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**“Improving Food Security :
The Challenges for Enhancing Resilience to Climate Change”**

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LIST OF CONTENTS

No	Title and Author	Page
1	FOOD SECURITY POTENTIALS OF AGROFORESTRY SYSTEMS IN SELECTED UPLAND FARMING COMMUNITIES IN THE PHILIPPINES Leila D. Landicho, Romnick S. Baliton, Rowena D. Cabahug, Roselyn F. Paelmo, Reynaldo A. Comia, Roberto G. Visco, Arnold Karl Castillo, Russel Son Cosico and Maryanne Abadillos	1-19
2	SHELF LIFE PREDICTION OF LOCAL ORANGES USING SPECTRAL INFORMATION IN UV-Vis-NIR REGION COMBINED WITH PLS REGRESSION Diding Suhandy, Dwi Dian Novita, Meinilwita Yulia, Arion Oktora, Yuni Kurnia Fitri	20-28
3	FOOD SECURITY UNDER PARTNERSHIP SCHEME AT PRODUCTION FOREST REGISTER 42 WAY KANAN, LAMPUNG PROVINCE Christine Wulandari And Pitojo Budiono	29 - 37
4	ANALYSIS FOR SELF-SUFFICIENCY OF RICE IN INDONESIA: FORECAST OF ITS PRODUCTION AND CONSUMPTION Agus Hudoyo, Dwi Haryono, Indah Nurmayasari	38 - 47
5	RISK MANAGEMENT AS A PILLAR IN FOOD SECURITY FOR PEPPER SMALLHOLDER FARMER Suci Wulandari	48 - 58
6	INDIRECT SELECTION OF SOYBEAN ELITE LINES DERIVED FROM WILIS X B3570 CROSSES Nyimas Sa'diyah, Viska Nurisma, Setyo Dwi Utomo, and Maimun Barmaw	59 - 65
7	A REVIEW ON FOOD SECURITY IN MALAYSIA: TOWARDS SUSTAINABILITY AND CHALLENGES Kamziahabd Kudus	66 - 74
8	SAVING IRRIGATION WATER AND ENERGY WITH LASER LEVELING EQUIPMENT TO MITIGATE EFFECTS OF CLIMATE CHANGE AND TO ENSURE FOOD SECURITY Phan Hieu Hien, Tran Van Khanh, Nguyen Duc Canh, Nguyen Thanh Nghi	75 - 94
9	FOOD PRODUCTION, RESURGENCE OF SHIFTING CULTIVATION SYSTEM, AND ITS ENVIRONMENTAL IMPLICATION: A CASE FROM PASAMAN DISTRICT, WEST SUMATRA PROVINCE, INDONESIA Yonariza	95 - 116
10	THE ROLE OF <i>Saccharomyces cerevisiae</i> AS MODIFICATION AGENT	117 - 124

