,17-Octadecadienal	-	1.49	-	-	-
26	35.645	12-Methyl-E, E-2,13-Octadecadien-1-ol	-	-	-	-	0.07
27	35.880	2-methyl-Z,Z-3,13-octadecadienol	0.42	1.56	-	3.07	-
28	35.911	Cycloeicosane	-	1.16	-	-	-
29	35.921	Methyl dehydroabietate	0.87	-	-	-	-
30	36.054	Cyclotetracosane	-	8.31	7.71	7.18	9.38
31	36.156	3-(Hexahydro-1H-azepin-1-yl)-,1,1-dioxide-1,2-benzisothiazole	0.97	-	1.42	0.63	-
32	36.156	7-Pentadecyne	-	-	-	1.42	-
33	36.156	1-Ethenyl cyclododecanol	0.37	-	-	-	-
34	36.442	(Z,Z)-9,12-Octadecadienoic acid	-	4.10	1.50	-	-
35	36.483	2-Heptadecenal	1.09	-	-	-	-
36	36.534	11-Dodecen-1-ol trifluoroacetate	-	-	2.08	-	-
37	36.646	Z,Z-10,12-Hexadecadien-1-ol acetate	-	1.76	1.01	-	-
38	36.646	(E)-9-Octadecenoic acid	-	-	-	0.70	-
39	36.718	Eicosane	1.86	-	-	-	-
40	36.748	Oleic acid	-	-	1.47	3.04	-
41	36.748	Methyl ester -11,14-eicosadienoic acid	-	1.50	-	-	-
42	36.789	*cis*-11-Hexadecenal	-	0.71	-	-	-
43	36.901	(E,E)-9,12-Octadecadienoic acid, methyl ester	0.20	-	1.74	-	-
44	36.942	1-Nonadecene	0.48	-	-	-	-
45	37.044	Stigmastan-3,5-diene	-	3.18	-	-	-
46	37.055	1-(Ethenyloxy)- octadecane	0.37	-	-	-	-
47	37.147	Diis ooctyl ester 1,2-benzenedicarboxylic acid	2.21	-	-	-	-
48	37.238	Homopterocarpin	1.76	-	-	-	-
49	37.361	1-Eicosene	2.81	4.68	4.23	6.18	-
50	37.361	9-Hexacosene	-	-	4.67	3.78	5.96
51	37.361	1-Hexacosene	6.05	-	-	-	-
		Total %	100	100	99.98	100	99.99

RT▲- Retention time

Table 2: Constituents present in control and salicylic acid treated callus

	Control (%)	200 µM (%)	300 µM (%)	400 µM (%)	500 µM (%)
Alkenes	83.5	65.39	79.68	68.91	84.12
Alkanes	5.28	11.29	8.76	-	-
Fatty acid	2.88	16.14	7.8	3.74	-
Others	8.34	7.18	3.76	27.35	15.88

