V. SIMPULAN DAN SARAN

A. Simpulan

1. Induksi kalus terbaik dihasilkan dari eksplan hipokotil cabai rawit putih yang ditanam pada medium 1 MS, walaupun untuk penghasilan capsaicin terbaik ditemukan pada medium 1½ MS.
2. Induksi kalus terbaik dihasilkan dari eksplan hipokotil cabai rawit putih yang ditanam pada medium dengan penambahan IAA + BAP, sedangkan untuk penghasilan capsaicin terbaik ditemukan pada medium dengan penambahan 2,4-D + Kin dan IAA + BAP.

B. Saran

1. Perlu dilakukan subkultur sebelum terjadi *browning* (minggu ke-3) dan dilakukan secara berkala.
2. Perlu ditambahkan *antibrowing agent* (antioksidan, adsorben) atau perlakuan gelap untuk menurunkan persentase *browning*.
3. Perlu ditambahkan prekursor untuk memacu biosintesis capsaicin.
4. Sebaiknya dilakukan kultur suspensi atau kultur imobilisasi sel untuk produksi capsaicin yang lebih besar.
5. Perlu dilakukan perhitungan potensial air medium MS untuk mengetahui hubungan potensial air terhadap peningkatan capasaicin.

DAFTAR PUSTAKA

Abdullah, M. A., Ali, M., Marziah, N. H., dan Arif, A. B. 1998. Establishment of Cell Suspension Cultures of *Morinda elliptica* for The Production of Anthraquinoes. *Plant Cell Tissue and Organ Cultures*. 54:173-182.

Aghabozorgi, M. 2006. The Study of Rooting Response of Some Horticulture Plants After Inoculation with *Agrobacterium rhizogenes*. *Thesis*. Ferdowsi University of Mashhad. Iran.

Agrawal, S. Chandra, N., Kothari, S. L. (1989). Plant Regeneration in Tissue Cultures of Pepper (*Capsicum annuum* L. cv. *mathania*). *Plant Cell, Tissue, and Organ culture*. 16:47-55.

Agüera, E., Cabello, Mata, L., Molina, E., and Haba, P. 2012. Metabolic Regulation of Leaf Senescence in Sunflower (*Helianthus annuus* L.) Plants. *Senescence*. InTech. Croatia.

Anogianaki, A., Negrev, N. N., Shaik, Y. B., Castellani, M. L., Frydas, S., Vecchiet, J., Tete, S., Salini, V., Amicis, D. D., Lutiis, M. A. D., Conti, F., Caraffa, A., and Cerulli, G. 2006. Capsaicin An Irritant Anti-inflammatory Compound. *Journal of Biological Regulators and Homeostatic Agents*. 1-4.

Andaryani, S. 2010. Kajian Berbagai Konsentrasi BAP dan 2,4-D Terhadap Induksi Kalus Jarak Pagar (*Jatropha curcas* L.) Secara *In Vitro*. *Skripsi S1*. Fakultas Pertanian Universitas Sebelas Maret. Surakarta.

Astawan, M. dan Kasih, A. L. 2008. *Khasiat Warna Warni Makanan*. PT. Gramedia Pustaka Utama. Jakarta.

Audus, L. J. 1972. *Plant Growth Substances*. Barnes and Noble Books. New York.

Behar, N., Kumar, P., and Chandel, G. 2011. Effect of Explant Type, Genotype, and Plant Growth Regulators on Morphogenetic Potential of Flax (*Linum usitatissimum* L.). *Journal of Cell Plant Sciences*. 2(1):13-18.

Blum, E., Mazourek, M., O'Connell, M., Curry, J., Thorup, T., Liu, K., Jahn, M., and Paran, I. 2003. Molecular Mapping of Capsaicinoid Biosynthesis Genes and Quantitative Trait Loci Analysis for Capsaicinoid Content in Capsicum. *Theor Appl Genet*. 103:79-86.

Bhojwani, S. S. and Razdan, M. K. 1996. *Plant Tissue Culture*. Elsevier Science B.V. Netherlands.

Cahyono, B. 2003. *Cabai Rawit: Teknik Budidaya dan Analisis Usaha Tani*. Kanisius. Yogyakarta.

Cairns, D. 2004. *Intisari Kimia Farmasi*. Penerbit Buku Kedokteran EGC. Jakarta.

Campbell, N. A., Reece, J. B., dan Mitchell, L. G. 2000. *Biologi.* Jilid II. Penerbit Erlangga. Jakarta.

Caribbean Export Development Agency. 2004. Hot Pepper Project Phase I Market Research The Viability and Export Potential of The Regional Hot Pepper Industry. *Final Report*. Barbados.

Chanda, S., Bashir, M., Babbar, S., Koganti, A. and Bley, K. 2008. In Vitro Hepatic and Skin Metabolism of Capsaicin. *Drug Metabolism and Disposition*. 36(4):670-675.

Chen, L. M., Cheng, J. T., Chen, E. L., Yiu, T. J., and Liu, Z. H. 2002. Naphthaleneacetic Acid Suppress Perioxidase Activity During The Induction of Adventitious Root in Soybean Hypocotyls. *J. Plant Physiol*. 159:1349-1354.

Dalimartha, S. 2006. *Atlas Tumbuhan Obat Indonesia Jilid 2*. Trubus Agriwidya. Jakarta.

Daradkeh, N. Q., Shibli, R. A., Makhadmeh, I. M., Alali, F., and Al-Qudah, T. S. 2012. Cell Suspension and In Vitro Production of Colchicine in Wild *Colchicum hierosolymitanum* Feib. *The Open Conference Proccedings Journal*. 3:52-59.

Departemen Kesehatan RI. 2000. *Parameter Standar Umum Ekstrak Tumbuhan*. Departemen Kesehatan Republik Indonesia. Jakarta.

Drewes, F. E., and Staden, J. V. 1995. Initiation of and Solasodine Production in Hairy Root Cultures of *Solanum mauritianum* Scop. *Plant Growth Regulation*. 17:27-31.

Fujiwake, H., Suzuki, T., Iwai, K. 1982. Intracellular Distribution of Enzymes and Intermediates Involved in Biosythesis of Capsaicin and Its Analogies in *Capsicum Fruits*. *Agric. Biol. Chem*. 46:245-355.

Fukui, H., Yoshikawa, N., and Tabata, M. 1983. Induction of Shikonin Formation by Agar in *Lithospermum erythrorhizon* Cell Suspension Cultures. *Phytochemistry*. 22:2451-2453.

Fukuoka, N. and Enomoto, T. 2001. The Occurrence of Internal Browning Induced by High Soil Temperature Treatment and Its Physiological Function in *Raphanus* Root. *Plant Science*. 161(1):117-124.

Gamborg, O. L. and Shyluk, J. P. 1981. *Nutrition, Medium, and Characteristics of Plant Cell and Tissue Cultures.* Academic Press. London.

Gaspersz, K. W. 1994. *Metode Perancangan Percobaan*. Penerbit CV Armico, Bandung.

Gati, E. dan Mariska, I. 1992. Pengaruh Auksin dan Sitokinin terhadap Kalus *Mentha piperita* Linn. *Buletin Littri*. 3:1-4.

George, E. F. dan Sherrington, P. D. 1984. *Plant Propagation by Tissue Culture*. Exegenetic Limited. England.

George, E. F., Hall, M. A., and Klerk, G. J. D. 2008. Plant Propagation by Tissue Culture 3rd Edition. *http://hos.ufl.edu/sites/default/files/courses/hos6373c/february%205/media2.pdf*. 24 Agustus 2012.

Goldsworthy, A. and Mina, M. G. 1991. Electrical Patterns of Tobacco Cells in Media Containing Indole-3-Acetic Acid or 2,4-D. *Planta*. 183 : 386-373.

Groll, J., Mycock, D. J., and Gray, V. M. 2002. Effect of Medium Salt Concentration on Differentiation and Maturation of Somatic Embryos of Cassava (*Manihot esculenta* Crantz). *Annals of Botany*. 89:645-648.

Gunawan, L. W. 1992. *Teknik Kultur Jaringan Tumbuhan*. PAU Bioteknologi IPB. Bogor.

Hanifah, N. 2007. Pengaruh Konsentrasi NAA dan BAP Terhadap Pertumbuhan Eksplan Jarak Pagar (*Jatropha curcas* L.) Secara *In Vitro*. *Skripsi.* Fakultas Pertanian UNS. Surakarta.

Harborne, J. B. 2006. *Metode Fitokimia: Penuntun Cara Modern Menganalisis Tumbuhan*. Penerbit ITB. Bandung.

Harpenas, A. dan Dermawan, R. 2010. *Budidaya Cabai Unggul*. Penebar Swadaya. Jakarta.