ON THE CASSAVA STARCH

Maria Erna Kustyawati, Azhari Rangga, Sri Setyani

- 11 DESIGNING AND CONSTRUCTING OF SUN DRYING WITH SEMI-AUTOMATIC MOVING RACKS** 125 - 133
Tamrin and Hendra Saputra
- 12 A REVIEW OF VARIOUS MATERIALS AND LEVELS MODULE ON THAI PEPPER (*Capsicum frutescens* L.) IN VERTICULTURE PATTERN OF LEVELS MODULE** 134 - 142
Sitawati, Agus Suryanto and Euis Elih Nurlaelih
- 13 ECONOMIC ANALYSIS OF SMALL RUMINANT MIXED FARMING TO THE FARMER'S INCOME IN YOGYAKARTA-INDONESIA** 143 - 148
Tri Anggraeni Kusumastuti
- 14 OXYGEN SATURATION OF LAMBS DURING ESTROUS CYCLE WITHIN DIETS WITH DIFFERENT CATION AND ANION RATIO** 149 - 155
Farida Fathuland Endang Linirin Widiastuti
- 15 DIFFERENCE OF CATION AND ANION DIETS ON LEUCOCYTES DIFFERENTIATION OF LAMB DURING ESTROUS CYCLE** 156 - 163
Farida Fathul and Endang Linirin Widiastuti
- 16 EFFECT OF SIGER RICE FROM CASSAVA ON BLOODGLUCOSE LEVEL AND THE PANCREAS IN MICE INDUCED ALLOXAN** 164 - 179
Subeki, Wisnu Satyajaya, Murhadi, Erwin Yuliadi
- 17 PHYSICOCHEMICAL CHARACTERISTICS OF CASSAVA STARCH PRODUCED BY ITTARA - A SMALL SCALE TAPIOCA INDUSTRY : A Case Study at PD Semangat Jaya , Lampung** 180 - 187
Siti Nurdjanah, Udin Hasanudin, Neti Yuliana ,Desy Silvianti
- 18 THE SHELF LIFE DETERMINATION OF CAPSAICIN ON RED CHILLI PASTE USING THE ARRHENIUS MODEL APPROACH** 188 - 196
Dharia Renate
- 19 ENHANCEMENT OF THE CAMERA CAPACITY MEASURING THE CATTLE WEIGHT** 197 - 202
H. Winoto, H. C. Pangestika, A. D. Putridinanti, A. Riyanto, G. Al Hadid and D. Samsudewa
- 20 BODY CONDITION SCORE AND NON RETURN RATE PERCENTAGE OF COW BEEF AFTER FLUSHING IN CENTRAL JAVA** 203 - 207
Daud Samsudewa, Marry Christiyanto, Mukh Arifin and Andri Hanindyoy
- 21 BODY MEASUREMENT OF TIMOR DEER (*Rusa timorensis*) IN CAPTIVITY OF CENTRAL JAVA** 208 - 132
Daud Samsudewa, Alfina Handayani, And Baroto Agus Aryadi

- 22 **THE ACCURACY LEVEL OF GOLD RING FOR SEX DETERMINING USING THE THEORY OF INTENSITY ELECTROMAGNETIC WAVES AGAINST ULTRASONOGRAPHY (USG)** 133 - 136
M. Anwar, A. Zabiq, M. Shobirin, Y. Fatikhaturrohmah, M. F. Ridho and D. Samsudewa
- 23 **EFFECTIVENESS OF POLISULFON MEMBRANE WITH NANOSILICA ADDITION OF BOILER ASH OF SUGAR INDUSTRY** 137 - 145
Elsa Windiastuti, Suprihatin, Nastiti Siswi Indrasti, and Udin Hasanudin
- 24 **INFESTATION OF MAJOR PESTS AND DISEASES ON VARIOUS CASSAVA CLONES IN LAMPUNG** 146 - 160
I G. Swibawa, F.X. Susilo, Purnomo, T.N. Aeny, S.D. Utomo & E. Yuliadi
- 25 **INTEGRATION OF OIL PALM PLANT AND ANIMAL IN LAMPUNG PROVINCE** 161 - 166
Muhtarudin, Kusuma Adhianto, Liman, Yusuf Widodo, and Apriansyah Marga
- 26 **BREAST ANTICANCER ACTIVITY OF BRUCEIN-A FROM MAKASAR FRUIT (*Brucea javanica*) AGAINST EXPRESSION OF GENE p53 IN RAT INDUCED DIMETILBENZAANTRAZENA** 167 - 176
Muhartono, Subeki
- 27 **ACCESSTO BIODIVERSITY TO ENHANCE RESILIENCEAND FOOD SECURITY FORLOCAL ANDINDIGENOUS PEOPLEIN HEART OFBORNEO (HOB)** 177 - 186
Yusurum Jagau, Hastin Ernawati Nur Chusnul Chotimah, Adri Aliayub, Cristina Eghenter, Didiek Surjanto, and Eri Panca Setyawan
- 28 **FETAL SKELETON DEVELOPMENT OF MICE (*Mus musculus* L) THREATED WITH NUTGRASS (*Cyperus rotundus*) EXTRACT** 187 - 194
Nuning Nurcahyani, Yan Wirasti, Jamsari, Djong Hon Tjong, Hendri Busman
- 29 **PERFORMANCE OF REPRODUCTION AND PRODUCTION BUFFALO (*Bubalus bubalis*) IN TULANG BAWANG REGENCY LAMPUNG PROVINCE** 195 - 199
M.Dima Iqbal Hamdani, Idalina Harris and Liman
- 30 **STUDY OF PHYSICAL, CHEMICAL, AND SENSORY CHARACTERISTICS OF MIXED FRUIT LEATHER SNAKEFRUIT (*Salacca edulis*) AND JACKFRUIT (*Artocarpus heterophyllus*) WITH VARIATIONS ON ARABIC GUM CONCENTRATION** 200 - 208
Nur Her Riyadi P, Ardhea Mustika Sari, Palupi D Respatiningrum
- 31 **THE ROLE OF *TRICHODERMA* SPP.ON CORN DOWNY MILDEW (*PERONOSCLEROSPORA MAYDIS*)** 209 - 217
J. Prasetyo

