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Evie Ratnasari

Attended

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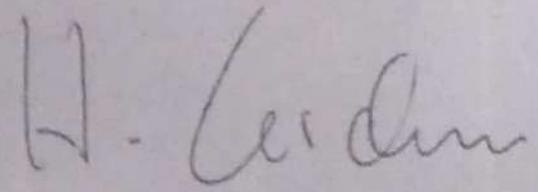
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PROCEEDING

ICOLIB

International Conference on Life Sciences and Biotechnology



EXPLORATION AND CONSERVATION OF BIODIVERSITY

The ICOLIB 2015 focuses on life sciences and biotechnology aspects to explore and conserve biodiversity by bringing together investigators from different fields such as health and medicine, agriculture, food technology and security, new and renewable energy, conservation and management including exploration of biodiversity

**Aston Jember Hotel
& Conference Center**
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**International Conference on Life Sciences and Biotechnology
(ICOLIB)**

Exploration and Conservation of Biodiversity

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Preface from the Editor

The explosive development of the sciences and its expansion into other disciplines such as the Life Sciences field is yielding groundbreaking discoveries from novel genes and bio-products to cutting-edge nanotechnology, resulting in a transformed science landscape with profound global applications in understanding life, eradicating diseases, securing a more equitable food and water supply distribution as well as creating novel bio-industries and products.

Based on these phenomena above, the ICOLIB 2015 with theme “**Exploration and Conservation of Biodiversity**”, provide an interdisciplinary platform of life sciences for researchers, academics, students, professionals, industries, and policy makers. This meeting also proposed to among scientists and professionals to stay at the leading edge of recent advances in life sciences and sustainability, act as a catalyst for further research, improve international collaboration while bridging the scientific and technological differences among scientists, and foster global health security. In order to disseminate to community more broadest, the articles were published as a proceeding.

The conference was organized by the Department of Biology, Faculty of mathematic and natural sciences, The University of Jember collaboration with the Flensburg University of Applied Sciences, Deutscher Akademischer Austausch Dienst (DAAD), Indonesian-German Network for Teaching, Training and Research Collaboration (IGN-TTRC), University of Kassel and IndoBIC (Indonesian Biotechnology Information Centre) The Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP). The conference participants from 5 countries and of which 9 lectures within the field health and medicine, agriculture, food technology and security, new and renewable energy, conservation and management including exploration of biodiversity. Presentation divided into plenary, oral and poster session. More than 150 researchers including students participated on this meeting.

On behalf of the organizing committee, i would like to thank all invited speakers and presenters for participating in the ICOLIB 2015 for giving valuable contribution to this conference. Also, acknowledgements are address to Rector University of Jember, Flensburg University of Applied Sciences, DAAD, Indonesian-German Network for Teaching, IGN-TTRC, University of Kassel and IndoBIC-SEAMO BIOTROP as well as all sponsors for the efforts. Finally, i would like to express deep appreciation to the member of the organizing committee for the good teamwork and the great effort to bring success to the conference.

Jember, September 2015

Kahar Muzakhar
Committee

CONTENTS

Preface.....	i
Contens.....	ii

KEYNOTE SPEAKER

Jumping DNA: Regulation and Application in Functional Gene Analysis Doreen Meier, Michael Friedrich, Isabelle Schuster, Wolfgang Nellen.....	1
Enzymes from Indonesian Biodiversity: Molecular Characterization and Their Potential Applications Debbie S. Retnoningrum.....	6
Bioconversion of Rare Sugars by Sugar Isomerase and Epimerase from Microorganisms Chang-Su Park.....	7
Molecular Study on Drought Stress Response to Improve Sugarcane Productivity by Genetic Transformation Bambang Sugiharto.....	10
Contribution of Integrated Pest Management in Enhancing Biodiversity in Tropical Vegetable Production R. Srinivasan.....	11
Risk Assessment of Silver Nano Particles Helmut Erdmann.....	15
Conservation of Threatened Tree Species on Ex-Mine Sites in Indonesia Irdika Mansur.....	16
Status and Challenges of Agriculture Biotechnology Development in Indonesia Bambang Purwantara.....	17

ORAL SESSION

Role of S100β Protein as Biomarkers Prenatal Ischemic Hypoxic Ratna Indriawati.....	18
Increasing Environmental Health Through Early Childhood Health Empowerment in Applying The PHBS in Gumuksari 3 Elementary School Kalisat Jember Muthmainah Farida Hanif, Eriena Melati Sukma, Mury Ririanty.....	20
Quality Evaluation and Preparation of Voluntary Acceptance of Jelly Candy Ketepeng China Leaf Extract (<i>Senna Alata L.</i>) Roxb as Anthelmintic Ratna Novita Maya Sari, Kartini.....	26
Structural and Functional Recovery of B-Cells Pancreas in Type 1 Diabetes Mellitus Induced Mesenhyal Stem Cell Conditioned Medium Widagdo Sri Nugroho, Dwi Liliek Kusindarta, Heru Susetya, Ida Fitriana,	

