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The Effect of Elisitor on Growth and Ginsenoside Level in Hairy Root Culture of *Panax ginseng* Cultivated in Shake Flasks

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Abstract. In recent years, plants have become an important part of traditional medicine. Although the medicinal potential of the plant looks very promising, there are great difficulties that inhibit products for the production of herbs on a large scale. *Panax ginseng* C.A. Mey. is a plant often used in traditional medicine in various countries because ginseng can cure various diseases in humans. The main bioactive component in ginseng is the triterpene saponin compound, namely ginsenosides. Ginseng needs to be cultivated at least 4 yr before the root can be harvested. In addition, a special environment is needed for ginseng to develop properly. One way to obtain ginsenosides is to use root hair culture. However, until now it has been reported that the accumulation of ginsenoside in root hair cultures is still low. Elicitation effective method to increase the production of secondary metabolites in vitro culture. The aim of this research was to observe the effect of elicitor in the form of yeast extract and coconut water to the media on the growth and levels of ginsenoside in flask scale *Panax ginseng* root hair culture. From the data obtained, it can be concluded that the addition of yeast extract from the start with a concentration of 20 mg L⁻¹ cannot increase the levels of ginsenoside in the hairy root culture of *P. ginseng*. The addition of coconut water from the beginning with a volume of 10 mL increases the biomass but cannot increase the ginsenoside levels in the hair culture of *P. ginseng*.

Keywords: Herbal product, *in vitro*, micropropagation, plant tissue culture, traditional medicine,

1 Introduction

In recent years, plants have become an important part of traditional medicine. Therefore, demand for herbal products is increased. Although the medicinal potential of the plant appears to be very promising, there are great difficulties that inhibit products for the production of herbs on a large scale. Some of these are lack of reproducibility in plant extracts, difficult standardization, genetic variation, and environmental conditions. In addition, secondary metabolites which have a therapeutic effect, are produced in small amounts, influenced by the physiological and developmental stages of the plant [1].

Panax ginseng C.A. Mey. is a plant often used in traditional medicine in various countries, such as Korea, China, and Japan [2]. *Panax* means "can cure all diseases" because ginseng can cure various diseases in humans. The main bioactive compound in ginseng is triterpene saponin, namely ginsenosides. Of the many species of ginseng, research focuses on examining the ginsenoside found in *Panax ginseng* (Korean ginseng), *Panax notoginseng* (Chinese ginseng), *Panax japonicum* (Japan ginseng), and *Panax quinquefolius* (American ginseng) [3]. Ginsenosides have many benefits for human health. Ginsenoside consists of two groups, namely the major ginsenosides consisting of the Rb group (Rb1,

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Rb2, Rc and Rd) and the Rg group (Re and Rg1) and minor ginsenosides such as Rh1, Rh2, and compound C. There have been many studies *in vivo* and *in vitro* which indicates that ginsenosides can act as anti-stress, anti-cancer, neuroprotective agents, and have benefits in the cardiovascular system and nervous system [4].

The main ginsenosides producer is the root where ginseng root need to be cultivated at least 4 yr before it can be harvested. In addition, cultivation requires special conditions where it is not exposed to direct sunlight. Ginseng is also sensitive to various diseases and requires quite expensive prevention costs. Therefore, plant tissue culture such as cell culture, callus culture, and root hair culture were developed to produce ginsenosides. Plant tissue culture is an alternative technique that can be used to produce high value secondary metabolites. Plant tissue culture are usually used on plants that have a high value but are difficult to develop conventionally, take a long time to harvest, and have low yields [5]. Several plant tissue culture that can be used to produce ginsenoside are adventitious shoot culture, callus culture, cell suspension culture, protoplast culture, bioreactor scale propagation, *in vitro* mutagenesis, polyploidization induction, and root hair culture [6, 7]. Root hair culture has a number of advantages, that is a stable growth phase, genetically stable, capable of producing secondary metabolites in greater numbers than the actual plant, and producing new secondary metabolites that are not found in the original plant [6]. However, until recently it was reported that the accumulation of ginsenoside in root hair cultures was still low. Therefore, several treatments are needed that can increase ginsenoside production, one of which is elicitation [4].

Elicitation is effective strategy to increase production of secondary metabolites in *in vitro* culture. This process involves adding an elicitor to the growth medium. Elicitor is a molecule that can stimulate the plant defense system so that it can increase the production of secondary metabolites. Elicitors can be classified into biotic and abiotic elicitors. Biotic elicitor is an elicitor derived from living things, either in the form of exogenous elicitor derived from pathogens or endogenous elicitor derived from plants. Biotic exogenous elicitor is an elicitor in the form of a compound released by a microorganism or a component of the microorganism such as a cell wall, whereas a biotic endogenous elicitor is an elicitor in the form of a degradation product from the plant cell wall, protein, or molecule synthesized by plants in response to stress from the attack of microorganisms and pathogens. Abiotic elicitors are elicitors that do not come from living things such as UV radiation, osmotic stress, drought, floods, heavy metals, fungicides, and herbicides [4].

One of the commonly elicitors that can be used to increase the production of secondary metabolites is yeast extract. A certain concentration of yeast extract in culture media will affect the biosynthesis of various metabolites and the optimum concentration of yeast extract is different for each plant species. It was reported that yeast extract has been able to increase the production of secondary metabolite compounds in several plants such as *Psoralea corylifolia* L., *Hyoscyamus niger* L., *Pueraria candollei* Wall. ex Benth., and *Portulaca oleracea* L. Coconut water (*Cocos nucifera* L.) is a beverage that is famous internationally and always increase in the number of requests every year. Coconut water has the main composition, namely sugar and minerals so that it can increase the ability of cell division. Coconut water can be used as an elicitor by accelerating the culture to reach its stationary phase to produce secondary metabolites [8].

From previous research [9], it was obtained that the formulation B with a medium volume of 13 L was able to produce a fresh weight of about 2.7 kg and after drying, the dry weight average 109 g. However, the accumulation of ginsenoside in the root hair culture was still very small, around 2%. Although the dry weight has not reached the target, optimization is also carried out to increase ginsenoside levels using elicitation. The aim of this research was to observe the effect of elicitor in the form of yeast extract and coconut water to the media from the start on the growth and levels of ginsenoside in flask scale root hair culture of *P. ginseng*. It is expected that the use of elicitors can increase ginsenoside levels by more than 5%. The concentration of yeast extract used was 20 mg L⁻¹ while the volume of added coconut water was 10 mL.

