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"RESEARCH, EDUCATIONAL SYSTEM AND MANAGEMENT OF BIOSCIENCE AND BIOTECHNOLOGY TOWARD THE INDUSTRIAL REVOLUTION 4.0"

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I Ketut Berata

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Prof. Dr. Ir. I Nyoman Rai, M.S.

Chairman of the
Committee

Dr. I Made Sukewijaya, S.P., M.Sc.

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THE 10TH INTERNATIONAL CONFERENCE ON BIOSCIENCE AND BIOTECHNOLOGY

"RESEARCH, EDUCATIONAL SYSTEM AND MANAGEMENT OF BIOSCIENCE AND BIOTECHNOLOGY TOWARD THE INDUSTRIAL REVOLUTION 4.0"

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Ni Wayan Febriana Utami
A.A. Keswari Krisnandhika
Rindang Dwiyani
I Nyoman Wijaya
I Gusti Alit Gunadi
I Made Agus Dharmadiatmika

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WELCOME NOTE

It has been an honor for faculty of Agriculture Udayana University to host the international conference on biosciences and biotechnology for the nine times. The ICBB is a yearly conference initiated by Asia Oceania Biosciences and Biotechnology Consortium (AOBBC). This year, the 9th ICBB is held at the same time with the celebration of the anniversary of Udayana University and the anniversary of Faculty of Agriculture Udayana University. The theme for the ICBB 2019 is "Research, Educational System and Management of Bioscience and Biotechnology toward the Industrial Revolution 4.0"

The conference had been attended by approximately 165 participants. They come from various science background, such as agriculture, health and medicine, veterinary, and animal husbandry. Participant from Australia, Japan, Korea and Indonesia itself – Java, Flores, Kupang, Sulawesi, and Bali. Their contribution on the advancement of the biosciences and biotechnology are documented partly in this proceeding book.

At this good moment, I especially would like to thank Rector of Udayana University for the financial support given. Thank you for all keynote and invited speakers, persenters, and especially writers whom have contributed their knowledge, science research and experience to the wider audience through this proceeding. To all participants for your enthusiasm during the conference that make this conference a success. I also would like to thank the conference organizer team and student volunteers for their untiring efforts to make this conference as one of the memorable ones. My best wishes to all of you.

Denpasar, 20 November 2019
Dean of Faculty of Agriculture Udayana University
Prof. Dr. Ir. I Nyoman Rai,M.S.

DAFTAR ISI

OPTIMIZATION OF URBAN AGRICULTURAL LAND THROUGH THE DEVELOPMENT OF RICE FISH CULTIVATION AS AN EFFORT TO SUSTAIN SUSTAINABLE AGRICULTURE
(Case study: Subak Umalayu, Penatih Village, Denpasar City),
Luh Putu Kirana Pratiwi[1] Ida Ayu Budhananda Munidewi[2]..... 1

BIOACCUMATION OF LEAD (Pb) AND CADMIUM (Cd) IN THE BLOOD AND TISSUES OF DUCKS REARED IN THE RICE FIELDS AROUND OF PETANU RIVER
I Ketut Berata[1], Ni Nyoman Werdi Susari[2], I Made Kardena[3],
I Wayan Sudira[4], Ida Bagus Oka Winaya[5].....20

COMPARISON OF EXAMINATION METHODS FOR IDENTIFICATION GASTROINTESTINAL PARASITES IN PIGS
Ida Ayu Pasti Apsari[1]; Nyoman Sadra Dharmawan[2] ; I Made Dwinata[3]; Ida Bagus Ngurah Swacita[4].....32

ABSTRACT
MECHANISM OF MOLD PROSPERITY OF Aspergillus aculeatus ON GROWTH OF FUNGUS Colletotricum gloeosporioides FUNGUS CAUSES OF ANTRACNOSA DISEASE IN ORANGE OF KINTAMANI CITRUS (CITRUS NOBILIS L.)
By: Ni Nyoman Darsini.....42

THE ROLES PARASITOID EGGS FOR CONTROLLING OF YELLOW RICE STEM BORERS*Scirpophagaincertulas*WALKER (LEPIDOPTERA: PYRALIDAE)
ABSTRACT By
I Nyoman Wijaya, I G P. Wirawan, Adiartayasa Wayan and Made Sritamin.....72

BIOLOGICAL CHARACTERISTICS AND VIRULENCY OF *Cucumber Mozaic Virus* (CMV) ISOLATES BALIHORTICULTURE PLANT BALIHORTICULTURE PLANT.