Guntari Titik Mulyani, Yuda Heru Fibrianto, Aris Haryanto, Teguh Budipitojo.....	29
Nanomedicine: A “Panacea” in Medicine	
Dito Anurogo, Taruna Ikrar.....	33
Toxicity of Mixturing of <i>Annona Squamosa</i> Seed and <i>Piper betle</i> Leaf Extract for The Mortality of <i>Aedes aegypti</i> Larvae (Preliminary Study)	
Dwi Wahyuni, Intania Loren, Muhammad Roy F.R.....	37
The Ant Plant (<i>Myrmecodia pendans</i>) Infuse as an Acute Diarrhea Medicine	
Yoni Astuti, Tantri Wahyu Utami, Idiani Darmawati.....	41
The Effects of Different Diluent Medium on Human Sperm Viability	
Isnawati, Tjandrakirana, Nur Ducha.....	45
Antibacterial Activity of <i>Pleurotus ostreatus grey oyster</i> Variety Against Pathogen Bacterial of <i>Salmonella typhi</i>	
Lita Meilina, Evi Hanizar, Dwi Nur Rikhma Sari.....	48
The Effect of Extract Thymoquinone Black Cumin Seed (<i>Nigella sativa</i>) of Against Neutrophils Number in Rats Socket Post Extraction with Traumatic Tissue	
Asri Dinar Pawestri, Abdul Rochim, Mei Syafriadi.....	52
Traditional Medication of Osing Tribe in Banyuwangi	
Novia Luthviatin, Pudjo Wahjudi, Siti Muslichah.....	56
Antihyperlipidemic Activity of The Combination of <i>Guazuma Ulmifolia</i> L. Leaves and <i>Hibiscus Sabdariffa</i> L. Flowers Extract in Rats Induced by High-Fat Diet	
Nuri, Ika Puspitasari, Mahmudatus Sholihah, Putu Argianti Meytasari, Novia Hilma..	60
The Effect of Tomato (<i>Lycopersicum pyriforme</i>) Extract on Lipid Peroxide and Cell Damage of Carbontetrachloride in Rat Liver	
Poncojari Wahyono.....	65
The Role of Water Clover (<i>Marsilia crenata</i>) on Estrogen Level and Uterine Histology in Rats (<i>Rattus norvegicus</i>)	
Pratiwi Trisunuwati, Nurina Titisari, Ahmad Fauzi, Anom Adnyana.....	69
Effect of Cadmium (Cd) Accumulation on Protein Levels of Gills and Kidneys Freshwater Mussel <i>Elongaria orientalis</i> (Lea, 1840)	
Selvi Ariyunita, Akhmad Syakur, Andhika Puspito Nugroho.....	75
Effects of Antioxidant Different in Cep Diluent on Sperm Quality of Brahman Bull During Storage at Refrigerator Temperature	
Nur Ducha, Tjandrakirana, Lisa Lisdiana.....	79
Effect of Emotional Intelligence and Physical Intelligence Attitudes Towards Healthy Living Leather Glove Factory Employees in Yogyakarta	
Tri Pitara Mahanggoro, Soemarno, Edi Widjajanto, Pratikto Prawoto	83
The Effect of Bases Toward Disintegration Time of Phyto-Capsules	
Esti Hendradi, Siti Wafiroh, Muji Harsini, Handoko Darmokoesoemo,	

Pratiwi Pudjiastuti.....	87
DBLβC2 Domain of Var Gene of Indonesian <i>Plasmodium falciparum</i> Had an Association with Severity of Malaria	
Erma Sulistyaningsih	89
Odonata of Island Garden City of Samal and Its Relation to Other Small Islands in The Philippines	
Milton Norman Dejadena Medina, Anlyn Anzano Cabras, Reagan Joseph Torayno Villanueva.....	93
Orchid Mycorrhizae Fungus: Identification of Rhizoctonia in West Borneo	
Rosa Suryantini.....	98
<i>Sauropus androgynus</i> (L.) Merr. Leaf Variation That Grows in The Area of Some Traditional Societies in East Java	
Ari Hayati, Estri Laras Arumingtyas, Serafinah Indriyani, Luchman Hakim.....	104
Characterisation of Symbiotic Bacteria Isolated From Sponge <i>Haliclona sp.</i>	
Sapto Andriyono, Bayu Jalasena, Wahyu Tjahtjaningsih, And Heru Pramono.....	110
Antibacterial Activity of Infusion and Ethanol Extract of Some Medicinal Plants as Antidiarrhea Based on Bangka Society's Knowledge	
Henny Helmi.....	116
Diversity and Distribution of Dragonflies (Odonata) in Bromo Forest Area (BKPH Lawu Utara : KPH Surakarta) Central Java	
Diagal Wisnu Pamungkas, Euis Citra Ayu Ruspendi, Inna Listri Ani.....	123
Giving of Protein Dietary Level Broodstock of Catfish (<i>Clarias Sp</i>) and Laserpuncture Induction to Estrogen Level and GSI	
Dyah Hariani, Pungky Slamet Wisnu Kusuma.....	128
Fish Diversity of <i>Cyprinidae</i> Family Based on DNA Barcodes in Harapan Rainforest, Jambi	
T. Sukmono, D. Duryadi, M.F Rahardjo, R. Affandi.....	134
Brown Planthopper Populations on Some Rice Varieties	
Retno Wijayanti, Supriyadi, Sholahuddin.....	139
Morphological Variation of Local Durian (<i>Durio zibethinus</i> Murr.) on The Ternate Island	
Sundari.....	143
Mealybugs and Their Natural Enemies Diversity on Cassava Crops (<i>Manihot esculenta</i> Crantz)	
Nurmasari, Purnomo, Purwatiningsih.....	149
Local Wisdom in The Making of Plants Based Biopesticides by Organic Rice Farmers in East Java	
Lisa Lisdiana, Yuliani.....	152
GC-MS Analysis of Phytocomponents in The Methanolic Extract of <i>Justicia gendarussa</i> Burm. f	
Hamidah, Dwi Kusuma Wahyuni, Noer Moehammadi.....	158