2 Material and methods

2.1 Media preparation

For this research, the composition of the media that was used was formulation B and the elicitor was added since the beginning of the media preparation. The control medium was prepared by mixing all the ingredients into the container then adding Reverse Osmosis (RO) water using Pure RO II. After the RO water was added, the media was stirred using a homogenizer at 400 rpm (1 rpm = 1/60 Hz) for 40 min. After being evenly mixed, the pH of the medium was adjusted 5.8 to 6.0. After adjusting the pH, the media was poured 100 mL into a 250 mL flask and then covered with aluminum foil. Media was sterilized using Autoclave Hankuk HK-AC200P at 121 °C, pressure of 1.5 atm for 15 min. Then let the media cool it self for at least 3 d before it can be used. The experimental medium had the same steps as the control medium, only the addition of 20 mg L⁻¹ of yeast extract (MERCK) or 10 mL of coconut water into the medium. The total medium that is prepared are 50 flasks, consisting of 10 flask of control medium, 20 flask of yeast extract medium, and 20 flask of coconut water medium.

2.2 Inoculation and incubation of hairy root culture in flask

Inoculation of hairy root was carried out in sterile Laminar Air Flow (LAF) and carried out aseptically. Hairy root was cut into small pieces (± 1 cm) before being put into the medium. After being cut, put ± 10 strands of root hair into the medium. The inoculation process was carried out two times where the first inoculation only used 30 flasks, that is 10 flask of control medium, 10 flask of yeast extract medium, and 10 flask of coconut water medium then next week with addition of 10 flask of yeast extract medium and 10 flask of coconut water medium. After the inoculation process, the flask is placed on the shaker and then stirred at 110 rpm for 7 wk.

2.3 Harvest and drying hairy root culture

After the incubation period is over, the hairy root were harvested. The media was removed and then the hairy root is collected in a container. The hairy root was rinsed using tap water 1 time. After rinsing using tap water, rinse the root hair using RO water 1 time. The root hair was drained first using a perforated container for 1 h to minimize the water attached to hairy root before being put into a container. The fresh weight was measured using Mettler Toledo MS16001L. The hairy root were dried using a Memmert UF750 oven which had been adjusted to the temperature at 50 °C, 60 % fan, 60 % flap, for 7.5 h. After drying, the root hair dry weight was weighed using the Mettler Toledo MS16001L. After the dry weight is obtained, the yield percentage was calculated using the Equation (1):

$$\% \text{ Yield} = \frac{\text{Dry weight}}{\text{Fresh Weight}} \quad (1)$$

2.4 Extraction and sample preparation

The dried hairy roots were extracted using the Soxhlet method. 5 g of dry hairy roots were extracted in 300 mL methanol 80 % for 2 h. Then the extract was dried using 1 set of rotary evaporator (BUCHI). The dry extract was dissolved in 20 mL of RO water. The extract was extracted again using water-saturated butanol in a ratio of 1:1. The mixture was centrifuged at 8 000 rpm for 15 min. The organic phase was taken and entered into a 250 mL erlemeyer. The extract was extracted using water-saturated butanol until the organic phase became clear. The butanol extract was then dried again using a rotary evaporator (BUCHI). The butanol extract was evaporated to obtain a dry extract which did not contain the butanol scent.

2.5 Ginsenosides analysis

Samples were analyzed using HPLC Agilent 1260 Infinity II. The dried extract was dissolved in 50 mL of methanol-HPLC grade. Then the sample was filtered using a 0.2 μm PTFE filter. The stationary phase used was Kinetex C18 with a size of 50 mm \times 4.6 mm and the mobile phase was an eluent with a gradient of acetonitrile (A) and water. The gradient eluent program is set as follows: 7 min 19 % A (isocratic); 7 to 11 min with 19 to 29 % A (isocratic), 11 to 14 min with 29 % A (isocratic); 30 to 31.5 min with 70 to 90 % A (isocratic); 31.5 to 34 min with 90 % A (isocratic); 34 to 34.5 min with 90 to 19 % A (isocratic); 34.5 min to 45 min with 19 % A (isocratic). The sample was injected as much as 5 μL with a sample flow rate of 0.6 mL min^{-1} . The sample was detected using a Diode Array Detector (DAD) at a wavelength of 203 nm. The quantitative calculation is done by comparing the peak area of the sample with the peak area of the reference standard.

2.6 Statistical analysis

All data were analyzed statistically using IBM SPSS statistics 25 software. The fresh weight, dry weight, % yield, and % ginsenoside will be tested using the One Way ANOVA test if the data is normal and homogeneous (parametric test) or tested using the Kruskal-Wallis test if the data is not normal or homogeneous (non-parametric test). Data were considered significantly different if the P value was < 0.05 [10].

3 Result and discussion

Elicitation is a effective strategy to increase the production of secondary metabolites in *in vitro* culture. This process involves adding an elicitor to the growth medium. Elicitors can be defined as compounds that can cause stress to plants when added in small amounts. Elicitors can also increase the biosynthesis of certain compounds because they are used by plants as precursor compounds. To maximize the use of elicitors, there are several things need to be considered, that is elicitor concentration, duration of exposure, age of culture, and media composition [1]. The amount of yeast extract added to the medium is 20 g L^{-1} , whereas because coconut water is a liquid, 10 mL are added. Yeast extract is not a strong elicitor, so it is added from the media preparation so the exposure occurs since the hairy root are inoculated into

the flask. Coconut water can be used as a component in growth media because it contains various kinds of carbohydrates, minerals, and amino acids which can support cell division so it is expected to increase the fresh weight of hairy root [8]. In addition, it is hoped that the addition of coconut water can also promote hairy root growth so that the hairy root enters the stationary phase faster. The stationary phase is the stage where cell division decreases but there is an accumulation of secondary metabolites in plant cells. Therefore, coconut water is also added to the media so that exposure occurs since the root hairs are cultured into the flask media. After incubation for 7 wk and based on the results of HPLC analysis, data obtained can be seen in **Figure 1**.