Hartman, H. T., Kester, D., E., dan Davis, F. T. 1990. *Plant Propagation : Principles and Practices*. Prentice Hall International Inc. New Jersey.

Hendaryono, D. P. S. dan Wijayani, A. 1994. *Teknik Kultur Jaringan : Pengenalan dan Petunjuk Perbanyakan Tanaman secara Vegetatif Modern*. Kanisius. Yogyakarta.

Herawan, T. dan Na'iem, M. 2006. *Teknik Kultur Jaringan, Laboratorium Kultur Jaringan Tanaman.* Pusat Antar Universitas (PAU) Bioteknologi. Institut Pertanian Bogor. Bogor.

Herbert, R. B. 1995. *Biosintesis Metabolisme Sekunder*. Edisi kedua. School of Chemistry. New York: University of Leeds.

Hilton, M. G., and Rhodes, M. J. C. 1994. The Effect of Varying Levels of Gamborgs B5 Salt and Temperature on The Accumulation of Strach and Hyoscyamine in Batch Culture of Transformed Roots of *Datura stramonium*. *Plant Cell Tissue Org. Cult*. 38:45-51.

Ignacimuthu, S. 1997. *Plant Biotechnology*. Science Publisher, Inc. New York.

Isaac, S. 1992. *Fungal Plant Interactions*. Chapman & Hall. London.

Jain, S., Kharya, M. D., Nayak, S., and Barik, R. 2008. Effect of Antioxidants on Callus Browning of *Glycyrrhiza glabra*. *Journal of Natural Remedies*. 44-47.

Johnson, E. L. dan Stevenson, R. 1991. *Dasar Kromatografi Cair*. Penerbit ITB. Bandung.

Johnson, T.S., Ravishankar, G.A., and Venkataraman, L.V. 1996. Biotransformation of Ferulic Acid and Vanillylamine to Capsaicin and Vanillin in Immobilized Cell Cultures of *Capsicum frutescens*. *Plant Cell. Tiss. Organ. Cult.* 44:117-121.

Kaga, H., Goto, K., Takahashi, T., Hino, M., Tokuhashi, T., Orito, K. 1996. A General and Stereoselective Synthesis of The Capsaicinoids Via The Orthoester Claisen Rearrangement. *Tetrahedron* **.** 52:8451-8470.

Kalidass, C., Mohan, V. R., and Daniel, A. 2010. Effect of Auxin and Cytokinin on Vincristine Production by Callus Culture of *Catharanthus roseus* L. (Apocynaceae). *Tropical and Subtropical Agroecosystems*. 12:283-288.

Karuppusamy, S. 2009. A Review on Trends in Production of Secondary Metabolites from Higher Plants by In Vitro Tissue, Organ, and Cell Cultures. *Journal of Medical Plants Research*. 3(13):1222-1239.

Katuuk, J. R. P. 1989. *Teknik Kultur Jaringan dalam Mikropropagasi Tanaman*. Departemen P dan K. Jakarta.

Kehie, M., Kumaria, S., and Tandon, P. 2012. Osmotic Stress Induced Capsaicin Production in Suspension Cultures of *Capsicum chinense* Jacq.cv. Naga King Chili. *Acta Physiol Plant*. 34(2).

Khadi, B. M., Goud J. V., and Patil V. B. 1987. Variation in Ascorbic Acid and Mineral Contentin Fruits of Some Varieties of Chilli (*Capsicum annuum* L.). *Plant Foods for Hum Nutri*. 37(1):9-15.

Kharisma, G. G. 2011. Pengaruh Suplemen Organik Terhadap Induksi Kalus dan Regenerasi Tunas pada Kalus Biji Padi (*Oryza sativa* L.) cv. Ciherang Secara *In Vitro*. *Skripsi*. Fakultas Teknobiologi Universitas Atma Jaya Yogyakarta. Yogyakarta.

Khosroushahi, A. Y., Naderi-Manesh, H., and Simonsen, H. T. 2011. Effect of Antioxidants and Carbohydrates in Callus Cultures of *Taxus brevifolia*: Evaluation of Browning, Callus Growth, Total Phenolics and Paclitaxel Production. *BioImpacts*. 1(1):37-45.

Kittipongpatana, N., Hock, R. S., and Porter, J. R. 1988. Production of Solasodine by Hairy Root, Callus, and Cell Suspension Cultures of *Solanum aviculare* Forst. *Plant Cell Tiss. Organ Cult*. 52:133-143.

Kittipongpatana, N., Maneerat, P., Pattanakitkosol, P., and Kittipongpatana, O. S. 2007. Effect of Some Factors on The Growth of *Capsicum annuum* L. Cell Suspension Culture and Biotransformation of Hydroquinone to Arbutin. *Journal Natural Science*. 6(2):207-218.

Kumar, O. A., Tata, S. S., and Rupavati, T. 2010. In Vitro Induction of Callusogenesis in Chili Peppers (*Capsicum annuum* L.). *International Journal of Current Research*. 3:42-45.

Kumari, M., Patade, V. Y., Ahmed, Z., 2012. Establisment of High Regeneration Potential in Sweet Pepper (Capsicum annuum L.) Cultivars Yolo Wonder and California Wonder. *World Journal of Science and Technology*. 2(7):26-35.

Kurz, W. G. W. dan Constabel, F. 1991. Produksi dan Isolasi Metabolit Sekunder. Dalam Wetter, L. R. dan Constabel, F. (eds). *Metode Kultur Jaringan Tanaman*. Penerjemah: Widianto, M. B. ITB Press. Bandung.

Lauterboom, D. P. 2011. Evaluasi Kualitas Hasil dan Analisis Genetik Kadar Capsaicin dan Vitamin C pada Cabai (*Capsicum annuum* L.). *Tesis*. Sekolah Pascasarjana Institut Pertanian Bogor. Bogor.

Lestari, E. G. dan Mariska, I. 2003. Pengaruh Berbagai Formulasi Media terhadap Regenerasi Kalus Padi Indica. *Prosiding Seminar Hasil Penelitian Rintisan dan Bioteknologi Tanaman*. 257-263.

Lestari, E. G. dan Yunita, R. 2008. Induksi Kalus dan Regenerasi Tunas Padi Varietas Fatmawati. *Bul.Agronomi*. 36(2) :106-110.

Loveless, A. R. 1991. *Prinsip-Prinsip Biologi Tumbuhan untuk Daerah Tropik*. PT. Gramedia Pustaka Utama. Jakarta.

Madhusudhan, R. 1998. Plant Cell Cultures for The Production of Phytochemicals – Anthocyanin and Capsaicin in Bioreactors. *Thesis*. University of Mysore.

Maliszewska, J. and Tegowska, E. 2011. Capsaicin as An Organophosphate Synergist Against Colorado Potato Beetle (*Leptinotarsa decemlineata* Say). *Journal of Plant Protection Research*. 52(1):28-34.

McKently, A. H., Moore, G. A., and Gardner, F. P. 1989. In Vitro Plant Regeneration of Peanut from Seed Explants. *Crop Sci*. 30:192-196.

Meyer, H. J. and Staden, J. V. 1995. The In Vitro Production of An Anthocyanin from Callus Cultures of *Oxalis linearis*. *Plant Cell Tissue Organ Cult*. 40:55-58.

Mohamed, N. B. 2007. Callus and Root Induction of *Capsicum frutescens*. *Thesis*. Faculty of Science University Technology Malaysia. Malaysia.

Mojica-Marín, V., Luna-Olvera, H. A., Sandoval-Coronado, C. F., Morales-Ramos, L. H., González-Aguilar, N. A., Pereyra-Alférez, B., Ruiz-Baca, E., and Elíaas-Santos, M. 2011. In Vitro Antifungal Activity of Gobernadora (*Larrea tridentata* (D. C.) Conville) Against *Phytophthora capsici* Leo. *African Journal of Agricultural Research*. 6(5):1058-1066.

Muryanti, S. dan Anggarwulan, E. 2005. Pertumbuhan dan Produksi Reserpin Kalus Pule Pandak (*Rauvolfia serpentia* (L.) Bentham ex. Kurz.) pada Pemberian Metil Jasmonat secara *In Vitro*. *Bioteknologi*. 2(2): 58-64.

Negi, R. S. 2011. Fast In-Vitro Callus Induction in *Catharanthus roseus* – A Medicinally Important Plant Used in Cancer Therapy. Research Journal of Pharmaceutical, Biological, and Chemical Sciences. 2(4):597-603.

Noggle G.R. and Fritz, G. J. 1983. *Introductory Plant Physiology*. Second edition. Pretince-Hall, Inc. New Jersey.