The Use of The Local Flora as Biopesticides by Organic Rice Farmers in East Java Yuliani, Lisa Lisdiana.....	162
Influence of The Kind of Vermicompost Material and Earthworm <i>Pontoscolex Corethrurus</i> Population on The Yield and Quality Of <i>Phak-Coi</i> Mustard (<i>Brassica Rapa</i> L.) with Organic Potting Media Nurhidayati, Usman Ali, Indiyah Murwani.....	168
Bat Species Richness of Order Chiroptera in The Sanctuary of Duasudara Mountain, North Sulawesi Henry Jefry Lengkong, Endang Arisoesilansih, Luchman Hakim, Sudarto.....	177
Growth Rate of Black Soldier Fly (<i>Hermetia illucens</i>) During Bioconversion of Restaurant Waste Ramadhani Eka Putra, Ida Kinasih, Ahmad Rizan Hadziqi, Finsa Firlana Gusmara.....	180
Mycorrhiza Diversity from Various Private Forest Ecosystem Types in South Sulawesi Gusmiaty, Muh. Restu, Samuel A. Paembonan, Astuti Arif, Siti Halimah L.....	185
The Environmental Security Perspective on The Governance of Herbal Medicine in Indonesia Arry Bainus, R. Widya Setiabudi, Satriya Wibawa, Affabile Rifawan, Wahyu Wardhana.....	189
Potential of Local Food Pumpkin (<i>Cucurbita moschata</i> Duch) as Diversification of Rice to Food Security Soewanto Rusman.....	193
Increasing Concentrations of The Biogas and Storage Conditions Mochammad Junus, Agung Widodo, Wahyono Suprpto, Windi Zamrudy.....	198
Proximate Analysys of Flours Derived From Peel and Kernel of Gedong Gincu Mango Tania Avianda Gusman, Arif Nurudin, Badawi.....	201
Effects of Aminoethoxyvinylglycine, Plastic Wrapping, and Storage Temperatures on Fruit Shelf-Life and Qualities of ‘Cavendish’ Banana Soesiladi E. Widodo, Zulferiyenni, Amelia Ekaprasetio.....	204
Amylase Activity of Fish Ventriculi after Various Storage Temperatures and Periods Erlix Rakhmad Purnama, Nur Kuswanti.....	209
Effect of PH and Fermentation Time on Patchouli Leaf Fermentation in a Stirred Fermentor Sri Rulianah, Prayitno.....	213
Fragment DNA 387BP Gene Lectin of Soybean (<i>Glycine max</i> (L.) Meriil) Varieties Detam 2	

Rini Puspitaningrum, Ria Amelia, Ernawati, Adisyahputra.....	217
Response of Six Genotypes Soybean on The Dose of NPK Fertilizer	
Achmad Yozar Perkasa, Utomo, Teguh Widiatmoko.....	220
Development and Validation Analysis Method of Sodium Cyclamate and Aceculfam-K in Supplements Drink Using High Performance Liquid Chromatography Method (HPLC)-UV	
M. Hatta Prabowo, Rochmy Istikharah, Ari Wibowo, Mardhiyah Fithriana Mufti.....	227
Development and Validation of Analytical Method of Sodium Cyclamate and Aspartame in Powder Beverage Using High Performance Liquid Chromatography UV-detector	
Anita Suryaningrum, Ari Wibowo, Rochmy Istikharah, M. Hatta Prabowo.....	234
The Using Woof is Composed of Fermented Eceng Gondok (<i>Eichhornia Crassipes</i>), Tahu Dregs and Dried Kangkung (<i>Ipomoea Aquatica</i>) as The Ruminant Livestock Woof Formulation	
Isnawati, Herlina Fitrihidajati, Gatot Suparno.....	239
The Effect of Lime, Phosphorus and Potassium Fertilizer on The Growth and Productivity of Black Soybean Under Saturated Soil Culture on Tidal Swamp	
Munif Ghulamahdi, Abdul Jabar.....	246
Effects of Aminoethoxyvinylglycine, Plastic Wrapping, and Storage Temperatures on Fruit Shelflife and Qualities of ‘Cavendish’ Banana	
Soesiladi E. Widodo, Zulferiyenni, Amelia Ekaprasetio.....	251
Characterization and Molecular Identification of <i>Lactobacillus</i> Spp. Isolated from Feces of Healthy Infants for Local Probiotic Development in Bali	
Y Ramona, I N Sujaya, K A Nocianitri, W R Aryantha, R Cintyadewi, I A S Maha Uni, N M Nursini.....	256
Potential of Biological Control for Oil Palm Beetle (<i>Oryctes rhinoceros</i>) with <i>Metarhizium anisopliae</i> and <i>Beauveria bassiana</i>	
Dyah Nuning Erawati, Irma Wardati.....	262
Effect of Soil Microbes Effect on Productivity Cayenne Pepper (<i>Capsicum frutescens</i> L).	
Agung Prasetyo Wibowo, Ismul Mauludin Al Habib, Dwi Sucianingtyas S.....	267
Potential Test Trichoderma Indegenus Southeast Sulawesi as to <i>Fusarium oxysporum</i> Biofungisida The in-Vitro	
Gusnawaty Hs, Muhammad Taufik, Asniah, La Ode Santiaji B, Faulika.....	270
Lipid Analysis of Some Potential Microalgae for Food Supplement Candidate	
Fitortin Chasanah, Sapto Andriyono.....	276
Encapsulation of Embryogenic Callus and Shoot Tips for Storage of Sugarcane (<i>Saccharum Officinarum</i> L.)	
Fitri Damayanti, Suharsono, Utut Widyastuti, Ika Mariska.....	279
Morphological Character of Mosses from Family <i>Bryaceae</i> from Mount Argopuro	
Fuad Bahrul Ulum, Galen Rahardian.....	283