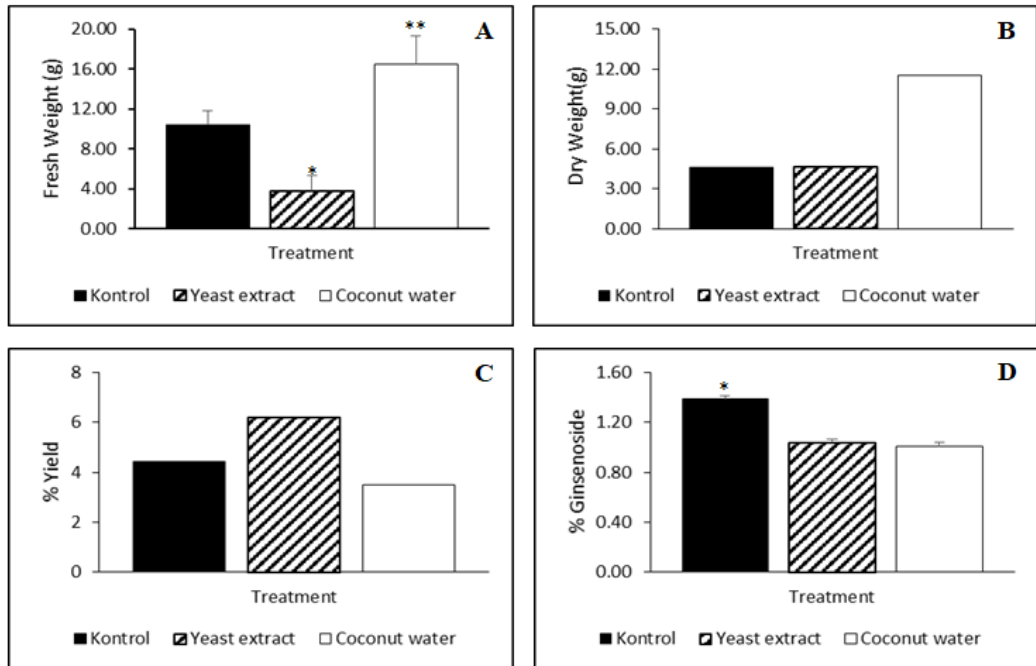


Fig 1. Bar chart showing average fresh weight (A), average dry weight (B), average % yield (C) average % ginsenoside (D) for each hairy root produced by each medium growth. Significant differences were indicated by the presence of signs (*) and (**) P value < 0.05

The average fresh weight produced by the control medium, the yeast extract medium, and coconut water medium were $10.42 \text{ g} \pm 1.38$, $3.80 \text{ g} \pm 1.54$, and $16.50 \text{ g} \pm 2.86$, respectively. Based on the results of statistical analysis, the fresh weight produced by each medium is significantly different from one another where the largest fresh weight produced by coconut water medium that is $16.50 \text{ g} \pm 2.86$, while the smallest fresh weight is produced by yeast extract medium, that is $3.80 \text{ g} \pm 1.54$. The fresh weight produced by the yeast extract medium has a smaller fresh weight produced by the control medium and coconut water medium. This can be caused by the yeast extract causes the hairy root become stress, thus inhibiting the cell division process. It is possible that carbon, minerals, and nitrogen in the medium are diverted to form secondary metabolites so that the biomass decrease. The biggest fresh weight was produced by coconut water medium. This is in accordance with the theory where the largest fresh weight will be produced by the media added with coconut water. This is because coconut water contains sugars in the form of sucrose, sorbitol, glucose, fructose, galactose, xylose, and mannose which can increase cell division. In addition, coconut water also contains various minerals such as potassium, calcium, sodium, magnesium, and many more where these minerals act as cofactors in an enzymatic reaction. Coconut water also contains amino acids such as Alanine, arginine, cysteine, and serine in higher amounts than cow's milk. This amino acid can be used as a nitrogen source to produce faster biomass growth [8]. In addition to increase the fresh weight, it is expected that hairy root that is inoculated in coconut water medium also have high ginsenoside because they enter the stationary phase faster.

After finished drying, the dry weight produced by each flask is very small. For analysis purpose, the dry hairy root produced by the control medium, yeast extract medium, and coconut water medium are each added together. Ten dry hairy root flasks were mixed together to obtain one dry weight data for control medium, two dry weight data for yeast extract medium, and two dry weight data for coconut water medium. The average dry weight produced by the control medium, the yeast extract medium, and the coconut water medium were 4.60 g, 4.70 g, and 11.50 g, respectively. Dry weight data cannot be analyzed statistically because the number of replications is very small. However, although it cannot be analyzed by statistical analysis, it can be seen that the dry weight produced by the coconut water medium is the largest and the dry weight produced by the control medium is the smallest. The dry weight produced by the coconut

water medium is the largest because it has the largest wet weight but the interesting part is the smallest dry weight is the dry weight produced by the control medium where the smallest fresh weight is owned by the yeast extract medium. This can be caused by difference in water content. Hairy root produced by yeast extract medium contains small amount of water so that even though the fresh weight is very small, it has a fairly large dry weight.

This can be seen in the average yield percentage produced by the control medium, yeast extract medium, and coconut water medium are 4.41 %, 6.18 %, and 3.48 %, respectively. From these data, it can be seen that the hairy root produced by yeast extract medium contains small amount of water, via versa, the hairy root produced by control medium and coconut water medium contains a lot of water so that it has a lower yield compared to the yield of the yeast extract medium.

The average levels of ginsenoside produced by the control medium, yeast extract medium, and coconut water medium were $1.39 \% \pm 0.02 \%$, $1.04 \% \pm 0.02 \%$, and $1.00 \% \pm 0.04 \%$, respectively. Based on the results of statistical analysis, the levels of ginsenoside produced by the control medium were significantly different from the levels of ginsenoside produced by the yeast extract medium and coconut water medium, while the levels of ginsenoside between the yeast extract medium and the coconut water medium were not significantly different. The levels of ginsenoside in hairy root extract produced by yeast extract medium and coconut water medium are not higher than control. In the case of yeast extract, this is inconsistent with the theory that the addition of an elicitor can increase the production of secondary metabolites. This can be caused by the use of the concentration of yeast extract that is not optimal. In a report reported by Kochan [4], the optimal concentration of yeast extract is 50 g L^{-1} can increase the ginsenoside level in American ginseng. In addition, the duration of exposure also affects the accumulation of ginsenoside in hairy root. In a report by Kochan [4], it was also reported that ginsenoside levels would decrease with the length of exposure duration, where the peak of ginsenoside accumulation is on the 3rd d since yeast extract is given. In the case of coconut water, the levels of ginsenoside produced are the lowest. From these data it can be concluded that coconut water can increase biomass but cannot increase ginsenoside levels. This is because the hairy root are not in the stationary phase for a long period so that the accumulation of ginsenoside is not as much as the others.