Nunez-Palenius, H. G., and Ochoa-Alejo, N. 2005. Effect of Phenylalanine and Phenylpropanoids on The Accumulation of Capasaicinoids and Lignin in Cell Cultures of Chili Pepper (*Capsicum Annuum* L.). *In Vitro Cell. Dev. Biol. Plant*. 41:801-805.

Ochoa-Alejo, N. and Gómez-Peralta, J. E. 1993. Activity of Enzymes Involved in Capsaicin Biosynthesis in Callus Tissue and Fruits of Chili Pepper (*Capsicum annuum* L.). *J. Plant Physiol*. 141**:**147-152.

Ochoa-Alejo, N. and Ramirez-Malagon, R. 2001. In Vitro Chili Pepper Biotechnology. *In Vitro Cell.* 37:701-729.

Palupi, A. D., Solichatun, dan Marliana, S. D. 2004. Pengaruh Asam 2,4-Diklorofenolsiasetat (2,4-D- dan Benziladenin (BA) Terhadap Kandungan Minyak Atsiri Kalus Daun Nilam (*Pogostemon cablin* Benth.). *BioSMART*. 6(2):99-103.

Pierik, R. M. L. 1987. *In Vitro Culture of Higher Plant*. Marthinus Mijhoff Pub. Netherlands.

Prasad, B. C. N., Gururaj, H. B., Kumar, V., Giridhar, P., Parimalan, R., Sharma, A., and Ravishankar, G. A. 2005. Influence of 8-Methyl-Nonenoic Acid on Capsaicin Biosynthesis in In Vivo and In Vitro Cell Cultures of *Capsicum* Spp. *Central Food Technological Research Institute*. India.

Prihatmanti, D. dan Mattjik, N. A. 2004. Penggunaan ZPT NAA (*Naphtaleine Acetic Acid*) dan BAP (*6-Benzil Amino Purine*) serta Air Kelapa untuk Menginduksi Organogenesis Tanaman Anthurium (*Anthurium andraeanum* Linn.). *Buletin Agronomi*. 32(1): 20-25.

Purwanto, A. 2008. Kajian Macam Eksplan dan Konsentrasi IBA Terhadap Multiplikasi Tanaman Manggis (*Garcinia mangostana* L.) Secara In Vitro. *Skripsi*. Fakultas Pertanian Universiatas Sebelas Maret. Surakarta.

Rahardja, P. C. dan Wiryanta, W. 2005. *Aneka Cara Memperbanyak Tanaman*. Agromedia Pustaka. Jakarta.

Rahayu, B., Solichatun, dan Anggarwulan, E. 2003. Pengaruh Asam 2,4-Diklorofenoksiasetat (2,4-D) Terhadap Pembentukan dan Pertumbuhan Kalus Serta Kandungan Flavonoid Kultur Kalus *Acalypha indica* L. *Biofrms*. 1(1):1-6.

Rajendran. L., Ravishankar, G. A., Venkataraman, L.V., and Prathiba, K. R. 1992. Anthocyanin Production in Callus Cultures of *Daucus carota* L. as Influenced by Nutrient Stress and Osmoticum. *Biotechnol. Lett*. 14:707-712.

Ramachandra Rao, S. and Ravishankar, G. A. 2000. Biotransformation of Protocatechuic Aldehyde and Caffeic Acid to Vanillin and Capsaicin in Freely Suspended and Immobilized Cell Culture of *Capsicum frutescens*. *J. Hiotechnol*. 76:137-146

Rashid, U., Ali, S., Ali, G. M., Ayub, N., Masood, M. S. 2009. Establishment of an Efficient Callus Induction and Plant Regeneration System in Pakistani Wheat (*Triticum aestivum*) cultivars. *Electronic Journal of Biotechnology*. 12(3):1-12.

Reyes-Escogido, M. L., Gonzalez-Mondragon, E. G. and Vazquez-Tzompantzi, E. 2011. Chemical and Pharmacological Aspect of Capsaicin. *Molecules*. 16:1235-1270.

Richmond, A. E. and Lang, A. 1957. Effect of Kinetin on Protein Content and Survival of Detached Xanthium leaves. *Science*. 15:650-651

Sahai, O. P. And Shuler, M. L. 1984. Environmental Parameters Influencing Phenolics Production by Batch Cultures of *Nicotiana tabacum*. *Biotechnol. Bioeng*. 26:111-120.

Sarin, R. and Bansal, N. 2011. Impact of Growth Regulators on Callus Production of Two Medical Plants viz. *Adhatoda vasica* and *Agretum conyzoides*. *International Journal of Research in Plant Science*. 1(1):1-8.

Salisbury, F. B. dan Ross, C. W. 2010. *Fisiologi Tumbuhan*. Penerbit ITB. Bandung.

Santoro, M. V., Nievas, F., Zygadlo, J., Giordano, W., and Banchio, E. 2013. Effects of Growth Regulators on Biomass and The Production of Secondary Metabolites in Peppermint (*Mentha piperita*) Micropopagated in Vitro. *American Journal of Plant Sciences*. 4:49-55.

Santoso, U. dan Nursandi, F. 2004. *Kultur Jaringan Tanaman*. Universitas Muhammadiyah Malang Press. Malang.

Schooley, J. 1997. Introduction to Botany. Delmar Publishers. New York.

Seitz, H. U. and Hinderer, W. 1988. *Anthocyanin in Cell Cultures and Somatic Cell Genetics of Plants*. Academic Press. San Diego.

Shaoxiong, Y., Kian, H. K., and Pauline, M. D. 1996. Effect of Sucrose, Exogenous Product Concentration, and Other Culture Conditions on Growth and Steroidal Alkaloid Production by *Solanum aviculare* Hairy Roots. *Enzyme and Microbial Technology*. 18:238-243.

Siregar, L. A. M., Keng, CL. dan Lim, BP. 2006. Pertumbuhan dan Akumulasi Alkaloid dalam Kalus dan Suspensi Sel *Eurycoma longifolia* Jack. *Jurnal Ilmiah Pertanian KULTURA*. 41(1):19-27.

Soebagio, Budiasih, E., Ibnu, M. S., Widiarti, H. R., dan Munzil. 2005. *Kimia Analitik II*. Penerbit Universitas Negeri Malang. Malang.

Soecipto, S. 1994. *Petunjuk Pelaksanaan Kegiatan Kultur Jaringan*. Departemen Kehutanan. Yogyakarta.

Solanelli, C., Allesandra, P., Elena, L., Amedeo, A., and Pierdominco, P. 2006. Sucrose Specific Induction of The Anthocyanin Biosynthetic Pathway in *Arabidopsis*. *Plant Physiol*. 140:637-646.

Stafford, A. and Warren, G. 1991. *Plant Cell and Tissue Culture*. John Willey and Sons. England.

Sudha, G. and Ravishankar, G. A. 2003. Influence of Methyl Jasmonate and Salicylic Acid in The Enhancement of Capsaicin Production in Cell Suspension Cultures of *Capsicum frutescens* Mill. *Current Science*. 85(8):1212-1217

Sudirga, S. K. 2002. Analisis Kandungan Senyawa Bioaktif Azadirachtin dalam Kultur Suspensi Sel Tanaman Mimba (*Azadirachta indica* A.Juss). *Jurnal Biologi.* VI(2):60-63.

Sukrasno, Kusmardiyani, S., Tarini, S., Sugiarso, N. C. 1997. Kandungan Kapsaisin dan Dihidrokapsaisin pada Berbagai Buah *Capsicum*. *JMS*. 2:28 – 34.

Sung, Y., Chang, Y. Y., and Ting, N. L. 2005. Capsaicin Biosynthesis in Water-Stresses Hot Pepper Fruits. *Botanical Bulletin of Academica Sinica*. 46:35-42.

Suryowinoto, M. 1991. *Budidaya Jaringan Terobosan Bermanfaat dalam Bioteknologi*. UGM Press. Yogyakarta.

Tabata, M. 1977. *Recent Advances in The Production of Medicinal Substances by Plant Tissue Culture.* In: W. Barz, E. Reinhard, and M.H. Zenk (eds). *Plant Tissue Cultures and It's Bio-Technological Applications*. Springer Verlag. Berlin.

Tang, W. and Newton, R. J. 2004. Increase of Polyphenol Oxidase and Decrease of Polyamines Correlate with Tissue Browning in Virginia Pine (*Pinus virginiana* Mill.). *Plant Sci*. 167:621-628.

Taiz, L. and Zeiger, E. 2002. *Plant Physiology*. Sinauer Associates. Massachusetts.

Turhan, H. 2004. Callus Industion and Growth in Transgenic Potato Genotypes. *African Journal of Biotechnology*. 3(8):375-378.

Umamaheswari, A. and Lalitha, V. 2007. In Vitro Effect of Various Growth Hormones in *Capsicum annuum* L. on The Callus Induction and Production of Capsaicin. *Journal of Plant Sciences*. 2(5):545-551.