- 32 **EXPLORATION OF THE PREDATORS OF SUGARCANE SCALE INSECT (*Aulacaspis tegalensis* Zehntn) AND TESTING THE DURABILITY OF THE PREDATOR WITH ALTERNATIVE FEEDS** 218 - 227
Sudi Pramono, Fx. Wagiman, Y. Andi Trisyono and Witjaksono
- 33 **POTENTIAL OF RICE ANALOGUES MADE FROM MODIFIED CORN FLOUR AND CASSAVA FLOUR PROCESSED BY GRANULATION METHOD AS FUNCTIONAL FOOD WITH LOW GLYCEMIC INDEX** 228 - 237
Beni Hidayat, Syamsu Akmal, Surfiana, and Bambang Suhada
- 34 **PERFORMANCE OF SINGLE-CROSS MAIZE HYBRIDS FROM DIVERSE CROSS COMBINATION OF PARENTAL INBRED LINES IN ACID SOIL CONDITIONS** 238 - 246
P.K. Dewi Hayati , Sutoyo, And Teguh Budi Prasetyo
- 35 **THE EFFECT OF MAGNETIC FIELD EXPOSURE TO MEDIUM ON PROTEASE PRODUCTION OF *Bacillus* sp. IN QUALITATIVE TEST** 247 - 255
Ajeng Pratiwi, Sumardi, and Rochmah Agustina
- 36 **PHYSICAL, CHEMICAL, AND COLOR SCORE CHARACTERISTICS OF SLICED PAPAYA (*Carica papaya* L.) PROCESSED BY OSMOTIC DEHYDRATION AND CONTINUED BY CONTROLLED ATMOSPHERE DRYING** 256 - 269
Rofandi Hartanto, Siswanti, Gagah Analdi
- 37 **ANALYSIS OF ENERGY INPUTS IN RICE PRODUCTION OF VARYING YIELD LEVELS AMONG SELECTED MUNICIPALITIES OF LAGUNA, PHILIPPINES** 270 - 290
Gyaw Shine Oo
- 38 **NUMBER OF ARBUSCULAR MYCORRHIZA FUNGI SPORE FROM TRAP CULTURE AFFECTED BY TYPE OF MEDIA USED** 291 - 297
Maria Viva Rini

NUMBER OF ARBUSCULAR MYCORRHIZA FUNGI SPORE FROM TRAP CULTURE AFFECTED BY TYPE OF MEDIA USED

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ABSTRACT

Trap culture is commonly technique used to develop fresh and viable spore of arbuscularmycorrhiza fungi (AMF) from field soil sample. In this study, soil sample for trap culture were collected from rhizosphere of 3 years old *Albizia* (Sengon) plantation. Two media were tested consisted of mixture of river sand and zeolite and mixture of malang sand and zeolite. Maize as the host plant was used in these media. The pot cultures were maintained for 3 months and let to dry for another 2 weeks. At the end of study, spore number were determine using wet sieving method and the result showed that media of mixture of river sand and zeolite had higher number of spore (91.0 spore/10 g media) compared to mixture of malang sand and zeolite which only had 41.0 spore per 10 g media. The AMF root infection in maize was also determined by using tryphan blue dye. The result obtained showed that there was no different rate of AMF infection between two tested media.