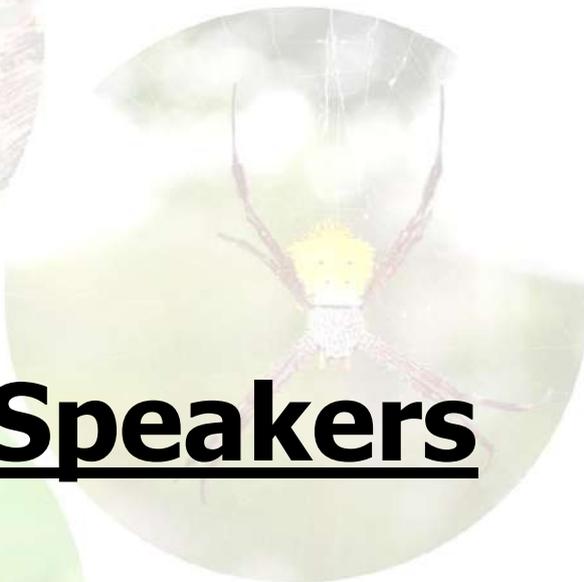
A Comparison of Effectiveness of <i>Acorus calamus</i> L. Extract and Neem-Based Insecticide in The Field Against Coffee Berry Borer, <i>Hypothenemus hampei</i> Ferrari (Coleoptera: Curculionidae) Purwatiningsih.....	286
Synthesis and Characterization of 1-(4-Trifluoro Methyl Benzoyl Oximethyl)-5-Fluorouracil Ika Oktavianawati, Nita Ernawati, Ayik Rosita Puspaningtyas.....	288
Fungal Isolation and Artificial Inoculation Induced Agarwood in Gaharu Tree (<i>Aquilaria malaccensis</i>) Rudju Winarsa.....	292
Kinship of Banteng (<i>Bos bibos</i>, d'Alton) and Bali Cattle (<i>Bos sondaicus</i>, Muller) Hidayat Teguh Wiyono, Arya Mahd.....	295
The Diversification of Food Consumption and The Improvement of Desirable Dietary Pattern (DDP) with The Application of Sustainable Reserved Food Model (SRFM) in Madiun Regency Amik Krismawati and PER. Prahardini.....	301
Effect of Propolis Coating on Albumin and Yolk Index of Local Indonesia Chicken's Egg Ida Kinasih, Ramadhani Eka Putra, Yani Suryani, Fitriyani Elia Purwati, Taufik Rizkiand	312
Modification Shredded as an Effort to Food Diversified to Reach Food Security Ninna Rohmawati	317
The Effectiveness of Stored Spltmnpv on Mortality and Normality of <i>Spodoptera Litura</i> Winarsih, Evie Ratnasari, Mahanani Tri Asri.....	320
Growth Performance of Some Varieties Chrysanthemum as a Mother Plant on in Vitro Propagation PER. Prahardini and Amik Krismawati	327
Preference Test of Sustainable Food Household Area (Krpl)'S Products in Sukorejo Village, Ponorogo Sri Satya Antarlina and Aniswatul Khamidah	334
Effect of Liquid Fertilizer Supplement (Ppc) and Anorganic Fertilizer Dosage on Growth and Yields of Cabbage (<i>Brassica oleraceae</i> L). Zunaini Sa'adah, Rohmad Budiono and Nurul Istiqomah.....	343
Hydrolysis Profile of Oil Palm Empty Fruit Bunch by An Extracellular Enzyme From <i>Aspergillus niger</i> Kahar Muzakhar, Ainul Latifah, Sutoyo, Siswoyo, and Rudju Winarsa.....	348
Investigating Cryotherapy Techniques to Eliminate Virus on Potato Shoot Tips	

Ida Ayu Astarini, Angel L. Chappell, Douglas C. Scheuring, Sean Michael Thompson, J. Creighton Miller, Jr	352
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POSTER SESSION

Agroforestry of <i>Apis arana</i> Honey Bees' Feed Plants in Right Forest Area in Panekan Sub-District, Magetan Regency Anang Susanto.....	357
Preference Test of Sustainable Food Household Area (KRPL)'S Products in Sukorejo Village, Ponorogo Sri Satya Antarlina And Aniswatul Khamidah.....	361
The Toxicity of Polar and Non-Polar Fraction of Rhizome Extract of <i>Acorus calamus</i> L. Against <i>Hypothenemus hampei</i> (Ferr.) Arminatul Jannah, Sri Mumpuni W. W., Purwatiningsih.....	368
Development of Transgenic Sugarcane Containing Double Overexpression (Stacked) of The Genes for Sucrose-Phosphate Synthase and Sucrose Transporter Protein Dwi Ratna P., Mohamad S. Aswan, Parawita Dewanti, Bambang Sugiharto.....	370
Mycorrhiza Diversity from Various Peoples Cultivation Forest Ecosystem Types in South Sulawesi Gusmiaty, Muh. Restu, Samuel A. Paembonan, Astuti Arif, Siti Halimah L.....	376
Response of Plant Growth and Yield of Kencur (<i>Kaempferia galanga</i> L.) by Applied Different Source of Organic Matter Per. Prahardini, Nurul Istiqomah.....	380
Development of Commercial Nurseries on Selling Plants and Flowers in Denpasar, Bali Made Ria Defiani.....	383
Morphological Characteristics of Some Varieties of Cucumber (<i>Cucumis Sativus</i> L.) Nurul Jadid, Nilna R. Mubarakah, Rizka Maziyah, Sri Nurhatika.....	386
Decomposition of Coffe Pulp Polysaccharides by <i>Aspergillus niger</i> Extraselluler Enzyme Syafiq Ubaidillah, Siswanto, Kahar Muzakhar.....	390
Production Single Cell Proteins <i>Saccharomyces cerevisiae</i> Using Product Hydrolysis <i>Jatropha Curcas</i> Cernel Cake Fermentation by <i>Aspergillus niger</i> Anis Barokah, Siswanto, Kahar Muzakhar.....	396
Author Index.....	401