The addition of yeast extract from the start with a concentration of 20 g L^{-1} cannot increase the levels of ginsenoside in hairy root culture of *P. ginseng*. The addition of coconut water from the beginning with a volume of 10 mL increase the biomass but cannot increase the ginsenoside levels in the hair culture of *P. ginseng*. For the next experiment, yeast extract can still be used but the addition is not in the media preparation but after 27 d of incubation and incubated again for 3 d to obtain the biggest ginsenoside level.

This research was conducted by a grant from the Ministry of Research and Ministry of Education, Culture, Research and Technology, Republic of Indonesia, and PT. Bintang Toedjoe, Republic of Indonesia (No. 3122/E3/ PKS.08/KL/2021).

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A Vision4Press website

[All issues](#) ▶ Volume 374 (2023)

◀ [Previous issue](#)

[Table of Contents](#)

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E3S Web of Conferences

Volume 374 (2023)

The 3rd International Conference on Natural Resources and Life Sciences (NRLS) 2020

Virtual, September 23-24, 2020

R.H. Setyobudi, A. Yaro, I. Zekker, M. Zahoor and T. Turkadze (Eds.)

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Vincēviča-Gaile

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PDF (1.910 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

Study of Biodiversity in Submontana of Kamojang Nature Reserve, West Java, Indonesia 00002

Afrisal Isfan Abdillah, Silva Eka Putra Utama and Nguyen Van Minh

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400002>

PDF (3.913 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

Improving the Capability of Corn Processing into Tortillas by Family Welfare Programme, In Gedong, Ngadirojo, Wonogiri Regency, Central Java, Indonesia 00003

Afriyanti Afriyanti, Novian Wely Asmoro, Retno Widyastuti, Catur Budi Handayani, Ira Liana Sari, Rahayu Relawati and Peeyush Soni

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400003>

PDF (5.133 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

The Awareness of Intellectual Property Rights (IPRs) Regimes on Small and Medium Enterprises (SMEs) of Agricultural Products Processing at Malang Area, East Java Province, Indonesia 00004

Aris Winaya, Maftuchah Maftuchah, Sofyan Arif, Leila Neimane and Ida Ekawati

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400004>

PDF (4.153 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

Buyer Decisions on Hydroponic Vegetable Products 00005

Asgami Putri, Jabal Tarik Ibrahim, Adi Sutanto, Syafrani Syafrani, Bambang Yudi Ariadi, Istis Baroh, Rahayu Relawati, Juris Burlakovs, Erni Hawayanti, Sri Utami Lestari et al. (6 more)

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400005>

PDF (4.188 MB) | [References](#) | [NASA ADS Abstract Service](#)

Utilization of Green Tea Extract on Anti-aging Cream With Butylated Hydroxytoluene (BHT) and Tertiary Butylhydroquinone (TBHQ): Physical Stability Aspect 00006

Cynthia Marisca Muntu, Yulianita Yuwono, Christina Avanti and Manar Fayiz Mousa Atoum

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400006>

PDF (5.577 MB) | [References](#) | [NASA ADS Abstract Service](#)

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The Effect of Pod Storage on Chemical and Microbiological Characteristics of Organic and Non-organic Balinese Cacao Pulps 00007

Christina Mumpuni Erawati, Ruth Chrisnasari and Peeyush Soni

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400007>

PDF (4.176 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

Characterization Properties of Banana Peel as a Promising Alternative for Bioplastic 00008

Dina Maria Abel, Juvencio de Castro Ruas, Adilson de Castro Ruas and Tjie Kok

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400008>

PDF (3.790 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

Assessment of *Aegle marmelos* Fruit Extracts as Organic Insecticide for *Spodoptera exigua* on *Allium ascolanicum* 00009

Dyah Roeswitawati, Teuku Ramzy, Praptiningsih Gamawati Adinurani, Roy Hendroko Setyobudi, Zahid Hussain, Irum Iqrar and Nguyen Ngoc Huu

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400009>

PDF (4.830 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

Sterilization of Ready to Serve Product for Special Needs of Hajj and Umrah: Skipjack Tuna in A Retort Pouch Package 00010

Elsa Azhari, Muhamad Subroto Aliredjo, Agus Heri Purnomo, Damat Damat, Maizirwan Mel, Satriyo Krido Wahono, Suherman Suherman and Erkata Yandri

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400010>

[Open Access](#)

Production and Nutrient Value of Elephant Grass in Agroforestry Systems in Indonesia 00011

Endang Dwi Purbajanti, Didik Wisnu Widjajanto, Praptiningsih Gamawati Adinurani, Zahid Hussain and Ida Ekawati

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400011>

[PDF \(3.940 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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Quality Assessment on Honey Produced from Six Months Old *Acacia crassicarpa* 00012

Eni Suhesti, Lili Zalizar, Joko Triwanto, Ervayendri Ervayendri, Roy Hendroko Setyobudi, Nugroho Tri Waskitho, Jabal Tarik Ibrahim, Maftuchah Maftuchah, Hadinoto Hadinoto, Zane Vincēviča-Gaile et al. (5 more)

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400012>

[PDF \(4.880 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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Incentive Strategy for Energy Efficiency Programs in Industries Consuming 6 000 TOE/year with Sustainable Energy Performance 00013

Satryo Martoyoedo, Priyadi Priyadi, Dewanto Fajrie, Ratna Ariati, Erkata Yandri, Roy Hendroko Setyobudi, Suherman Suherman, Juris Burlakovs, Maizirwan Mel, Satriyo Krido Wahono et al. (4 more)

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400013>

[PDF \(4.577 MB\)](#) | [References](#) | [NASA ADS Abstract Service](#)

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Bird Diversity, Abundance, and Evenness Rates in Ecotone Area of Sutan Syarif Hasyim Forest Park, Riau, Indonesia 00014

Hadinoto Hadinoto, Lili Zalizar, Joko Triwanto, Ervayenri Ervayenri, Roy Hendroko Setyobudi, Muhammad Chanan, Nugroho Tri Waskitho, Jabal Tarik Ibrahim, Eni Suhesti, Nguyen Van Minh et al. (3 more)

Published online: 21 March 2023

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The Length-Weight Relationships and Condition Factors of *Potamocorbula faba* Hinds., 1843 in the Permisan Bay, East Java, Indonesia 00015

Hariyadi Hariyadi, Aris Winaya, Muhammad Zainuri, Norma Afiati, Lachmudin Sya'rani and Olga Anne

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400015>

PDF (5.818 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

Biodiesel Industrial Waste based on *Jatropha curcas* as a Fungicide to Control *Fusarium oxysporum* and *Alternaria solani* 00016

Henik Sukorini, Dyah Erni Widyastuti, Dini Kurniawati, Sawita Suwannarat, Maizirwan Mel and Roy Hendroko Setyobudi