Verma, A. K., Singh, R. R., and Singh, S. 2012. Improved Alkaloid Content in Callus Culture of *Catharanthus roseus*. *Botanica SERBICA*. 36(2):123-130.

Voigt, R. 1995. *Buku Pelajaran Teknologi Farmasi*. Gadjah Mada University Press. Yogyakarta.

Wagner, H. and Bladt, S. 2009. *Plant Drug Analysis*. Springer. New York.

Wetter, L. R. dan Constabel, F. 1991. *Metode Kultur Jaringan Tanaman*. ITB Press. Bandung.

Wattimena, G. A. 1991. *Bioteknologi Tanaman*. PAU Bioteknologi IPB. Bogor.

Yusnita. 2004. *Kultur Jaringan Cara Memperbanyak Tanaman Secara Efisien*. Agro Media Pustaka. Jakarta.

Zeyrek, F. Y. and Oguz, E. 2005. In Vitro Activity of Capsaicin Against *Helicobacter pylori*. *Annals of Microbiology*. 55(2):125-127.

Zhou, B., Wei, X. F., Wang, R. T., and Jia, J. M. 2010. Quantification of The Enzymatic Browning and Secondary Metabolites in The Callus Culture System of *Nigella glandulifera* Freyn et Sint. *Asian Journal of Traditional Medicine*. 5(3):109-116.

Lampiran 1. Komposisi Medium MS

Jenis Larutan *Stock*	**Larutan *Stock***	**Bahan**	**Berat (g/L) dalam 100 mL**	**Volume (ml) untuk 1L medium MS**
A	Makro	NH_4NO_3 (Merck)	8,25	20
B		KNO_3 (Merck)	9,5	20
C		$CaCl_2.H_2O$ (Merck)	4,4	10
D		$MgSO_4.7H_2O$ (Merck)	3,7	10
		KH_2PO_4 (Merck)	1,7	
E	Besi	$Na_2EDTA.2H_2O$ (Merck)	0,745	5
		$FeSO_4.7H_2O$ (Merck)	0,557	
F	Mikro	$MnSO_4.H_2O$ (Merck)	0,338	5
		$ZnSO_4.4H_2O$ (Merck)	0,172	
		H_3BO_3 (Merck)	0,124	
		KI (Merck)	0,0166	
		$NaMoO_4.2H_2O$ (Merck)	0,005	
		$CoCl_2.6H_2O$ (Merck)	0,0005	
		$CuSO_4.5H_2O$ (Merck)	0,0005	
Vitamin dan Asam Amino	Vitamin	Tiamin HCl (Merck)	0,01	1
		Asam Nikotinat (Merck)	0,05	
		Piridoksin (Sigma Chemical)	0,05	
	Asam Amino	Glisin (Merck)	0,2	
Mioinositol	Mioinositol	Mioinositol (Merck)	1	10

Lampiran 2. Hasil Pengamatan Waktu Inisiasi Kalus Eksplan Hipokotil Cabai Rawit Putih (hari)

Kadar Nutrien	Ulangan	Kombinasi ZPT				Rerata
		A	B	C	K	
V	1	6	6	5	6	
	2	7	7	7	7	
	3	7	7	7	7	
Rerata		**6,6667**	**6,6667**	**6,3333**	**6,6667**	**6,5833**
W	1	7	6	6	7	
	2	6	7	6	7	
	3	6	7	5	7	
Rerata		**6,3333**	**6,6667**	**5,6667**	**7,0000**	**6,4167**
X	1	5	7	6	5	
	2	6	6	5	6	
	3	6	6	6	6	
Rerata		**5,6667**	**6,3333**	**5,6667**	**5,6667**	**5,8333**
Y	1	5	6	6	7	
	2	6	5	5	6	
	3	6	6	6	7	
Rerata		**5,6667**	**5,6667**	**5,6667**	**6,6667**	**5,9167**
Z	1	6	7	6	8	
	2	7	7	6	7	
	3	6	8	6	8	
Rerata		**6,3333**	**7,3333**	**6,0000**	**7,6667**	**6,8333**

Keterangan: V = ½ MS; W = ¾ MS; X = 1 MS; Y = 1¼ MS; dan Z = 1½ MS
A = 2,4-D + BAP; B = 2,4-D + Kin; C = IAA + BAP; dan K = Kontrol

Lampiran 3. Hasil Pengukuran Berat Basah Kalus Eksplan Hipokotil Cabai Rawit Putih pada Minggu Ke-9 (gr)

Kadar Nutrien	Ulangan	Kombinasi ZPT				Rerata
		A	B	C	K	
V	1	0,28140	0,0879	0,2173	0,0586	
	2	0,02416	0,1022	0,2625	0,0532	
	3	0,14566	0,0948	0,2209	0,0737	
Rerata		**0,1504**	**0,0950**	**0,2336**	**0,0618**	**0,1352**
W	1	0,1680	0,14724	0,1345	0,0762	
	2	0,1692	0,1535	0,2512	0,1381	
	3	0,1577	0,1525	0,3175	0,0786	
Rerata		**0,1650**	**0,1511**	**0,2344**	**0,0976**	**0,1620**
X	1	0,3633	0,1227	0,5494	0,1569	
	2	0,2064	0,1164	0,9469	0,0622	
	3	0,3567	0,1850	0,5085	0,1649	
Rerata		**0,3088**	**0,1414**	**0,6683**	**0,1280**	**0,3115**
Y	1	0,2341	0,1761	0,4850	0,0543	
	2	0,2408	0,215	0,2976	0,0518	
	3	0,2360	0,1060	0,2361	0,2771	
Rerata		**0,2370**	**0,1657**	**0,3396**	**0,1277**	**0,2175**
Z	1	0,1900	0,0813	0,2637	0,1068	
	2	0,2451	0,1821	0,2367	0,0939	
	3	0,1486	0,1014	0,2772	0,1141	
Rerata		**0,1946**	**0,1216**	**0,2592**	**0,1049**	**0,1701**

Keterangan: V = ½ MS; W = ¾ MS; X = 1 MS; Y = 1¼ MS; dan Z = 1½ MS
A = 2,4-D + BAP; B = 2,4-D + Kin; C = IAA + BAP; dan K = Kontrol

Lampiran 4. Perhitungan Persentase (%) Pertumbuhan Kalus Eksplan Hipokotil Cabai Rawit Putih pada Minggu Ke-1,2,3,4

Perlakuan		Minggu ke-			
		1	2	3	4
MS	ZPT	Persentase (%)	Persentase (%)	Persentase (%)	Persentase (%)
½	2,4-D + BAP	64,71	100,00	100,00	100,00
	2,4-D + Kin	47,62	52,38	80,95	85,71
	IAA + BAP	90,00	100,00	100,00	100,00
	Kontrol	31,58	47,37	73,68	78,95
¾	2,4-D + BAP	87,50	100,00	100,00	100,00
	2,4-D + Kin	52,94	64,71	70,59	70,59
	IAA + BAP	71,43	100,00	100,00	100,00
	Kontrol	40,00	73,33	73,33	73,33
1	2,4-D + BAP	78,95	94,74	100,00	100,00
	2,4-D + Kin	50,00	75,00	80,00	80,00
	IAA + BAP	90,48	100,00	100,00	100,00
	Kontrol	31,82	68,18	72,73	72,73
1¼	2,4-D + BAP	65,00	100,00	100,00	100,00
	2,4-D + Kin	60,87	69,57	91,30	91,30
	IAA + BAP	88,89	100,00	100,00	100,00
	Kontrol	52,94	52,94	52,94	52,94
1½	2,4-D + BAP	58,82	100,00	100,00	100,00
	2,4-D + Kin	27,78	66,67	66,67	77,78
	IAA + BAP	66,67	80,95	80,95	100,00
	Kontrol	20,00	60,00	60,00	65,00

Lampiran 5. Analisis Varian dan Uji Duncan Parameter Waktu Inisiasi Terbentuknya Kalus dari Hipokotil Cabai Rawit (*Capsicum frutescens*) Putih

Uji Antara Efek Subjek

Variabel terikat: Waktu Inisiasi Terbentuknya Kalus

Sumber Keragaman	Jumlah Kuadrat Tipe II	Derajat Bebas (db)	Kuadrat Tengah	F hitung	Sig.
KadarNutrien	8,900	4	2,225	6,357	0,000
KombinasiZPT	6,850	3	2,283	6,524	0,001
KadarNutrien*KombinasiZPT	5,233	12	0,436	1,246	0,288
Galat	14,000	40	0,350		
Total	2429,000	60			

DMRT

1. Kadar Nutrien

Waktu Inisiasi Terbentuknya Kalus

Duncan[a,b]