Keynote Speakers



THE EFFECTIVENESS of STORED *SpltMNPV* on MORTALITY and NORMALITY of *Spodoptera litura*

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Abstract

The storage of *Spodoptera litura* multiple nucleopolyhedrosis virus biopesticide is an important stage and often performed before the virus was applied, to maintain the purity of the virus. This study aims to determine *SpltMNPV* in vitro effectiveness which have been stored for 20 months at -80°C on mortality and normality *S. litura*. *SpltMNPV* in vitro at doses of A = 5.95×10^8 PIBs / ml, B = 5.95×10^7 PIBs / ml, C = 5.95×10^6 PIBs / ml and D = Control was infected to third instar Larvae of *S. litura*. This research was conducted in the laboratory with repeatedly for fifth times and each replication used 10 animals caterpillar. Infected Larvae has been incubated for 17 days and had been given an artificial feed. The parameters observed in the form of mortality and normality *S. litura*. Research result shows that in *S. litura* infected with *SpltMNPV* in vitro dose of 5.95×10^8 PIBs / ml caused high mortality of 44%, *S. litura* abnormal approximately 14% and the rest managed to become a moth normal life. Lowest mortality at doses C = 5.95×10^6 PIBs / ml; the mortality of *S. litura* is 38% and abnormalities *S. litura* is about 12%. *S. litura* abnormal morphology was observed in the Larvae stage, PrePupae and Pupae.

Keywords: Effectiveness, *Spodoptera litura* Multiple Nucleopolyhedrosis virus (*SpltMNPV*), mortality, abnormalities, *Spodoptera litura*

Introduction

Spodoptera litura multiple nucleopolyhedrosis virus (*SpltMNPV*) is biopesticide made from the virus. The effort has been made to propagate the virus in vitro by culturing cells midgut epithelial cells or ovarian cells of various insect Larvae such as *S. litura*, *S. frugiperda*, *Pseudaletia unipuncta*, *M. sexta* and *Heliothis virescens*. According to a preliminary test by [1], cultured midgut epithelial cells of *S. Litura* Larvae grow very fast growing / divide rapidly, reaching 975 times / 24 hours. The culture can be used to produce a wide range of bioproducts such as biopesticide virus production virus (baculovirus) and certain protein product that is typically use for genetic engineering.

The Pathogenicity of *SpltMNPV* biopesticide a result of in vitro propagation in midgut epithelial cell culture that was recultured second time and formulated with photo-protectant and infected to *S. litura* second instar Larvae, resulting 100 % of *S. litura* Larvae mortality at the fifth day [1] According to [2], latent virus in the shape of polyhedral or prism in epithelial cells cultured *S. litura* Larvae that have been saved (in the freezer in Grace's medium temperature $\pm 20^{\circ}\text{C}$) for at least 2 years then pathogenicities were 10 percent. However when the virus were infected repeatedly to the original host Larvae of *S. litura* their pathogenicitas increased to 14% after the first infection, and 63% in the second infection, and 90% in the third infection.

The factors that determine the level of effectiveness of the virus, among others, are the nature, concentration, application mode, storage, and persistence level of the virus. NPV inoculum are resistant when they are stored in a refrigerator in the form of dry polyhedra.

Mixture polyhedrosis usually last for four to five years, granulosis last for two to three years, and cytoplasmic polyhedrosis last for one year [3] Once applied on the field, the virus will survive for along time if it is not exposed to direct sunlight. If it is directly exposed constantly to the sunlight, then its virulence will reduce by 50 percent each day. According to Pramono (2000), the NPV pathogenicity remains unchanged after 1 year of storage. This is supported by Balitsa Lembang's study. He has packed NPV mixed with talc and then placed it in a plastic bottle. The result showed that the NPV can be stored for more than one year. In addition, based on a study conducted by Klaten research center, NPV was also quite effective in maintaining the stability and effectiveness up to 6 months when placed in a dry room temperature.

Based on the background this study will examine the effectiveness of in vitro propagated *SpltMNPV* on epithelial cells of *S. litura* Larvae that had been stored for 20 months in Ex-cell medium at -80°C , on the mortality and normality of *Spodoptera litura*.

Materials And Methods

A. Equipment and Materials Research

The tools used in this study include the main equipment and ancillary equipment. The main equipment consists of incubator, light microscope, Laminar Air Flow (LAF), incubator, centrifuge, magnetic stirrer, and blender. The support equipment consists of capsule bottles, brushes, gauze box, centrifuge tubes, syringe, aluminum foil, knife, and label.

The materials needed in the study are SpltMNPV Wonosobo, Central Java isolates that are propagated in vitro, third instar Larvae of *Spodoptera litura*, artificial feed for Larvae of *S. litura*, Bayclin, Alcohol 70% as a sterilizing agent, and distilled water.

A. Research Design

This research is an experimental study using a complete randomized design, one factor is the dose SpltMNPV propagated in vitro in the midgut epithelial cells of Larvae of *S. litura* which has been stored at a temperature of - 80 ° C for 20 months, infected Larvae fed by using artificial feed (Balitas, Malang).

This study aimed to test the pathogenicity of in vitro SpltMNPV that has been stored for 20 months on the mortality and normality of *S. litura*. The study was repeated 5 times and used 10 Larvae in each repetition. Larvae of *S. litura* were given SpltMNPV in vitro treatment with a dose: A = 5.95 x 10⁸ PIBs / ml; B = 5.95 x 10⁷ PIBs / ml; C = 5.95 x 10⁶ PIBs / ml; D = Control (akuadest). The results of research in the form of mortality and infected normality *S. litura* were analyzed descriptively.