Ni Putu Pandawani[1], Cokorda Javandira[2], Farida Hanum[3].....83

ANALYSIS OF LIMONENE AND OTHER ANTIOXIDANTS IN COMMERCIAL ESSENTIAL OIL PRODUCTS COMPARED TO HOMEMADE VIRGIN COCONUT OIL.

Ni Made Suaniti[1], I Wayan Bandem Adnyana[2], Manuntun Manurung[3], Oka Ratnayani[4], Raisyah Anjani[5].....94

RETURN COST RATIO AND VALUE ADDED AGRIBUSSINES OF SWEET CORN "F1" *PRIMA* "ORGANIC HYBRID

Ni Putu Sukanteri, , SP., M.Agb.....102

Theme: The Sustainable Development of Agriculture

OPTIMIZATION OF URBAN AGRICULTURAL LAND THROUGH THE DEVELOPMENT OF RICE FISH CULTIVATION AS AN EFFORT TOSUSTAIN SUSTAINABLE AGRICULTURE (Case study: Subak Umalayu, Penatih Village, DenpasarCity)

Luh Putu Kirana Pratiwi[1] Ida Ayu Budhananda Munidewi[2]
Agribussiness Study Program Agriculture Faculty,
Accounting Study Program, Economics Faculty,
Mahasaraswati Denpasar University, Jl Kamboja No 11A
Denpasar, Bali 80233

*Corresponding author: kirana.pratiwi@unmas.ac.id

ABSTRACT

The existence of subak as an institutional that is socio-agriculture-religious began to be threatened due to the degradation of urban agricultural land which is increasing every year. Therefore, the Denpasar City Government has designed the establishment of a Subak Lestari Zone to create a sustainable food agriculture land. This study aims: (1) To analyze differences in rice farming income after the development of fish rice; (2) To identify alternative strategies that are effectively developed in fish farming for sustainable agriculture in urban areas. The analytical method in this study was carried out quantitatively namely feasibility analysis and qualitatively through SWOT analysis. The results showed that the rice mina farming has a higher feasibility compared to the previous rice farming. Net income from rice mina is IDR 7,165,250 (35.29%), with an R / C ratio of 2.97. The alternative strategies developed are the SO (Strength Opportunities) Strategy by: (a) Increasing production and quality according to market demand, (b) Establishing market institutions, (c). Implement environmentally

friendly cultivation and handling of product yields, (d) Organism Control Integrated Control as early as possible in accordance with economic threshold, and (e) Development of Human Resources quality skills.

Keywords: Optimization, Rice, Sustainable Agriculture, Urban Subak

CHAPTER I.
BACKGROUND

Subak is a Balinese farmer organization that manages irrigation water in paddy fields with socio-agrarian-religious characteristics. The existence of subak began to be a threat both directly and indirectly that originated from Bali tourism, namely the condition of agricultural land which is increasingly shrinking. The conversion of paddy land to non-agricultural land in Bali from 85,776 ha in 2000 to 81,144 ha in 2010 with an average of more than 660 ha or 0.77% per year (Department of Agriculture of Food Crops of Bali Province, 2010). Budiasa (2012) states that several factors for the conversion of paddy fields are: (1) The attractiveness of other sectors in the form of higher income in the non-food crops sub-sector (such as plantations), industry, and services that also encourages farmers to switch professions; (2) Socio-economic conditions can also encourage owners to sacrifice their fields to be sold to investors to cover living costs (including health) and / or to invest in children's education or work in other sectors; (3) The existence of policies and regulations which actually become a disincentive for farmers to maintain their paddy fields such as the stipulation of Land and Building Tax based on NJOP; and (4) Weak control and enforcement functions of regulations related to spatial planning and unfair land use change by related institutions.