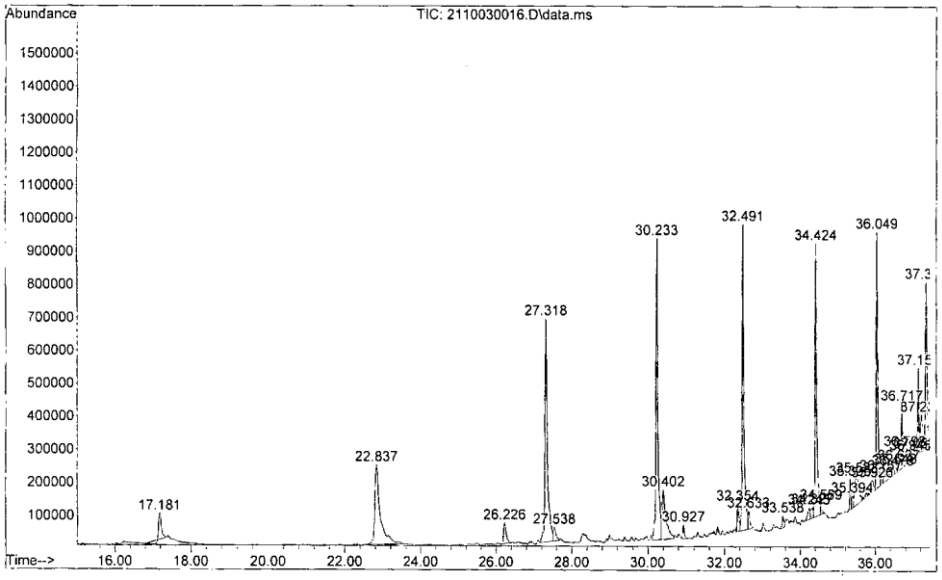

Fig. 1: GC-MS chromatogram of ethyl acetate extract of *in vitro* control callus

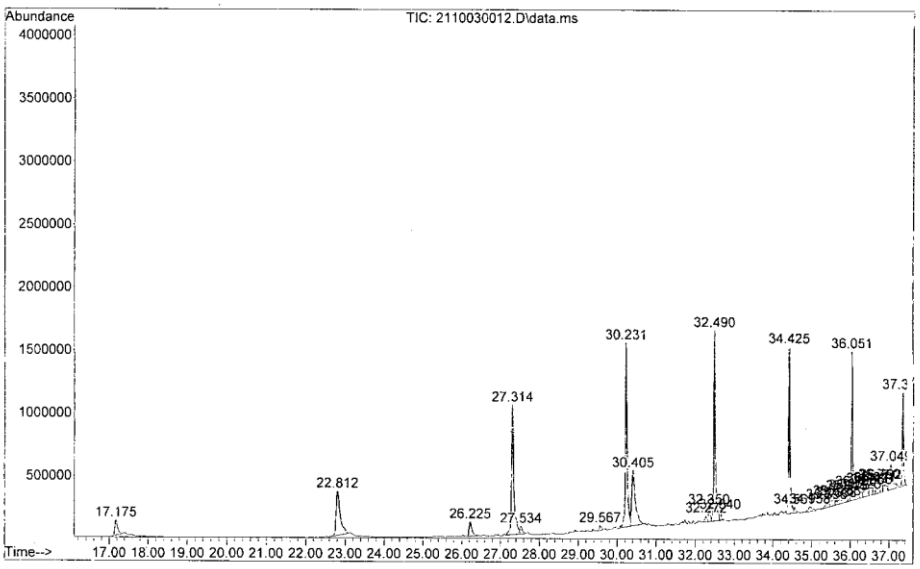

Fig. 2: GC-MS chromatogram of ethyl acetate extract of *in vitro* callus elicitated with 200 µM salicylic acid

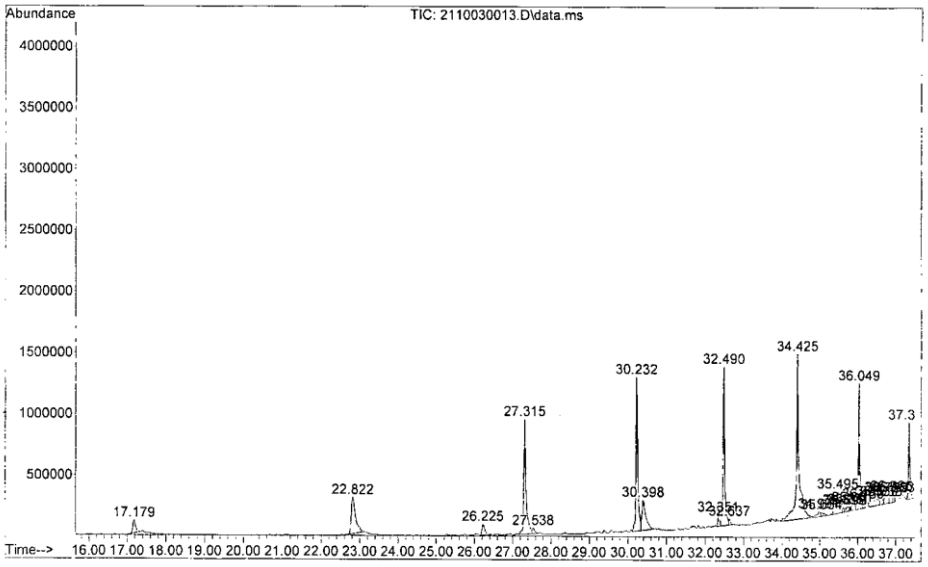

Fig. 3: GC-MS chromatogram of ethyl acetate extract of *in vitro* callus elicitated with 300 µM salicylic acid