B. Research Procedure

1. Preparation: The calculation of the concentration of SpltMNPV

SpltMNPV which are newly stored in the refrigerator in the temperature of 80oC adapted beforehand to the same temperature as the room. Virus concentration is calculated using haemocytometer. SpltMNPV concentration used is A. 5.95 x 10⁸ PIBs / ml; B. 5.95 x 10⁷ PIBs / ml and C 5.95 x 10⁶ PIBs / ml.

2. Propagation test insect (*Spodoptera litura*)

F0 *S. litura* Larvae captured in the area

around the jatropha and tobacco plantation in Malang, and subsequently maintained in the Laboratory of Microbiology UNESA up to the stage of imago and feeded using jatropha leaves as natural food. Imago were moved into screen box with the male and female ratio of 1: 3. Imago were allowed to marry and fed using liquid honey 10%. Eggs produced in this step is an F1 generation. Eggs obtained were subsequently maintained until they hatch into Larvae F1. The Larvae then immediately transferred into bottle maintenance/ bottle capsules with a volume of 50 ml. Feed given to the 1st and 2nd instar caterpillar is the natural feed in the form of jatropha leaves, and artificial feed used to feed instar 3 to 5 use. The composition of 1 liter of artificial feed includes: soybean flour (42 g), agar-agar powder (14 g), yeast (42 g), Wheat germ 36 grams, salt Wesson (10 g), vitamin mix (9.6 gr), parabernsoat (1 g), sorbic acid (1 g), aureomycin (1 g), streptomycin (0.2 g), Benlate (1 g) and formaldehyde (2 ml) that were cooked and printed as gelatin to size 1 x 1 cm square. *S. litura* Larvae used in this study was the third instar Larvae of *S. litura*.

C. Test of SpltMNPV pathogenicity in vitro which have been kept for 20 months on the Larvae of *S. litura*

Larvae of *S. litura* were infected orally by dipping Larvae feed on virus suspension. The infected Larvae were incubated in the capsule bottles individually for 17 days (Larvae become moth). Furthermore, the infected Larvae were observed in terms of their mortalities and morphological changes to see normality. Morphological changes of the Larvae were observed every day starting from initial infection until caterpillars' death.

Results And Discussion

A. The mortality and normality of *S. litura* infected with SpltMNPV

The result of this study is the effectiveness of SpltMNPV which were reproduced on midgut epithelial cells of *S. litura* Larvae on the mortality and normality of *S. litura*, with different SpltMNPV oncentrations and were infected with food dipping method. The treatment was repeated three times and there were 10 Larvae in each repetition. The percentage of mortalities, abnormalities and normalities of *S. litura* infected by SpltMNPV presented in Table 1, as follows:

Table 1. The percentage of mortalities, abnormalities and normalities of *Spodoptera litura* by SpltMNPV in vitro after 20 months of storage (observed at day 17)

Treatment	Imago	Replay					Average
		1	2	3	4	5	
A	Dead	40	50	50	40	40	44
	Abnormal	0	10	20	20	20	14
	Normal	60	40	30	40	40	42
B	Dead	50	20	40	30	60	40
	Abnormal	0	10	10	20	20	12
	Normal	50	70	50	50	20	48
C	Dead	10	50	50	50	30	38
	Abnormal	20	10	20	10	0	12
	Normal	70	40	30	40	70	50
D	Dead	20	10	20	30	10	18
	Abnormal	10	10	20	0	0	8
	Normal	70	80	60	70	90	74

Descriptions :

A = 5.95×10^8 PIBs / ml; B = 5.95×10^7 PIBs / ml; C = 5.95×10^6 PIBs / ml; D = Control

Data described as the percentage of mortalities, abnormalities and normalities of *S. litura* that died because of being infected by SpltMNPV (Table 1) can be seen at the seventeenth post infection. Based on the data in Table 1 above, it appears that the highest mortality achieved in SpltMNPV in vitro with a dose of 5.95×10^8 PIBs / ml were 44%, the next row at a dose of 5.95×10^7 PIBs / ml and 5.95×10^6 PIBs / ml with a mortality of 40% and 38%. Compare to the research conducted by [1] the same, but still new cultured, virus which were infected on second instar Larvae of *S. Litura* achieved 100% mortality on the fifth day. The mortality obtained by this study is lower than that was resulted by the new cultured virus. This is caused by the fact that the virus in this study has undergone sufficiently long time storage, that was 20 months.

According to Mangundihardjo et al (1992), the weakness of NPV stored for a long time is the virus does not get live cells so that its activity stops and crystallizes and it needs some time to be active when it find the host. Evidence that the virus which has been stored for a longer period shapes crystal/like a prism has been successfully photographed by [2] using scanning electron microscope, while the NPV which is still isolated from the new host shapes polyhedra (hexagon like dice). In addition, strains of SpltMNPV used were less virulent because the virus has undergone propagation in different environments i.e. in vitro cell culture conditions are not the same when SpltMNPV attack in vivo. In the in vitro conditions, the host's immune system does not exist so that the virus is not adaptive when inserted into the original host body.

According To Pramono, at the virus storage period for 1 year, the pathogenicity of the virus applied in the field reaches the mortality of *S. litura* as much as 49.33% on the tenth day. Thus, the virus storage for 20 months caused mortality of *S. litura* was slightly lower

(44%) than that of 1 year virus storage. %. According to Suwandi (2007), NPV stored in a dark room for 24 hours and infected on caterpillar H. Talaka (inch caterpillar on tea leaves), its pathogenicity decreased by 26.66%. In the in vitro conditions, the host's immune system does not exist so that the virus is not adaptive when inserted into the original host body.