Kadar Nutrien	Jumlah	Kelompok	
		1	2
1 MS	12	5,8333	
1¼ MS	12	5,9167	
¾ MS	12		6,4167
½ MS	12		6,5833
1½ MS	12		6,8333
Sig.		0,732	0,110

2. Kombinasi ZPT

Waktu Inisiasi Terbentuknya Kalus

Duncan[a,b]

Kombinasi ZPT	Jumlah	Kelompok		
		1	2	3
IAA+BAP	15	5,8667		
2,4-D+BAP	15	6,1333	6,1333	
2,4-D+Kin	15		6,5333	6,5333
Kontrol	15			6,7333
Sig.		0,224	0,71	0,360

Lampiran 6. Analisis Varian dan Uji Duncan Parameter Berat Basah Kalus dari Hipokotil Cabai Rawit (*Capsicum frutescens*) Putih

Uji Antara Efek Subjek

Variabel terikat: Berat Basah Kalus

Sumber Keragaman	Jumlah Kuadrat Tipe II	Derajat Bebas (db)	Kuadrat Tengah	F hitung	Sig.
KadarNutrien	0,231	4	0,58	8,234	0,000
KombinasiZPT	0,527	3	0,176	25,023	0,000
KadarNutrien*KombinasiZPT	0,244	12	0,20	2,900	0,006
Galat	0,281	40	0,07		
Total	1,284	60			

DMRT

1. Kadar Nutrien

Berat Basah Kalus

Duncan[a,b]

Kadar Nutrien	Jumlah	Kelompok		
		1	2	3
½ MS	12	0,1352		
¾ MS	12	0,1620	0,1620	
1½ MS	12	0,1701	0,1701	
1¼ MS	12		0,2175	
1 MS	12			0,3115
Sig.		0,344	0,133	1,000

2. Kombinasi ZPT

Berat Basah Kalus

Duncan[a,b]

Kombinasi ZPT	Jumlah	Kelompok		
		1	2	3
Kontrol	15	0,1040		
2,4-D+Kin	15	0,1349		
2,4-D+BAP	15		0,211	
IAA+BAP	15			0,3469
Sig.		0,318	1,000	1,000

3. Interaksi Antara Kadar Nutrien dengan Kombinasi ZPT

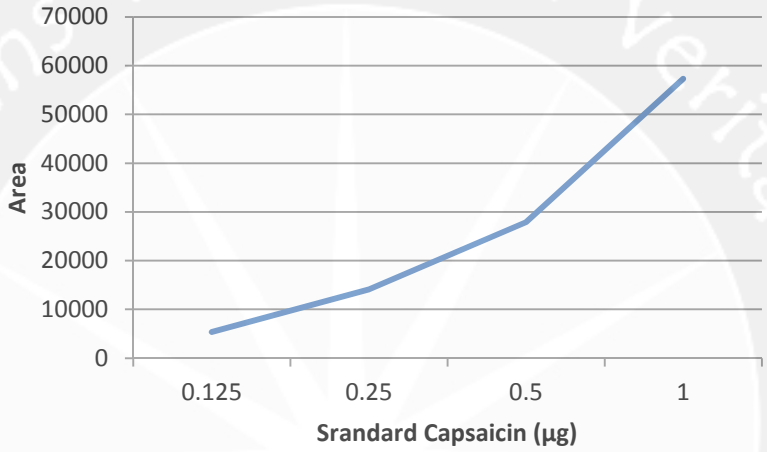
Berat Basah Kalus

Duncan[a,b]

Perlakuan	Jumlah	Kelompok				
		1	2	3	4	5
½ MS Kontrol	3	0,0618				
½ MS 2,4-D+Kin	3	0,0950	0,0950			
¾ MS Kontrol	3	0,0976	0,0976			
1½ MS Kontrol	3	0,1049	0,1049			
1½ MS 2,4-D+Kin	3	0,1216	0,1216			
1¼ MS Kontrol	3	0,1277	0,1277			
1 MS Kontrol	3	0,1280	0,1280			
1 MS 2,4-D+Kin	3	0,1414	0,1414			
½ MS 2,4-D+BAP	3	0,1504	0,1504	0,1504		
¾ MS 2,4-D+Kin	3	0,1511	0,1511	0,1511		
¾ MS 2,4-D+BAP	3	0,1650	0,1650	0,1650		
1¼ MS 2,4-D+Kin	3	0,1657	0,1657	0,1657		
1½ MS 2,4-D+BAP	3	0,1946	0,1946	0,1946	0,1946	
½ MS IAA+BAP	3		0,2336	0,2336	0,2336	
¾ MS IAA+BAP	3		0,2344	0,2344	0,2344	
1¼ MS 2,4-D+BAP	3		0,2370	0,2370	0,2370	
1½ MS IAA+BAP	3		0,2592	0,2592	0,2592	
1 MS 2,4-D+BAP	3			0,3088	0,3088	
1¼ MS IAA+BAP	3				0,3396	
1 MS IAA+BAP	3					0,6683
Sig.		0,113	0,053	0,056	0,072	1,000

Lampiran 7. Kurva Regresi Standard Capsaicin

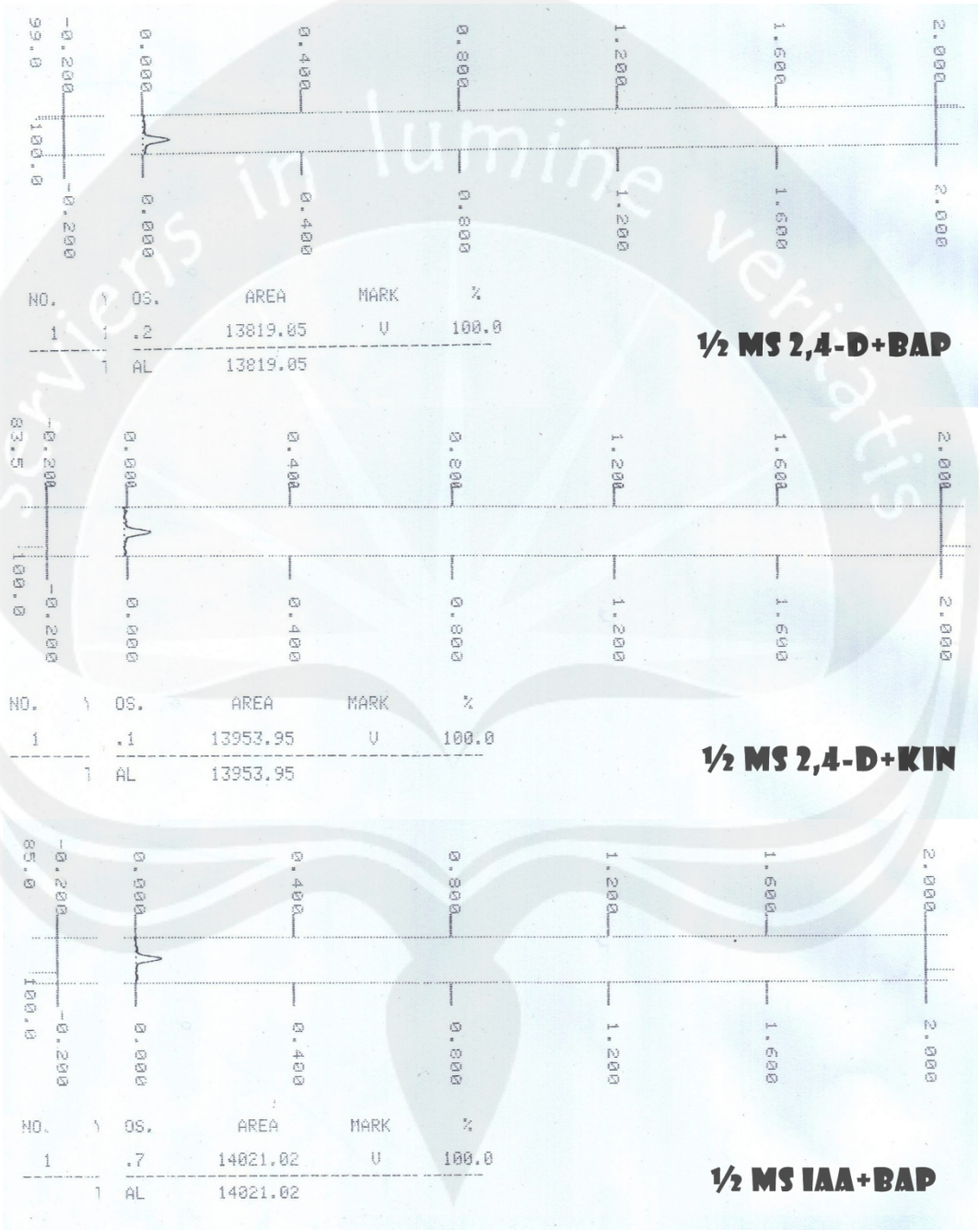
Standar (µg)	Area
0,125	5365,00
0,25	14116,25
0,5	27850,13
1	57283,62