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400016>

PDF (6.054 MB) | [References](#) | [NASA ADS Abstract Service](#)

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Population Development and Disease Incidence of Virus Disease Transmitted by Brown Planthopper on the Paddy Field Applied with Biofertilizers and Biopesticides 00017

I. Nyoman Widiarta, Ety Pratiwi, I. Putu Wardana and Oky Dwi Purwanto

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400017>

PDF (5.254 MB) | [References](#) | [NASA ADS Abstract Service](#)

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Moringa oleifera L. Microgreens and their Antioxidant Activity 00018

Ida Bagus Made Artadana and Edward Pandji

Published online: 21 March 2023

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PDF (4.458 MB) | [References](#) | [NASA ADS Abstract Service](#)

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The Effect of Elisitor on Growth and Ginsenoside Level in Hairy Root Culture of *Panax ginseng* Cultivated in Shake Flasks 00019

Johan Sukweenadhi, Stefan Pratama Chandra, Leonardo Satriono Putra, Yoanes Maria Vianney, Theresia Liliani, Merlyn Wongso, Melisa Widjaja, Sari Pramadiyanti, Pissa Christanti, Kim-Jong Hak et al. (2 more)

[Open Access](#)

Zeolite-microfragmenting Media: A Potential Strategy to Accelerate Coral Growth 00020

Khaulah Mujahidah, Aolia Ramadan, Veryl Hasan, Sahri Yanti, Izzul Islam and Irum Iqrar

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400020>

PDF (7.021 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

Prevalence of Gastrointestinal Helminthiasis in Beef Cattle During Dry Season in Bangkalan Regency, Madura, Indonesia 00021

Lili Zalizar, Aris Winaya, Yusuf Ridwan, Eka Arif Hardiansyah and Ravindran Jaganathan

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400021>

PDF (4.045 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

The Effect of Mangosteen Varieties as Dyes and ZnO Nanostructures Mixture to DSSC - Dye-sensitized Solar Cell Characteristics 00022

Lizda Johar Mawarani, Ratna Puspitasari, Doty Dewi Risanti and Luqman Ali Shah

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400022>

PDF (5.064 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

Bidens pilosa Linn.: Beautiful Weed for the Healthy Mouth – A Mini Review 00023

Mariana Wahjudi, Gracelynn Meira, Hadinata Santoso and Assidiq Zidane Irwansyah

Published online: 21 March 2023

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PDF (4.719 MB) | [References](#) | [NASA ADS Abstract Service](#)

[Open Access](#)

Hematological Features of Cihateup's Duck Blood that are Given Natural Isotonic in Dry Systems 00024

Nurul Frasiska, Putri Dian Wulansari, Novia Rahayu, Abdul Razak Alimon, Wahyu Widodo and

Nguyen Ngoc Huu

Published online: 21 March 2023

Open Access

Influence of *Hibiscus tiliaceus* Extract and Suspension on *Echerichia coli* and *Staphylococcus aureus* Growth 00025

Oktavina Kartika Putri, Lina Oktavia Rahayu, Gardiani Febri Hadiwibowo, Yuly Kusumawati and Asma Nisar

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400025>

PDF (4.960 MB) | [References](#) | [NASA ADS Abstract Service](#)

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Keiki induction by cytokinin on *Phalaenopsis* spp. 00026

Popy Hartatie Hardjo, Ida Bagus Made Artadana, Sulistyo Emantoko Dwi Putra and Asad Jan

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400026>

PDF (4.351 MB) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

The Pro-Environmental Producer Behavior on Food Small and Medium Enterprises in Malang, Indonesia 00027

Rahayu Relawati, Bambang Yudi Ariadi, Harpowo Harpowo, Bambang Hadi Prabowo, Leila Neimane and Ida Ekawati

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400027>

PDF (3.858 MB) | [References](#) | [NASA ADS Abstract Service](#)

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Antioxidant Activity Between Defatted and Different Solvent Temperature in Rice Bran var. IR-64 Extract 00028

Retno Widyastuti, Rahmat Dwi Irwanto, Enny Purwati Nurlaili, Sri Hartati and Irum Iqrar

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400028>

PDF (4.346 MB) | [References](#) | [NASA ADS Abstract Service](#)

Open Access

Inhibition of Carica Solid Soap to the Growth of *Staphylococcus epidermidis* Bacteria 00029

Roisatul Ainiyah, Cahyaning Riniutami and Muhannad Illayan Massadeh

Published online: 21 March 2023

-
- [Open Access](#)
Pest Control using Bark Pesticide Applicator (BPA) in Citrus Plants 00030
Rudi Cahyo Wicaksono, Otto Endato, Susi Wuryantini and Zahid Hussain
Published online: 21 March 2023
DOI: <https://doi.org/10.1051/e3sconf/202337400030>
PDF (5.466 MB) | [References](#) | [NASA ADS Abstract Service](#)
-
- [Open Access](#)
Food Waste Product for Overcoming Heat Stress in Broilers 00031
Rusli Tonda, Manar Fayiz Mousa Atoum, Roy Hendroko Setyobudi, Lili Zalizar, Wahyu Widodo, Mohammad Zahoor, David Hermawan, Damat Damat, Ahmad Fauzi, Asgami Putri et al. (12 more)
Published online: 21 March 2023
DOI: <https://doi.org/10.1051/e3sconf/202337400031>
PDF (5.053 MB) | [References](#) | [NASA ADS Abstract Service](#)
-
- [Open Access](#)
Thermostable Chitinase Producing Bacterium from Ijen Hot Spring – Indonesia: Isolation, Identification, and Characterization 00032
Ruth Chrisnasari, Liony Priscilla Sutanto, Dian Paulina, Alicia Wahjudi and Tjandra Pantjajani
Published online: 21 March 2023
DOI: <https://doi.org/10.1051/e3sconf/202337400032>
PDF (5.407 MB) | [References](#) | [NASA ADS Abstract Service](#)
-
- [Open Access](#)
Infestation of *Dendrophthoe pentandra* (L.) Miq. on Various Canopy Shading and Plants Diversity in Purwodadi Botanic Garden, Indonesia: A study on *Cassia fistula* L. 00033
Solikin Solikin, Melisnawati Hamza Angio, Tri Handayani and Nguyen Van Minh
Published online: 21 March 2023
DOI: <https://doi.org/10.1051/e3sconf/202337400033>
PDF (6.480 MB) | [References](#) | [NASA ADS Abstract Service](#)
-
- [Open Access](#)
The Impact of Madden - Julian Oscillation and Sea Surface Temperature Process Interaction on Rainfall Variability During Rainy Season: A Case Study in East Nusa Tenggara, Indonesia 00034
Sudirman Sudirman, Amir Mustofa Irawan, Dzikrullah Akbar, Peeyush Soni and Leila Neimane
Published online: 21 March 2023
-