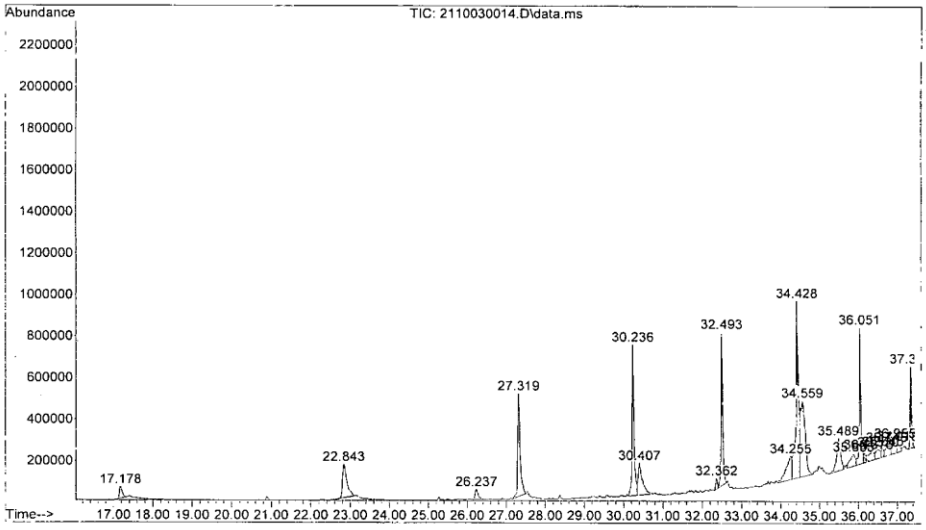

Fig. 4: GC-MS chromatogram of ethyl acetate extract of *in vitro* callus elicitated with 400 µM salicylic acid

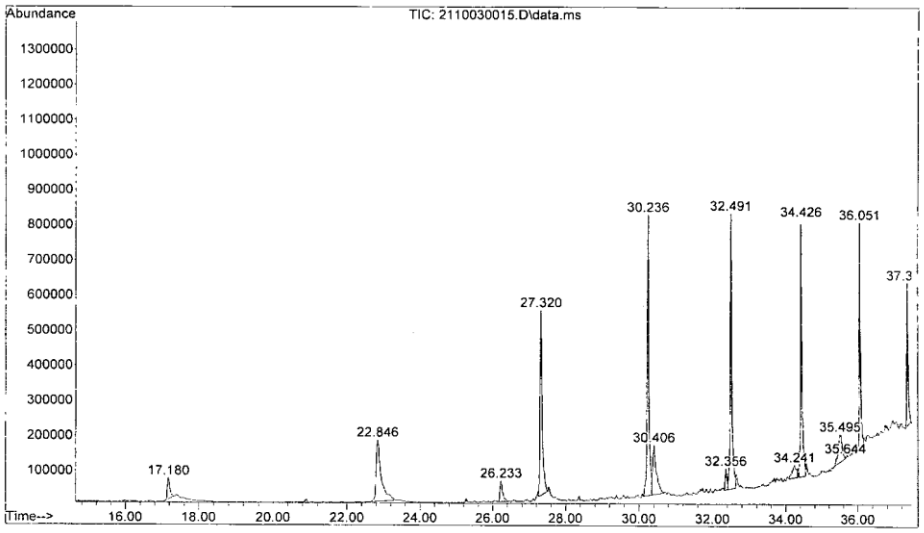

Fig. 5: GC-MS chromatogram of ethyl acetate extract of *in vitro* callus elicitated with 500 µM salicylic acid

The total number of compounds only present in control (13), 200 µM (9), 300 µM (4), 400 µM (3) and 500 µM (2) salicylic acids treatments. The compounds present only in control are 6,10-dimethyl-2-undecanone, propyl tetradecyl ester oxalic acid, heptadecane, methyl dehydroabietate, 1-ethenyl cyclododecanol, 2-heptadecenal, eicosane, 1-nonadecene, 1-(ethenyloxy)-octadecane, diis ooctyl ester 1,2-benzenedicarboxylic acid, homopterocarpin and 1-hexacosene. Compounds present only in 200 µM salicylic acid treatment are 1-tetradecene, nonahexacontanoic acid, 3-tridecyl ester-3-methyl-2-butenoic acid, Z,E-3,13-octadecadien-1-ol, (Z)-9,17-octadecadienal, cycloeicosane, methyl ester-11,14-eicosadienoic acid, *cis*-11-hexadecenal and stigmastan-3,5-diene. The following compounds were present only in salicylic acid treatment (300 µM) are 3- tetradecene, 1-undecene and 11-dodecen-1-ol trifluoroacetate. The compounds present only in 400 µM salicylic acid treatment are methyl ester-3-oxocyclohexanecarboxylic acid, 7-pentadecyne and (E)-9-octadecenoic acid. salicylic acid (500 µM) treatment only produced 5-tetradecene and 12-methyl-E, E-2,13-octadecadien-1-ol.