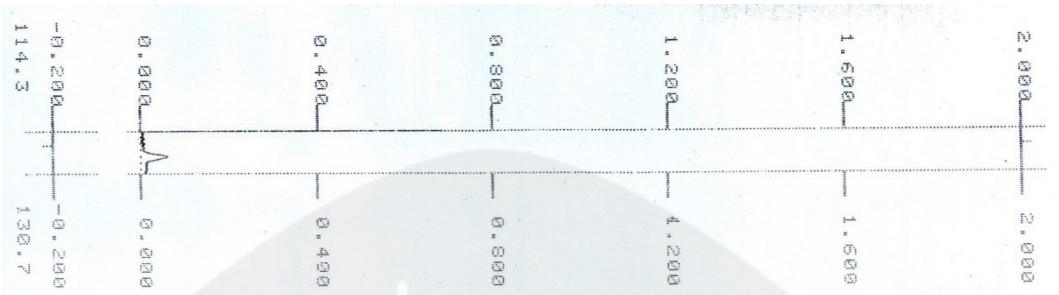


Lampiran 8. Hasil Uji Kuantitatif Capsaicin Kalus Eksplan Hipokotil Cabai Rawit Putih pada Medium dengan Variasi Kadar Nutrien MS dan Kombinasi ZPT

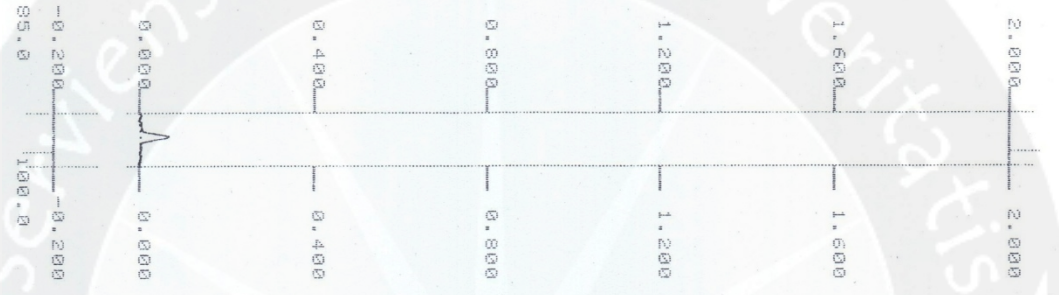
Sampel		Berat Sampel (g)	Area	Capsacin dalam Sampel (µg)
MS	Kombinasi ZPT			
½	2,4-D + BAP	0,04	13819,05	0,259
	2,4-D + Kin	0,04	13953,95	0,261
	IAA + BAP	0,04	14021,02	0,262
	Kontrol	0,04	11074,99	0,212
¾	2,4-D + BAP	0,04	14120,05	0,264
	2,4-D + Kin	0,04	14251,73	0,266
	IAA + BAP	0,04	15057,45	0,279
	Kontrol	0,04	12807,55	0,241
1	2,4-D + BAP	0,04	15150,74	0,281
	2,4-D + Kin	0,04	15307,09	0,283
	IAA + BAP	0,04	15361,08	0,285
	Kontrol	0,04	12891,70	0,242
1¼	2,4-D + BAP	0,04	15423,46	0,286
	2,4-D + Kin	0,04	15987,58	0,296
	IAA + BAP	0,04	17553,25	0,322
	Kontrol	0,04	13323,25	0,250
1½	2,4-D + BAP	0,04	18176,39	0,333
	2,4-D + Kin	0,04	19222,52	0,351
	IAA + BAP	0,04	19249,14	0,351
	Kontrol	0,04	13340,06	0,250

Lampiran 9. Kromatogram Capsaicin Kalus Eksplan Hipokotil Cabai Rawit Putih pada Medium dengan Variasi Kadar Nutrien MS dan Kombinasi ZPT