-
- [Open Access](#)
Bioelectrochemical System Application for Pesticides Removal: A mini-review 00035
Theresia Desy Askitosari and Amanda Larasati
Published online: 21 March 2023
DOI: <https://doi.org/10.1051/e3sconf/202337400035>
PDF (4.084 MB) | [References](#) | [NASA ADS Abstract Service](#)
-
- [Open Access](#)
Mini-Review: Extraction of Patchouli Oil from *Pogostemon cablin* Benth. Leaves 00036
Tjie Kok, Natasha Florenika, Mangihot Tua Gultom, Popy Hartatie Hardjo and Muhannad Illayan Massadeh
Published online: 21 March 2023
DOI: <https://doi.org/10.1051/e3sconf/202337400036>
PDF (4.277 MB) | [References](#) | [NASA ADS Abstract Service](#)
-
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Herbs as A Feed Additive in the Broilers for the Sustainability of Local Products 00037
Wahyu Widodo, Adi Sutanto, Imbang Dwi Rahayu, Apriliana Devi Anggraini, Trisakti Handayani, Roy Hendroko Setyobudi, Maizirwan Mel and Nguyen Ngoc Huu
Published online: 21 March 2023
DOI: <https://doi.org/10.1051/e3sconf/202337400037>
PDF (3.889 MB) | [References](#) | [NASA ADS Abstract Service](#)
-
- [Open Access](#)
Bioremediation in the Marine Environment: Challenges and Prospective Methods for Enhancement 00038
Watumesa Agustina Tan, Gabrielle Celina and Stephanie Pranawijaya
Published online: 21 March 2023
DOI: <https://doi.org/10.1051/e3sconf/202337400038>
PDF (3.980 MB) | [References](#) | [NASA ADS Abstract Service](#)
-
- [Open Access](#)
Characterization of Endophytic Bacteria Isolated from *Avicennia marina's* Leaf Tissue Collected from Ekowisata Mangrove Wonorejo Surabaya, Indonesia 00039
Wina Dian Savitri, Marvel Lewi Santoso, Yulanda Antonius, Popy Hartatie Hardjo and Asad Jan
Published online: 21 March 2023

Open Access

Identification of DNA G–quadruplex Forming Sequence in Shrimp White Spot Syndrome Virus (WSSV) 00040

Yoanes Maria Vianney, Priscilla Kandinata, Klaus Weisz and Maria Goretti Marianti Purwanto

Published online: 21 March 2023

DOI: <https://doi.org/10.1051/e3sconf/202337400040>

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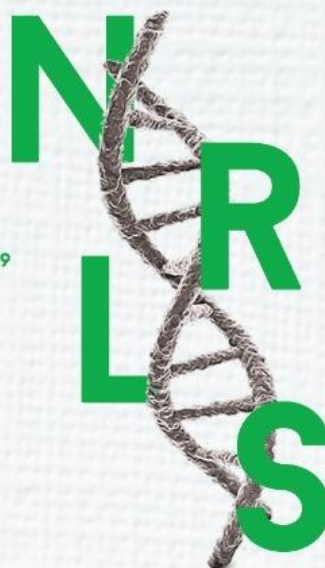
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PREFACE: the 3rd International Conference on Natural Resources and Life Sciences (NRLS) 2020

Nowadays, the sustainability of energy generation and environmental wellness are two of the big challenges in the world. Ecological disturbance and depletion of non-renewable energy in most countries urge the need for development and exploration of sustainable bioenergy resources. A proper approach to implement biotechnology for converting and conserving resources will be of great importance. Ideally, the conversion of natural resources into a certain form of energy should be parallel with the bioproduction of valuable compounds. Furthermore, environmental wellness does encourage us to employ proper habits that promote a healthy environment for a healthy life.

The bioproduction of valuable compounds originating from renewable resources and carbon-neutral waste materials as substrates is a promising approach for a sustainable environment and healthy life. Recent studies on genetic, epigenetic, protein and metabolic engineering offer significant improvement strategies in the bioproduction of many valuable compounds, such as biosurfactants, biofuels, bioinsecticides, bioplastic precursors, biopharmaceuticals, functional food, etc. Hence, in the 3rd International Conference on Natural Resources and Life Sciences (NRLS) themed Green technology to promote sustainable clean energy, environmental wellness, and healthy life, we will highlight the latest developments in biotechnological research and its applications, consisting of Clean Energy and Environmental Sustainability, Sustainable Agriculture, and Health Care.

Following the successful program of the 1st & 2nd NRLS, we intend to make the two-day meeting followed by a one-day workshop in the 3rd NRLS, as a global forum for scientific and industrial communities to discuss the recent advances in biotechnological research and its application. It is our great pleasure to welcome you to the virtual 3rd NRLS from September 23rd to 24th, 2020.

After a rigorous selection process, the Scientific & Editorial Board (S.E. Board) of the virtual 3rd NRLS decided to publish 40 papers in the E3S Web of Conferences, an open-access proceeding

in environment, energy, and earth sciences, managed by EDP Sciences, Paris, France, and indexed on Scopus, Scimago, Conference Proceedings Citation Index-Science (CPCI-S) of Clarivate Analytics's Web of Science, and DOAJ (Directory of Open Access Journals). As a result, E3S Web of Conferences is a conference proceeding with the highest SJR (Scopus and Scimago) score compared to other conference proceedings.

The proceeding of the 3rd NRLS comprises 40 selected papers compiled by 253 authors from 72 institutions. To improve the quality of manuscripts, S.E. Boards apply for a "guidance program" for several manuscripts. The impact there is joint research by Indonesian and overseas scholars. In the collaboration research, 22 institutions were involved, of which were from abroad Indonesia. The overseas institutions are from Germany, India, Jordan, Pakistan, the Republic of Korea, Latvia, Lithuania, Malaysia, the Netherlands, Poland, Taiwan - ROC, Thailand, Timor Leste, the United Kingdom, and Vietnam. Each manuscripts submitted in the E3S Web of Conferences was reviewed by at least two experts using the double-blind system (list of the experts in S.E. Board). As a result, the published articles have passed all necessary improvement requirements following the Web of Conferences standard, reviewer's comments, S.I. (*Système International d'Unités*), similarity tests by the Turnitin program (with the highest threshold of 20 %), meet the standard value of 85 % of the Premium Grammarly program, amount 90 % of references must be at least dated from 15 years and reflected on Google, as well as editing procedures by professional editors from five countries (Indonesia, Estonia, Georgia, Pakistan, and the United Kingdom).