Six compounds common in control and salicylic acid treatments are 1-dodecene, 2,4-bis(1,1-dimethylethyl) phenol, 1-hexadecene, 1-octadecene, dibutyl phthalate and 1-docosene. Homopterocarpin and cyclotetracosane compounds disappeared in salicylic acid treatments. 1-eicosene was absent in higher concentration of salicylic acid (500 µM) treatment but present in all salicylic acid treatments and control. 1-methyl-1-(1-methylethyl)-2-nonyl-cyclopropane was present in control, 200 µM salicylic acid, 300 µM salicylic acid and absent in 400 and 500µM salicylic acid. 1,2-diethyl cyclohexadecane were present in control, 200, 300, 500 µM salicylic acid not present in 400 µM salicylic acid. 1,2-diethyl cyclohexadecane and (E)-3-eicosene was present in control, salicylic acid (200, 400 µM). Cyclotetracosane was present only in salicylic acid treatment and absent in control. Major compounds in salicylic acid treatments are 1-docosene, 1-octadecene, (E)-5-eicosene and 1-hexadecene, which is reported to be used as pheromone against *Aphis gossypii* [18] and also exhibit antibacterial activity [19].

Alkenes content decreased at lower concentration of salicylic acid treatment, but increased at higher concentration. Alkanes and fatty acid contents increased with increasing concentration of salicylic acid but at higher concentration (500 µM) production of alkanes and fatty acid content was not found (Table 2).

Alkenes production decreased at lower concentration but increased at higher concentration of SA 500 µM. Alkenes are widely used in the petroleum industry [20], as chemical markers for estimation of diet composition in herbivores [21,22]. Stigmastan-3,5-diene was newly synthesized from 200 µM SA treatment and is a botanical steroid, exhibiting fibrinolytic and antiflammatory activities [23]. 9-hexacosene was newly synthesized in SA treatments ranges between 3.78-5.96% and is reported to express analgesic and anti-inflammatory activities [24].

Alkanes production increased to higher level at 200 µM SA followed by 300 µM SA treatments compared to control. Increasing concentration of SA led to absence of production of alkanes. Cyclotetracosane was newly synthesized, increased with increasing salicylic acid concentration ranging from 7.18 to 9.38%. Alkanes are important for the manufacture of plastics, paints, insecticides, cosmetics, detergents [25].

Higher production of fatty acids was seen at lower concentration of SA 200 µM followed by 300 µM and 400 µM treatments. Fatty acids are utilized in the manufacture of fuel, lubricating oil, polishes, textile chemical, insecticides, germicides, paper, candles, inks, metalloic soaps and food products [26]. Oleic acid, synthesized at 200 µM (1.47%) and 300 µM (3.04%) of salicylic acid elicitation is reported to be a major component of soap as an emulsifying agent, emollient [27], and also used in pharmaceuticals, solubilising agent in aerosol products [28].

CONCLUSION

The use of elicitors induced compound from plants has wide application in crop production and pest management. The present experiment achieved production of secondary metabolites from *in vitro* callus of *Jatropha curcas* using salicylic acid elicitation and has important industrial applications.

Abbreviations

Murashige and Skoog medium [MS], double distilled water [DDH_2O], naphthaleneacetic acid [NAA], kinetin [KN], micromolar [µM], gas chromatography-mass spectrometry [GC-MS], retention time [RT], salicylic acid [SA]