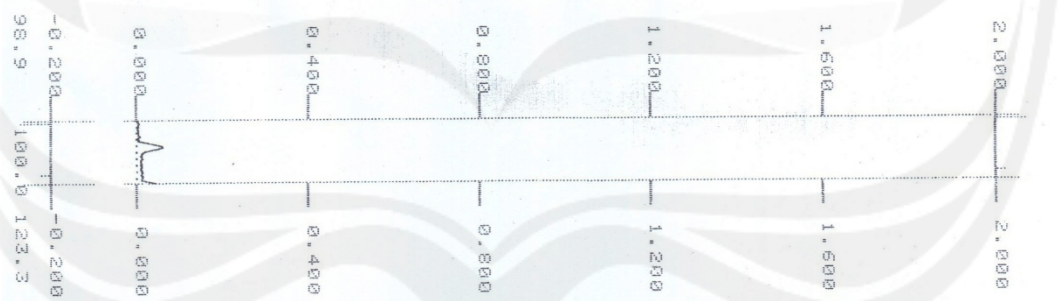




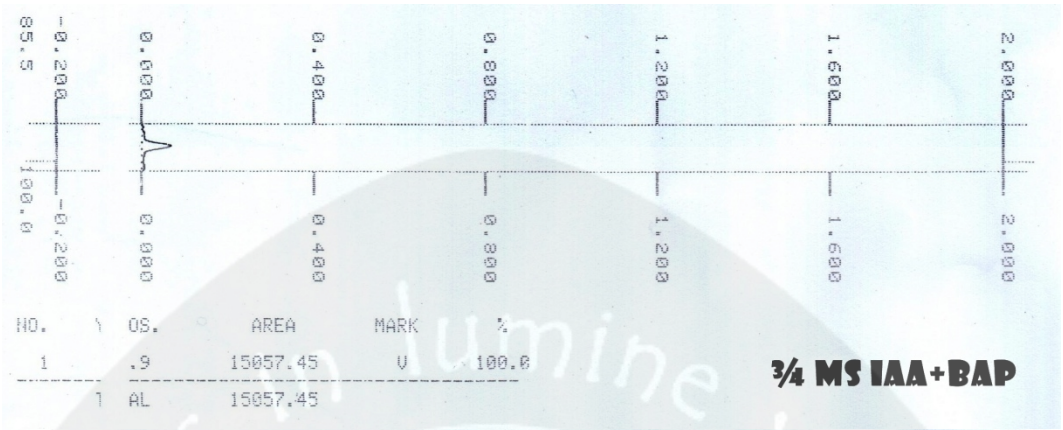
½ MS KONTROL



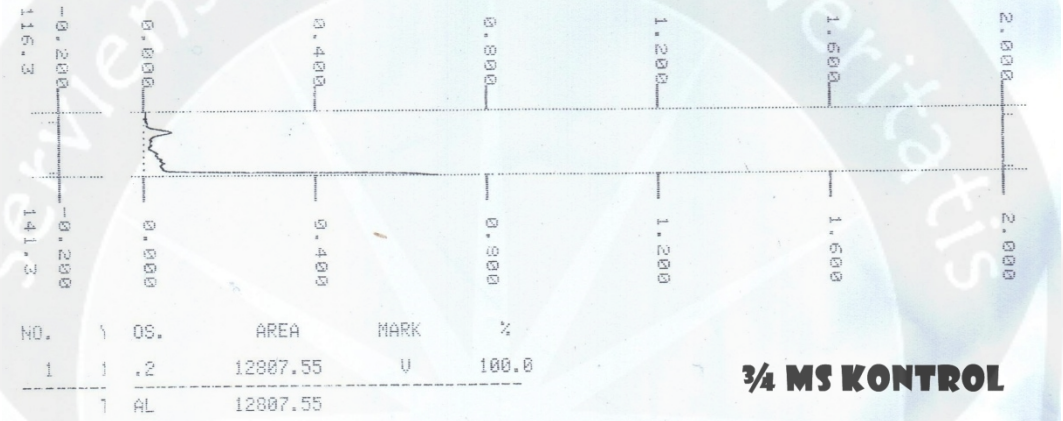
¾ MS 2,4-D+BAP



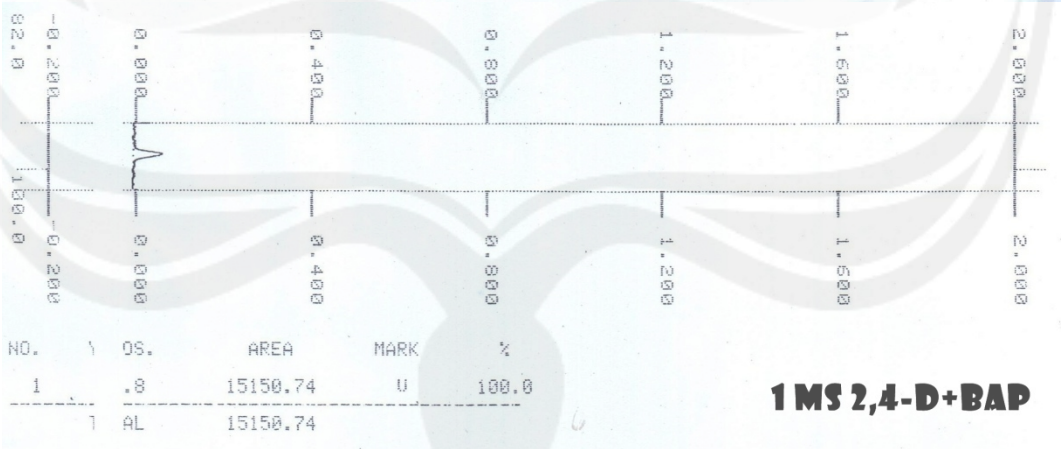
¾ MS 2,4-D+KIN



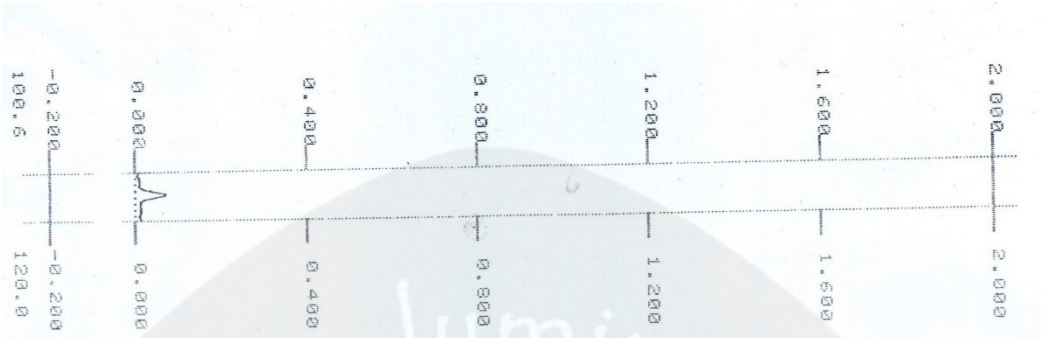
¾ MS IAA+BAP



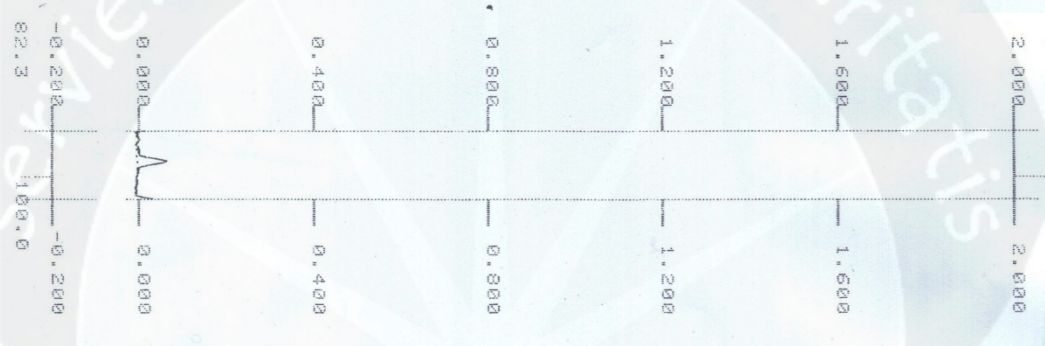
¾ MS KONTROL



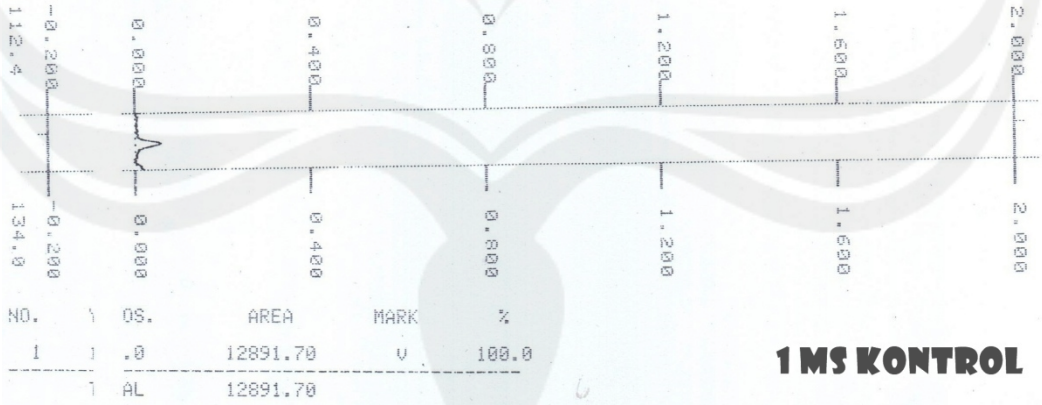
1 MS 2,4-D+BAP



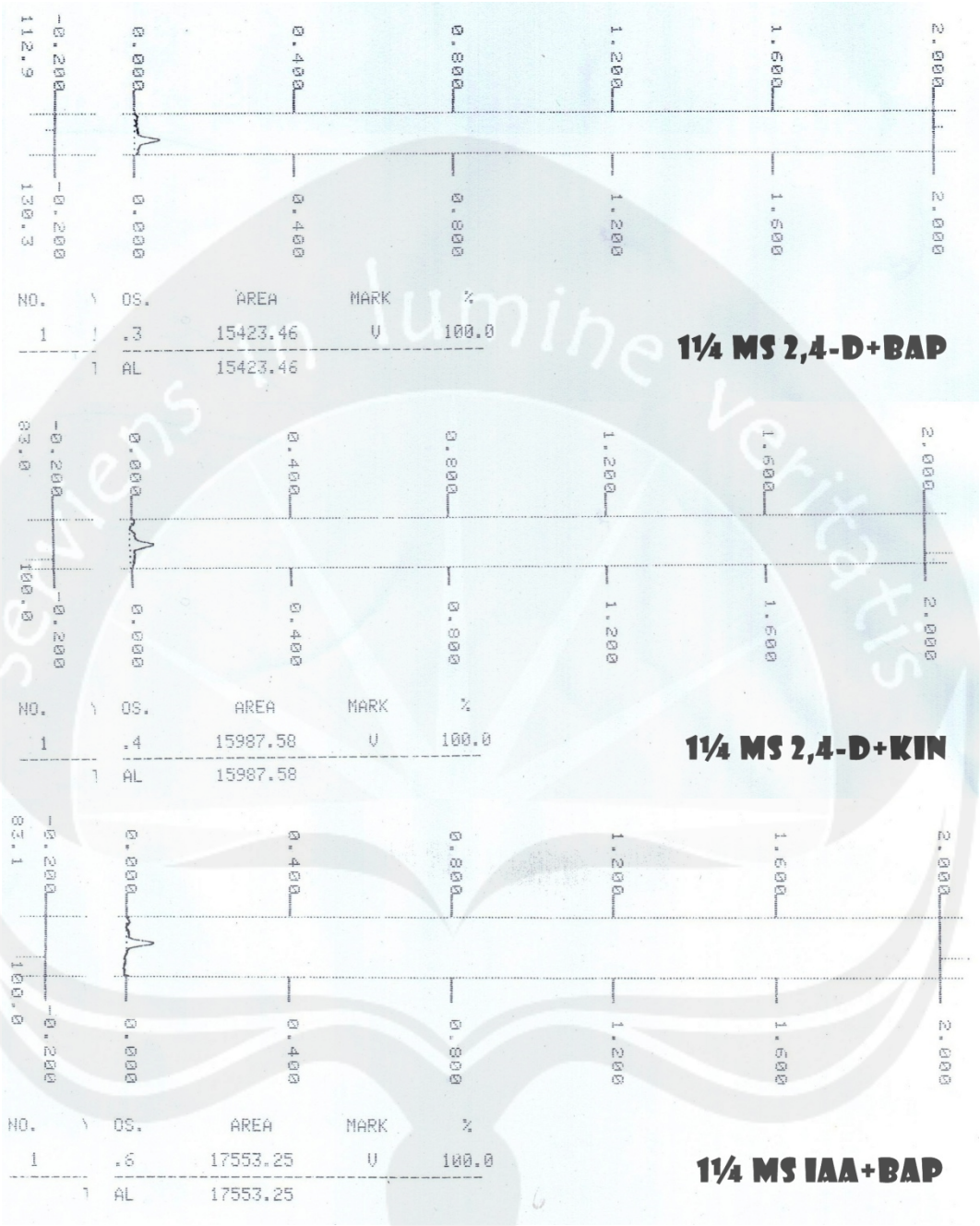
1 MS 2,4-D+KIN

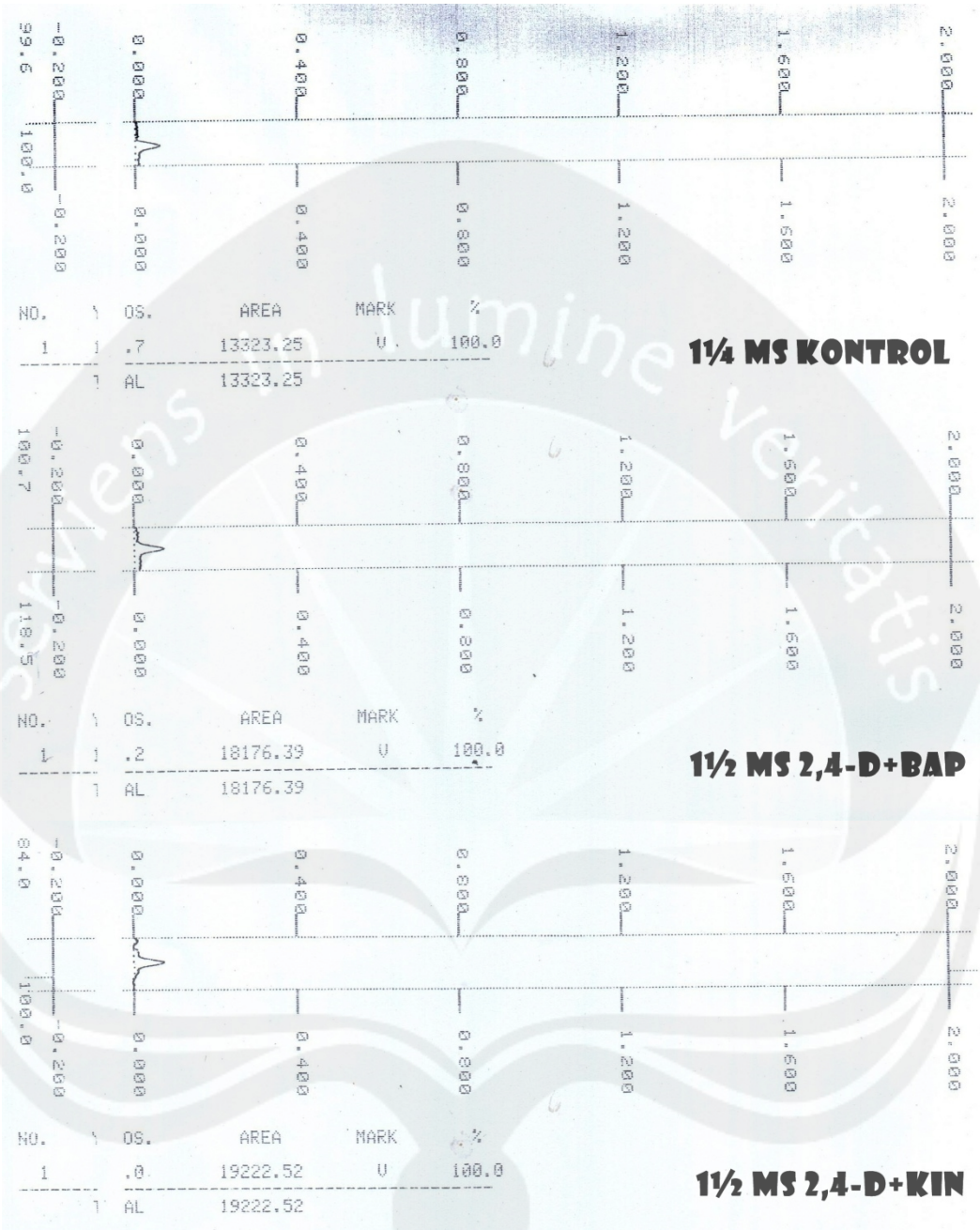


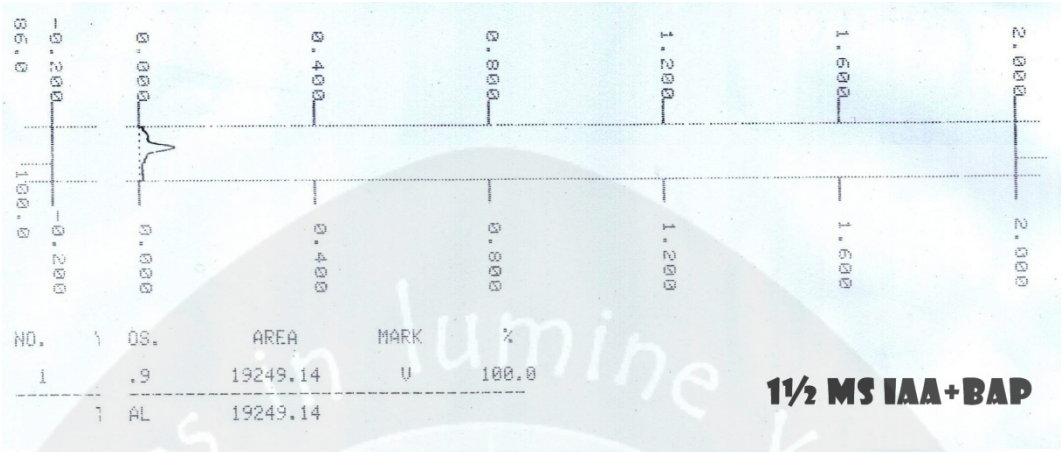
1 MS IAA+BAP



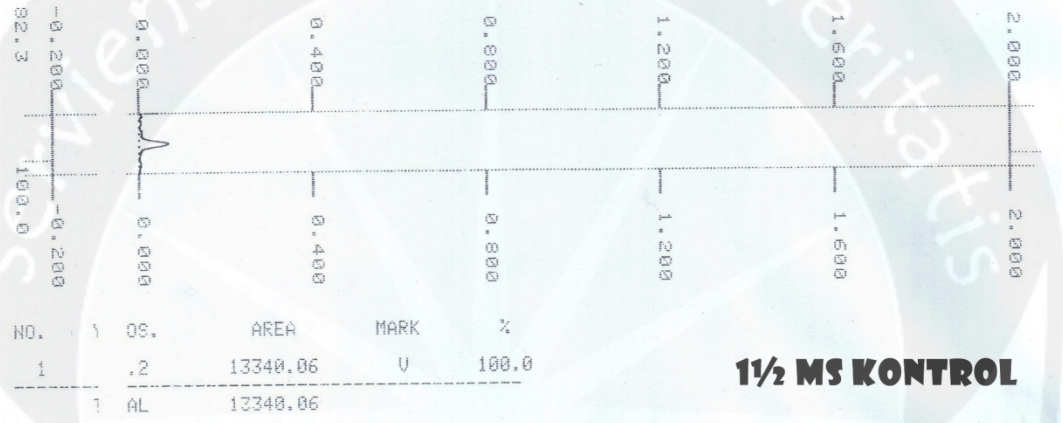
1 MS KONTROL


112.9
130.3
-0.200
-0.200
0.000
0.000
0.400
0.400
0.800
0.800
1.200
1.200
1.600
1.600
2.000
2.000
NO. \ OS. AREA MARK %