Besides, the relatively low mortality was also obtained at post infection on the seventeenth day. In the long period of death, according to Granados and Federici (1986, in Suwandi, 2007), NPV is said to be virulent if it can kill *S. litura* within 2-5 days, and is said to be less virulent if the NPV can only kill it within 2 to 3 weeks. So, based on the opinion, SpltMNPV in vitro which have been stored at -80°C for 20 months will be classified as less virulent. The use of SpltMNPV resulted from in vitro propagation causes the growth of *S. litura* at various abnormal stages (Figure 1,2 and 3).

It can be seen from the evidence that *S. litura* virus were dying in the stage of PrePupae, Pupae and imago. In the Larvael stage, virus rarely died. This shows that in the Larvael stage, the Larvae are able to make high defense against infection of SpltMNPV. Only in the intermediate stage of the Larvae become PrePupae, PrePupae into a Pupae and imago stages.

At this stage, *S. litura* are less able to form a high defense against viral infection. PrePupae is a critical stage because the Larvae feeding activity has stopped. The energy stored when the Larvae are widely used to convert the organs in the body of the Larvae into other organs whose functions change.

For example, the digestive organs was in the form of mouth /chewing food in the form of solids (leaf) will turn into fluid/ honey-sucking mouth. This applies to other organs such as the formation of the wings to fly which did not occur in the Larvae l stage.

B. The Morphology of SpltMNPV *S. litura* infected in vitro

The morphology of *Spodoptera litura* infected with SpltMNPV at various stages was observed to see the abnormalities produced.

Observations on abnormalities done at the Larvae 1, PrePupae, Pupae and imago stages.

Table 2. Characteristics of Larvae, PrePupae, Pupae and imago of *S. litura* infected with SpltMNPV in vitro.

No	Stages <i>S. litura</i>	The characteristics of <i>S. litura</i> abnormal	The characteristics of <i>S. litura</i> normal
1.	Larvae	The infected Larvae tend to has bright and shiny colors, especially in the lower abdomen, the mandible when it was touched its response is slow, the Larvae tend to rise to the top (closed bottle), his movements is slow, lazy to feed, time needed by instar to turnover tends to be longer, her body swelled and when died the virus body are easily broken when we touch, it discharged white milk containing viral polyhedra (Figure 1 A).	Color matches the color of the Larvae feed, on the day of the beginning of each instar Larvae tend to be bright colors (beige / brown) but on days 2-3 ahead instar change color becomes darker. The texture /line on the Larvae is clear. When being touched Mandible Larvae will respond quickly. Larvae at Instar 5 tend to be darker. The Larvae eat actively (Figure 1.B, C)
2.	PrePupae	PrePupae color is darker / blackish brown, sometimes the color is uneven (half brown and half dark brown), its body segments are irregularly, if we touch its response was slow, the formation of candidates for the head imago is not perfect, some PrePupae failed to shrink his body, even enlarged/swelling, diabdomen leg is still visible, diabdomen segment of the middle part shrink, soft body and when we touched it discharge milk chocolate which contains many polyhedra (Figure 2A)	PrePupae color is still like instar 5 Larvae that is dark brown, the length and width of the Larvae shrink nearly half the normal size, PrePupae eat inactively, make a nest by collecting leftover food and feces are made such that the whole body covered. Food remains and excrement held together with a kind of yarn produced by saliva. When we touch, PrePupae response is slow, and the body tends to curved (Figure 2B)
3.	Pupae	The size of the Pupae is smaller than the normal Pupae (<2 cm), Pupae color is uneven (there are several bulkhead colored younger), head of the Pupae enlarged, abdominal shrinking, the legs are still seen, bulkhead abdomen retracts but not hardened, its color is dark brown tends to black, head arched, some abdomen / head is soft and easily broken when we touch and issuing white liquid filled with polyhedra. (Fig 3 A)	Pupae normal size (\pm 1.5 to 2 cm), the color of the Pupae from young to old Pupae terraced ranging from light brown to dark brown. When touched, its response is fast (Figure 3B).
4	Imago	Imago wing curling, sometimes broken and small, when we touch the response is slow, passive and not actively foraging.	The shape and size of a normal imago (\pm of 1 - 1.5 cm), grayish-brown color with distinctive spots. When attached to the substrate it resembles an isosceles triangle. Fast response when touched. Tend to be nocturnal, actively approaching the feed in the form of liquid honey.

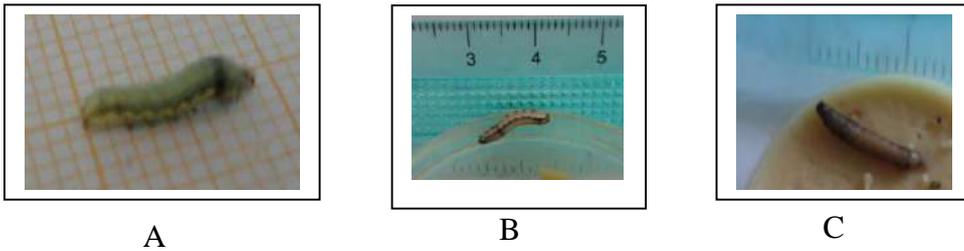


Figure 1. A third instar Larvae of *S. litura* normal, and B, C third instar Larvae of *S. litura* infected SpltMNPV (abnormal)

Based on Table 2, the changes in morphology and behavior of (healthy) normal *S. litura* Larvae and *S. litura* Larvae infected with SpltMNPV in vitro (abnormal) can be observed. Healthy *S. litura* Larvae movement is still active, feeds actively, the color of caterpillar is normal, that is cream- brown, dark brown

(accordance with the color of the feed)., the color is clear, when it is touched mandible is still moving. Colors on the body of *S. litura* Larvae is in accordance with the color of the food he ate.