Key words: Arbuscularmycorrhiza fungi, trap culture, spore, root infection

INTRODUCTION

Arbuscularmycorrhizal fungi (AMF) are fungi that belong to the Phylum Glomeromycota that form symbiosis with the roots of most plant. The AMF are believed to support plant growth by increasing the supply of immobile nutrient and water and improving plant tolerance to soil pathogen as well as a-biotic stresses (Babajet *al.*, 2014; Faceliet *al.*, 2009; Siddiqui and Pichtel, 2008). It is generally accepted that AMF are non-specific in their selection of host, since in nature they have been found to infect plant species belonging to different genera, family, or class (Smith and Read, 2008; Hijri, 2006). Because of the AMF advantage, most of bio-fertilizer based on AMF spore is now commercially available.

Naturally, AMF present in the soil from savanna to forest ecosystem in form of spore, external hyphae, or infected root. However, the population and their diversity is vary depend on host type, soil fertility, humidity, soil chemistry, and climate (Smith and Read, 2008; Jieet *al.*, 2013). However, not all AMF spores present in the soil sample were in good condition

physically nor high germination rate. Some spores were broken or parasitized by other soil microorganisms. To develop AMF inoculum as bio-fertilizer, trap culture of field rhizosphere soil can be used to develop AMF starter inoculum (Doudset *al.*, 2000; Sieverding, 1991). Soilless media which have lower bulk density, providing better aeration and allowing to control over the substrate chemical composition have successfully been used for mycorrhizal propagation such as sand, perlite, expanded clay, peat and vermiculite (Sharma *et al.*, 2000; Corkidi, 2008). The majority of researchers used sterilized sand augmented with a nutrient solution as a growth media for propagation of AMF (Ridgway *et al.*, 2006), however this media is too coarse with very low water holding capacity which effect the growth of the host plant. Therefore, in this study two type of sand (river sand and malang sand) were mixed with zeolite which have high water holding capacity and cation exchange capacity were used to elaborate their effect on AMF spore production.

MATERIAL AND METHOD

Study sites

Soil sample was collected from area with 3 years old *Albizia falcataria* (Sengon) plantation at Jember - East Java - Indonesia (8° 10' S, 113° 42' E and 133 m abl). The *Albizia* were planted at rectangle 2 x 1 m planting distance and the space between *Albizia* stand were covered by *Pueraria javanica* and some grass species. No fertilizer was applied for the last two years.

Sampling Procedure

Soil samples were collected on 8th August 2011. Twenty *Albizia* trees were randomly sampled. Soils were taken from 8 points at a circle with radius of 1 m from the tree and using soil core to 20-25 cm depth. The soils plus roots were uniformly bulked to form a composite sample and 3 kg of it then was taken and stored in sealed plastic bags for further study. About 250 kg of soil from each soil sample were air dried for chemical and physical analysis. The soil chemical and physical characteristics were presented at Table 1.

Table 1. Soil chemical and physical characteristics at sampling sites

Soil Characteristic	Unit	Value
Chemical properties		
pH H ₂ O (1:1)		5.2
C-org (Walkey& Black)	%	1.60
N-Total (Kjeldal)	%	0.17
P-HCl 25%	ppm	155.3
P- Bray	ppm	16.0
K – Bray	ppm	46.3
Physical Properties		
Sand	%	7.83
Clay	%	57.79
Silt	%	34.4

Trap Culture

Trap culture of soil sample is usually used to enrich the AMF propagule including spore from the soil sample. In this study, trap culture was done by using two different media (1) mixture of river sand: zeolite (V:V=1:1) and (2) mixture of malang sand and zeolite (V:V=1:1). Maize as a trap plants were used with 10 replications. Clean pots (1 L volume) were filled with 400 g sterilized river sand and zeolite or river sand and malang sand, according to treatment at the bottom and about 300 g soil sample from rhizosphere of *Albizia* was placed on top of sterilized media. Seeds of maize were surface sterilized with 30% clorox for 15 minutes and washed several times. Four seeds were planted and the pots were kept in the glass house for 3 months. The trap pot cultures were watered daily and fertilized with 20 ml red hyponex (2 g/L) per pot every two days for two months. Three months after planting, no water was added for another two weeks in order to stimulate the trap plants to dry and the AMF produce spores. Two weeks after drying periods, the media from pot cultures were taken out and sterilized media at the bottom of pot media and the roots of the trap plant were separated and mixed thoroughly. Fifty gram of sterilized media was then sieved (using 45 and 500 μ m sieves) by wet sieving method (Brundrett *et al.*, 1996) to isolate AMF spores. Spore counting was done manually under stereo microscope. The roots of host plants were randomly sampled about 2 gram and stain with trypan blue according to the method of Brundrett *et al.*,