1 .3 15423.46 V 100.0
AL 15423.46
1¼ MS 2,4-D+BAP
83.0
100.0
-0.200
-0.200
0.000
0.000
0.400
0.400
0.800
0.800
1.200
1.200
1.600
1.500
2.000
2.000
NO. \ OS. AREA MARK %
1 .4 15987.58 V 100.0
AL 15987.58
1¼ MS 2,4-D+KIN
83.1
100.0
-0.200
-0.200
0.000
0.000
0.400
0.400
0.800
0.800
1.200
1.200
1.600
1.500
2.000
2.000
NO. \ OS. AREA MARK %
1 .6 17553.25 V 100.0
AL 17553.25
1¼ MS IAA+BAP


-0.200
0.000
0.400
0.800
1.200
1.600
2.000
99.6
100.0
NO. OS. AREA MARK %
1 .7 13323.25 V 100.0
AL 13323.25
1¼ MS KONTROL
100.7
118.5
NO. OS. AREA MARK %
1 .2 18176.39 V 100.0
AL 18176.39
1½ MS 2,4-D+BAP
84.0
100.0
NO. OS. AREA MARK %
1 .0 19222.52 V 100.0
AL 19222.52
1½ MS 2,4-D+KIN



1½ MS IAA+BAP



1½ MS KONTROL