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REFERENCES

1. Makkar HP, Becker K. *Jatropha curcas*, a promising crop for the generation of biodiesel and value-added coproducts. Europ J Lipid Sci Technol 2009; 111(8): 773-87.
2. Oskoueian E, Abdullah N, Saad WZ, Omar AR, Ahmad S, Kuan, WB, Zolkifli NA, Hendra R, Ho YW. Antioxidant, anti-inflammatory and anticancer activities of methanolic extracts from *Jatropha curcas* Linn. J Med Plants Res 2011; 5(1): 49-57.
3. Gupta MB, Nath R, Srivastava N, Shanker K, Kishor K, Bhargava, KP. Anti-inflammatory and antipyretic activities of β-sitosterol. Planta Medica 1980; 39(2): 157-63.
4. Awad AB, Chen YC, Fink CS, Hennessey T. Sitosterol inhibits HT-29 human colon cancer cell growth and alters membrane lipids. Anticancer Res 1996; 16(5A): 2797-804.
5. Ajaiyeoba EO, Onocha PA, Nwozo SO, Sama W. Antimicrobial and cytotoxicity evaluation of *Buchholzia coriacea* stem bark. Fitoterapia 2003; 74(7-8): 706-9.
6. Sparg SG, Light ME, Van Staden J. Biological activities and distribution of plant saponins. J Ethnopharmacol 2004; 94(2): 219-43.
7. Makkar HPS, Siddhuraju P, Becker K. In: Methods in molecular biology: Plant secondary metabolites. Totowa: Humana press; 2007. p. 93-100.
8. Goel G, Makkar HPS, Francis G, Becker K. Phorbol esters: structure, biological activity, and toxicity in animals. Inter J Toxicol 2007; 26(4): 279-88.
9. Saw NMMT, Reidel H, Kutuk O, Ravichandran K, Smetanska I. Effect of elicitors and precursors on the synthesis of anthocyanin in grape *Vitis vinifera* cell cultures. Energy Res J 2010; 1(2):189-92.
10. Kumar A, Sopory, S. Recent advances in plant biotechnology and its applications. New Delhi: I. K. International; 2010. 718 p.
11. Ghorbanpour M, Hatami M, Khavazi K. Role of plant growth promoting rhizobacteria on antioxidant enzyme activities and tropane of *Hyoscyamus niger* under water deficit stress. Turkish J Biol 2013; 37(3): 350-60.
12. Hussain MS, Fareed S, Saba Ansari M, Rahman A, Ahmad IZ, Saeed, M. Current approaches toward production of secondary plant metabolites. J Pharm Bioallied Sci 2012; 4(1):10-20.
13. Mathur L, Yadav RK. Effect of salicyclic acid on trigonelline production in *Trigonella foenum-graecum* L cell suspension culture. Inter Referred Res J 2011; 1(17): 137-38.
14. Ajungla L, Patil PP, Barmukh RB, Nikam TD. Influence of biotic and abiotic on accumulation of hyoscyamine and scopolamine in root cultures of *Datura metel* L. Indian J Biotechnol 2009; 8(3): 317-22.
15. Sudha G, Ravishankar GA. Influence of methyl jasmonate and salicylic acid in the enhancement of capsaicin production in cell suspension cultures of *Capsicum frutescens* Mill. Curr Sci 2003; 85(8): 1212-17.
16. Satdive RK, Fulzele DP, Eapen S. Enhanced production of azadirachtin by hairy root cultures of *Azadirachta indica* A. Juss by elicitation and media optimization. J Biotechnol 2007; 128(2): 281-89.
17. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant 1962; 15(3): 473-97.
18. Li YY, Zhou XR, Pang BP, Chang J. Effects of hosts plants on the life table parameters of experimental populations of *Aphis gossypii*. Chinese J Biotech 2013; 24(5): 1435-38.
19. Yogeswari S, Ramalakshmi S, Neelavathy R, Muthumary J. Identification and comparative studies of different voltatile fractions from *Monochaetia kansensis* by GC-MS. Global J Pharmacol 2012; 6 (2): 65-71.
20. Moss GP, Smith PAS, Tavernier D. Glossary of class names of organic compounds and reactive intermediates based on structure (IUPAC Recommendations. Pure Appl Chem 1995; 67(8): 1307-75.
21. Barcia P, Bugalho MN, Campagnolo ML, Cerdeira JO. Using n-alkanes to estimate diet composition of herbivores. A novel mathematical approach. Animal-Cambridge University Press 2007; 1(1): 141-49.
22. Dove H, Mayes RW. The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: A review. Aust J Agri Res 1991; 42(6): 913-52.
23. Zhang YQ. Studies on acaricidal action mechanism and bioguided isolation of bioactive substance from *Artemisia annua* L. Chongqing Southwest University, PhD Dissertation, China. 2008.
24. Githinji CG, Mbugua PMM, Kanui TI, Kariuki DK. Analgesic and anti-inflammatory activities of alpha-hexacosene and stigmasterol isolated from *Mondia whytei*. Phytopharmacol 2012; 2(1): 212-23.
25. Shipman C, Tong SL, Smith SH, Katlama NB, Drach JC. Establishment of a murine cell line resistant to arabinosyladenine and devoid of adenosine deaminase activity. Antimicro Agents and Chemother 1983; 24(6): 947-49.
26. Ruston NA. Commercial use of fatty acids. J Ameri Oil Chem Soc 1952; 29(11): 495-98.
27. Carrasco F. Ingredientes Cosmeticos. Diccionario de Ingredientes, 4th ed. Malaga; 2009. 428 p.
28. Smolinske SC. Handbook of food, drug, and cosmetic excipients. CRC Press INC; 1992. 439 p.