Figure 2. A. PrePupae abnormal because infected SpltMNPV and B. The development of normal uninfected PrePupae SpltMNPV

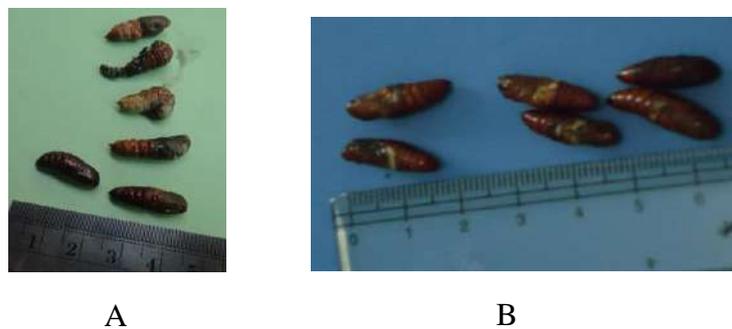


Figure 3. A. B. Pupae normal abnormal

In this study, the feed used is in the form of artificial feeding (formula of Balitas Malang), cream / yellow colored, so that most of the Larvae color is also cream. The older the instar Larvae, the darker the color of their bodies. Their skin color then fade when the change their skin as a marker of instar stages of change. The color change is due at the time of the skin change, cuticle that has melanin pigment was released. The cuticle layer will be replaced with a new cuticle that has little melanin.

The *S. litura* larvae infected with SpltMNPV in vitro showed changes in morphology and behavior. The changes can be seen in the Larvae body at the beginning of the infection caused by SpltMNPV begin damaging cells it attacked. The initial infection usually begins in the digestive tract. This causes the larvae will not eat, because many cells in the gastrointestinal tract begins to rupture / lysis. This resulted in the production of digestive enzymes are inhibited (eg proteases), metabolism becomes impaired, the energy derived from the metabolism decreases as a result of passive larvae (slow motion). Larvae look began to stop eating, and tends to rise to the end of the bottle as in their natural habitat, that is in the plant, which tend to rise to the top of the plant. The infected Larvae look swollen, especially on the back / dorsal, while it looks redness at the abdominal / ventral. The swelling is caused by the many larvae were infected by lysis so that the cells secrete fluid mixes with hemolimfa. In the abnormal Larvae that died, the color of reddish brown caterpillar can be seen in the abdomen, and at the back has white colored cream milk or destroyed at the end of the bottle with the liquid that comes out milky white. Soft and when it was touched, it broke and secrete a milky white liquid. Mandible did not move. Caterpillar size is smaller. One characteristic of *S. litura* larvae infected with the virus is dead in a dependent position as the letter "V" inverted with abdominal leg as a footstool. The fluids which broke from the body of dead caterpillars infected SpltMNPV SpltMNPV containing polyhedra which, if left in a few moments, will cause a bad smell. This odor is due to the fermentation of the remnants of larvae metabolites that were out of the lysis cell or because of the degradation of the remnants of lysis cells / tissues / organs.

At normal PrePupae, PrePupae color is still like the 5 instar Larvae, that is dark brown, the length and width of the Larvae shrink nearly half the normal size, PrePupae eat inactively, make a nest by collecting leftover food and feces are made such that the whole body covered. Food and excrement remnants held together with a kind of yarn produced by saliva. When

PrePupae is touched, its response is slow, the body tends to curve.

Meanwhile, abnormal PrePupae because of being infected by darker / blackish brown virus, sometimes the color is uneven (half brown and half dark brown), its body segments are irregularly, if it was touched its response was slow, the formation of candidates for the head of imago is not perfect. Some PrePupae failed to shrink his body even enlarged / swollen, diaphragm legs are still visible, diaphragm segments of the middle part shrink, the body is soft and when it was touched it discharged milk chocolate contains a lot of polyhedral.

The normal growth Pupae showed the characteristics of normal size (± 1.5 to 2 cm), the color of the Pupae from young to old Pupae terraced ranging from light brown to dark brown. When it was touched, the response was fast (Figure 3B). Meanwhile, abnormal Pupae has specific characteristics, namely size of the Pupae is smaller than the normal Pupae (<2 cm), Pupae color is uneven (there are several bulkhead colored younger), head of the Pupae enlarged, abdominal shrinking, legs are still visible, abdomen bulkhead retracts but not hardened, dark brown color tends to black, curved head, some of its abdomen / head was soft and easily broken when it was touched and issued white liquid filled with polyhedra. (Figure 3 A).

In the imago stage formed after 8 days old Pupae, morphology imago. The shape and size of imago is normal (\pm of 1 - 1.5 cm), its color is grayish-brown with distinctive spots. When attached to the substrate, it resembles an isosceles triangle. It has fast response when was touched. Tend to be nocturnal, actively approaching the feed in the form of liquid honey. Conversely, abnormal imago has characteristics of curly wings, sometimes broken and small, when it is touch the response is slow, passive and not actively foraging.

Conclusion

Based on the data above it can be seen that SpltMNPV propagated on epithelial cells of *S. litura* Larvae, and had been maintaining in the storage at -80° C for 20 months caused the mortality of third instar *S. litura* Larvae highest achieved at doses SpltMNPV 5.95×10^8 PIBs / ml by 44 %, the next row at a dose of 5.95×10^7 PIBs / ml and 5.95×10^6 PIBs / ml with a mortality of 40% and 38%. The mortality was observed for 17 days after treatment Mortalitas tersebut diamati 17 hari setelah perlakuan. Stadia *S. litura* in the forms of Larvae, PrePupae, and Pupae or imago which were infected with SpltMNPV showed the characteristics of specific abnormalities that are

different from normal. The percentage of abnormal imago decreased is equivalent with the decrease of SpltMNPV concentrations given.

Acknowledgment

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