1996) and AMF root infection were determined. Data obtained were subjected to t-test analysis

RESULTS AND DISCUSSION

The results from the trap culture experiment for rhizosphere soil of *Albizia* showed that the number of spore harvested were significantly higher in mixture of river sand with zeolite as the growing media when compared to mixture of malang sand with zeolite (Figure 1). Mixture of river sand with zeolite gave spore number of 91.1 per 10 g media while the mixture of Malang sand and zeolite only gave 14.0 spores per 10 g media.

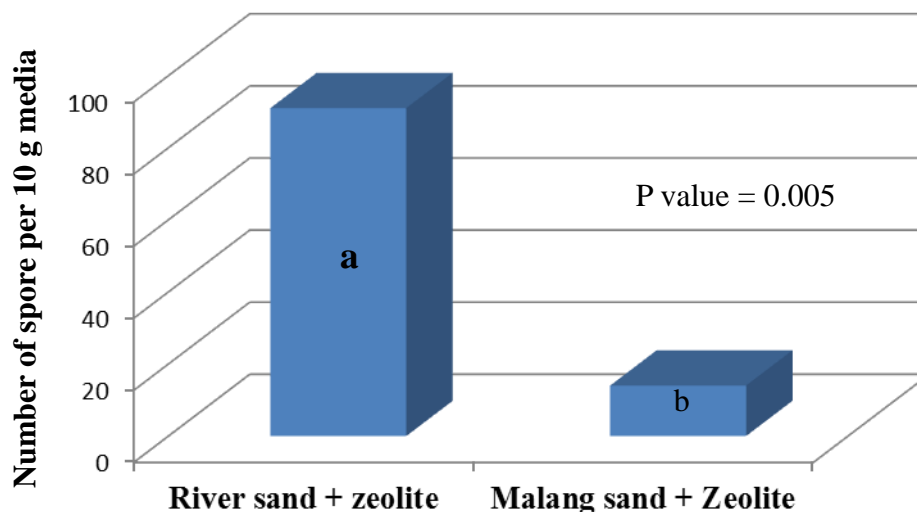


Figure 1. Spore number of AMF from trap culture using two different media.

Based on the result obtained, mixture of zeolite with the river sand was a better growing media to propagate AMF spore in trap culture. The river sand size is smaller than zeolite, so the sand particle covered the macro pores formed between particles of zeolite which have bigger size. This combination can hold more water for the plant and give good condition for the root of host plants to grow as well as the external hyphae of the AMF, hence the production of AMF spore in the pot culture was better than the other media. In addition, zeolite also has higher CEC (Balai Penelitian Tanah, 2010) which can retain the nutrient added into the media and available for the host plant root. The better host plant growth support AMF growth and development because they provide organic carbon to the fungi (Treseder, 2013; Giovannetti *et al.*, 2010). The malang sand size is higher than river sand and

almost the same with zeolite. This combination of growing media had a lot of macro pores which make the media very porous with low water holding capacity. Therefore, the host plant could not grow well consequently affected the development of AMF hyphae and AMF spore production.

In contrast to number of spore, no significant difference was obtained in root infection between the two media tested (Table 2). This result indicated that the host plant used was suitable for the AMF present in the soil. The AMF infect the root and develop along the root system massively as the rate of root infection was above 90%. Although the root infection was very high (>90%), it can be assumed that the hyphae of AMF growth outside of the root are affected by the growing media used (Figure 1). This result also in agreement with other result that the rate of root infection is not always has correlated with the external hyphae growth in the growing media (Rini, 2001)

Table 2. Maize root infection by AMF from trap culture of Albiziarhizosphere soil

Media	Root Infection (%)
River sand:zeolite	96.0
Malang sand:zeolite	94.0
P value	Not significant

From the results of this study, it can be concluded that the mixture of river sand and zeolite is a suitable media for the trap culture of AMF as this media produced significantly higher AMF spore when compared to the mixture of malang sand and zeolite.

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