We thank all presenters and attendees for the rigorous participation in this conference to share scientific ideas, inspire new studies for closer co-operations. We hope you are encouraged for further collaboration in order to explore natural resources and life sciences in future. We look forward to inviting you for the next 4th NRLS.

Surabaya, Februari 28, 2023



Dr.rer.nat. Theresia Desy Askitosari, S.Si., M.Biotech.
3rd NRLS Chairwoman

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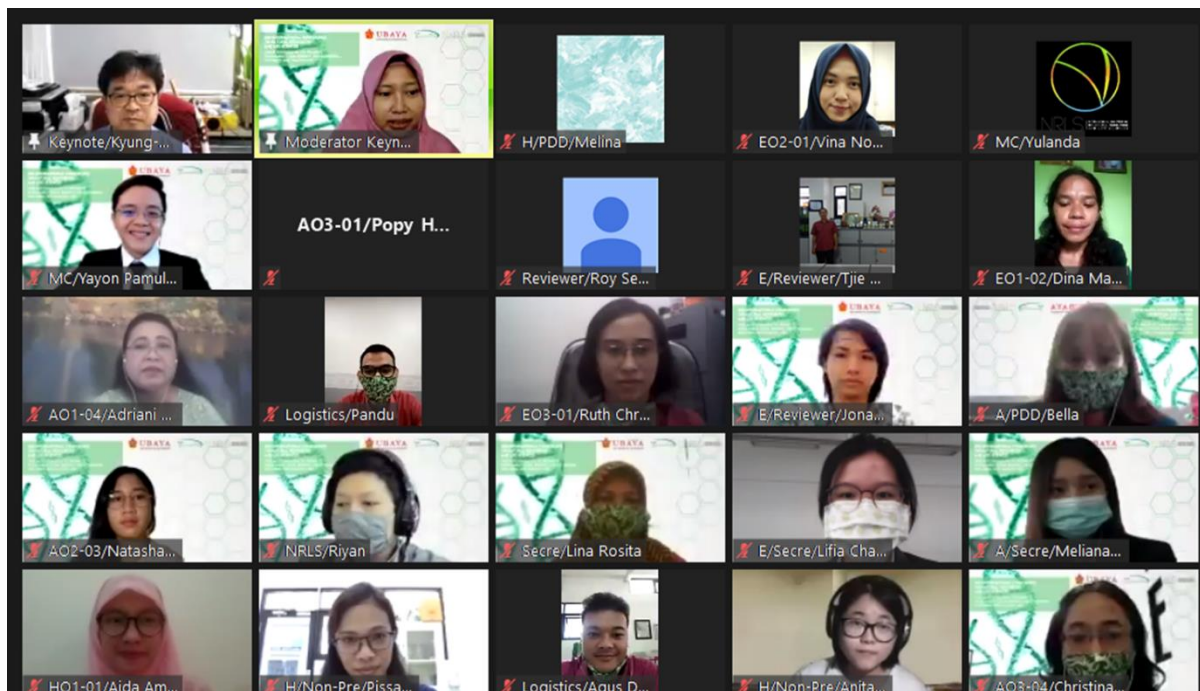
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- Trias Agung Pakarti, University of Brawijaya, and RP Editing & Proofreading Servives (IDN).
- Tsitsino Turkadze, Department of Chemical and Environmental Technologies, Akaki Tsereteli State University, Kutaisi (GEO).
- Yahya Jani, Division of Sustainable Environment and Construction, School of Business Society and Engineering, Mälardalen University (SWE).
- Yusilawati Ahmad Nor, Department of Biochemical-Biotechnology Engineering, Kuliyyah of Engineering, International Islamic University Malaysia (MYS).

The VIRTUAL 3rd NRLS: CONFERENCE PHOTOS



The VIRTUAL 3rd NRLS: Invited Speakers

Dr. Henry Budi Utari
THANK YOU FOR SHARING YOUR KNOWLEDGE



Dr. rer. nat. Theresia Desy Askitosari
THANK YOU FOR SHARING YOUR KNOWLEDGE



Prof. Berthold Hocher
THANK YOU FOR SHARING YOUR KNOWLEDGE



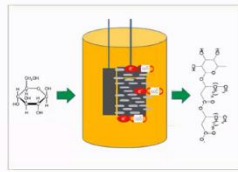
Prof. Kyung Min Kim
THANK YOU FOR SHARING YOUR KNOWLEDGE



Prof. Yashwant Pathak
THANK YOU FOR SHARING YOUR KNOWLEDGE



<https://www.google.com/search?q=nrls+ubaya+2020&oq=nrls&aqs=chrome.0.69i59l2j69i57j69i59j46i10i433i512j0i10i512j69i61l2.4512j0j7&sourceid=chrome&ie=UTF-8#fpstate=ive&vld=cid:4c89479a,vid:YvnRnLbw17s>



Bioelectrochemical System Application: An Insight into Green Technology

THERESIA DESY ASKITOSARI



Ruprecht-Karls-Universität Heidelberg



1. Can Plant Viruses Cross the Kingdom Border

Introduction

- It is currently accepted that a strict separation exists between plant and vertebrate viruses regarding their host range and pathogenicity, and plant viruses are believed to infect only plants.
- Some plant and animal viruses are closely related; humans are considerably exposed to plant viruses; plant viruses may trigger events in mammalian cells and elicit immune responses in invertebrates, non-human vertebrates and humans, and was recently associated with clinical symptoms.
- The distribution of plant and animal viruses within the currently defined indicates that some plant and animal viruses belong to same viral families.
- In 1988, CGMMV, a tobamovirus, was recovered in cow manure three days after feeding with infected cucumbers.

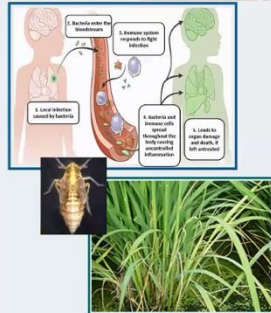


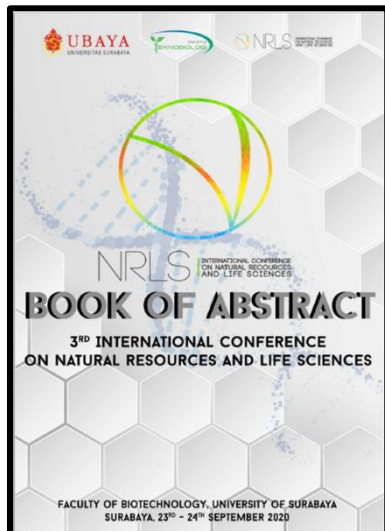
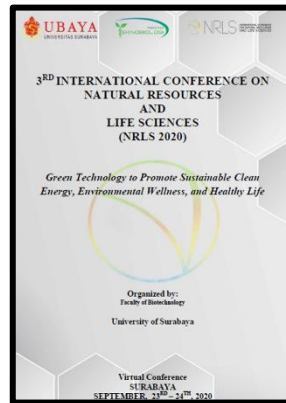
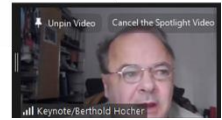
Figure. Sapovirus occurs when a local infection spreads to the entire body. Find out what causes sapovirus and how sapovirus is treated. <https://www.dailymotion.com/video/x7j2k1k>

Fetal Programming - from Epidemiology to Epigenetics

Berthold Hocher

E-Mail: berthold.hocher@medma.uni-heidelberg.de

Department of Medicine (Nephrology/Endocrinology/Rheumatology), University Medical Centre Mannheim, University of Heidelberg, Heidelberg, Germany
School of Medicine, Hunan Normal University, Changsha, China; Reproductive Genetic Hospital of CITIC-Xiangya, Changsha, China;



Stress and CVD: How to reduce the stress? Understand the pathways of the stress

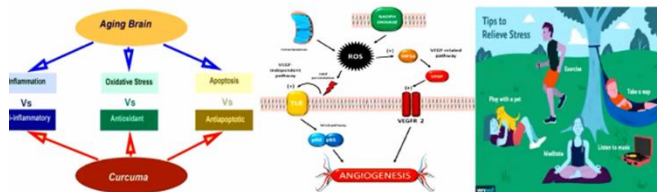
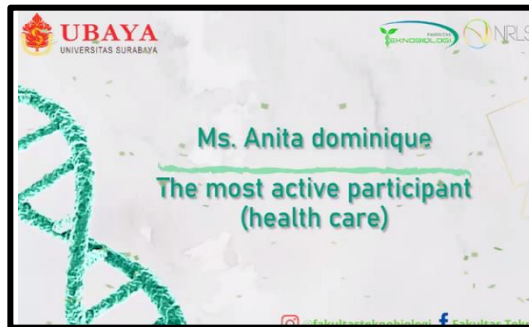
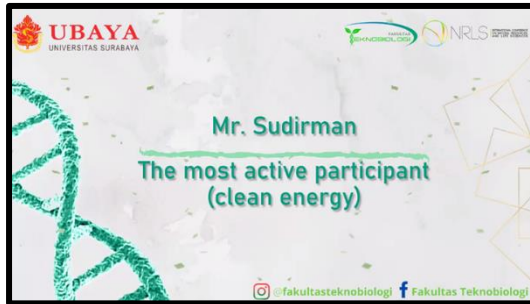


Figure taken from https://www.researchgate.net/figure/Oxidative-stress-and-angiogenesis-The-two-sources-of-oxidative-stress-mitochondria_fig1_284180117


<https://manage.digiseminar.id/public/nrls/nrls3/boa.pdf>



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The VIRTUAL 3rd NRLS: Sponsors



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ON NATURAL RESOURCES
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**FACULTY OF BIOTECHNOLOGY, UNIVERSITY OF SURABAYA
SURABAYA, 23RD - 24TH SEPTEMBER 2020**

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2. All articles have been subjected to peer review administered by the proceedings editors.
3. Reviews have been conducted by expert referees, who have been requested to provide unbiased and constructive comments aimed, whenever possible, at improving the work.
4. Proceedings editors have taken all reasonable steps to ensure the quality of the materials they publish and their decision to accept or reject a paper for publication has been based only on the merits of the work and the relevance to the journal.

Title, date and place of the conference

Title: The 3rd International Conference on Natural Resources and Life Sciences (NRLS) 2020

Date: September 23rd to 24th, 2020.

Place: Virtual.

Website: <https://www.youtube.com/watch?v=YvnRnLbwI7s>

<https://www.facebook.com/817522545315115/posts/nrlsnrls-day-1-is-still-on-going-appreciate-to-all-participant-who-join-to-our-i/1150840888649944/>

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Muhammad Zahoor (Peshawar, PAK), and Tsitsino Turkadze (Kutaisi, GEO).

Date and editor's signature

Malang, Indonesia, Februari 22, 2023

Dies Cinerum/Ash Wednesday,

(Roy Hendroko Setyobudi)

Ps. Thank you to our God because E3S has been covered again by Scopus. Congrats!



Natural Resources Life Sciences <nrls@unit.ubaya.ac.id>

Letter of Acceptance Abstract

1 message

Natural Resources Life Sciences <nrls@unit.ubaya.ac.id>

Mon, Aug 24, 2020 at 10:46 PM

To: sukwee@staff.ubaya.ac.idSurabaya, August 24th 2020

To

Mr./ Mrs. Johan Sukweenadhi

ABSTRACT ACCEPTANCE LETTER

Dear Johan Sukweenadhi

We are pleased to inform you that submitted abstract as specified below :

Title: The Effect of Elicitor on Growth and Ginsenoside Level in Hairy Root Culture of *Panax ginseng* Cultivated in Shake Flasks

Author: Johan Sukweenadhi*, Stefan Pratama Chandra, Leonardo Satriono Putra, Yoanes Maria Vianney, Theresia Liliani, Merlyn Wongso, Melisa Widjaja, Sari Pramadiyanti, Pissa Christanti, Kim-Jong Hak, Deok-Chun Yang

Has been officially accepted for **oral presentation** in International Conference on Natural Resources and Life Sciences which held online on September 23th -24th ,2020

Please make bank transfer payment payable to:

Bank Name : Central Bank Asia (BCA – Indonesia)

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Please send the proof of your payment to nrls@unit.ubaya.ac.id.Regarding the complete registration payment, we would like to clarify our previous e-Mail (notification of abstract acceptance). You can still complete your registration payment until August 28th at a early bird rate.

A handwritten signature in purple ink, appearing to read 'Desy'.

Yours Sincerely

Dr. rer. nat. Theresia Desy Askitosari

Executive Chief of NRLS Committee 2020