Basically the agricultural sector in Bali is in a very weak condition. and marginalized. According to Windia (2001) it indicates that the growth of the agricultural sector is the lowest compared to other economic sectors, which is around 2.1% per year. The contribution of the agricultural sector to Bali's GRDP continues to decline and is now at 19%. The labor force working in the agriculture sector turned out to be the highest, at more than 40%, while the growth of workers who wanted to enter the agricultural sector

was estimated at 2% per year. Without rice fields, subak is impossible to exist and without subak sustainability for sustainable agricultural development in Bali will be threatened.

Due to very high land degradation, the Denpasar City Government designed the establishment of a Subak Lestari Zone to create a sustainable food agriculture land. In an effort to reduce degradation of agricultural land, the government made a Green Line Regulation in the Subak Urban Area. In principle, it is permitted to sell agricultural land, but it is not permitted to transfer land, but is still managed as agricultural land, and is able to increase the productivity of farmers in synergy with the distribution network of rice sellers and rice processing, and protect farmers' grain production in the city of Denpasar. (Department of Agriculture, Food Crops, Hoticulture and Plantation, 2016).

In the context of optimizing land narrow agriculture in urban areas is carried out by technological innovations from only rice farming to mina-rice. From an economic point of view, mina rice cultivation is an integrated cultivation that can increase the productivity of paddy fields, that is, besides not reducing rice yield, it can also produce fish. In terms of ecology, rice mina directs agriculture towards organic. Paddy fields become fertile with fish manure containing various nutrients, so as to reduce the use of fertilizer. Fish can also limit the growth of other plants that are competitive with rice in the utilization of nutrients, so that it can also reduce the cost of weeding wild plants. Minapadi cultivation is carried out in 2 (two) cropping patterns, namely interrupts and intercropping. The cropping pattern of tenders is the maintenance of fish in the paddy fields ahead of rice planting, while waiting for the results of the rice seedlings to be planted. With intensive rice mina development techniques it is expected to be able to optimize agricultural land, streamline production costs through the use of organic

dasticide fertilizers from fish dung, as well as efforts increasing farmers' income and preserving sustainable agriculture in Denpasar City.

CHAPTER II. MATERIAL AND METHODS

This research was conducted on Subak Umalayu farmers in Penatih Village, East Denpasar District, Denpasar City. The location selection method is purposive, with the main reason that Subak Umalayu is one of the pilot subaks in the Subak Lestari Area of Denpasar City which cultivates mina-rice. The population in this study were 75 farmers, the sample selected based on cencus sampling were all farmers in Subak Umalayu of 75 people. This is because the number of respondents is below 100 people (Sugiyono, 2012). The method of collecting data through structured interviews with the help of questionnaires to farmers in Subak Umalayu. This study also conducted in-depth interviews with key informants such as: Head of the Penatih Village, Pekaseh Subak Umalayu, as well as local community leaders. This research data collection method is also equipped with documentation and literature study.

The analysis in this study was carried out quantitatively using income and feasibility analysis to find out the increase in farmers' income in the usina rice farmers measured using R / C ratio calculation analysis. Whereas qualitatively to determine effective internal, external and alternative strategies in the development of sustainable micro-rice farming through SWOT analysis.

The IFAS matrix is used to evaluate internal factors for developing mina-rice cultivation, which are related to strengths and weaknesses. While the EFAS Matrix is used to evaluate external factors relating to opportunities and threats. After the difference between the score of

strengths with weaknesses, opportunities and threats obtained, then the results of this score difference are applied to the Cartesius Diagaram SWOT Analysis, to determine the quadrant position occupied by the company based on the difference in score. Then the SWOT(Strength-Weakness-Opportunitty-Threaths) matrix is used to match the results obtained on the IFE and EFE matrices. The results obtained from the SWOT matrix are alternative strategies that are feasible to use in developing strategies for effective rice cultivation as an effort to increase income and sustainable agriculture. This matrix can produce four possible alternative cell strategies, namely the S-O (Srenghts-Opportunity) strategy, W-O (Weakness-Opportunity) strategy, W-T (Weakness Threats) strategy, and S-T strategy (Strenghs-Threaths) (Rangkuti, 2002).

CHAPTER III. RESULTS AND DISCUSSION

3.1. Income and Business Feasibility of Mina Padi Farmers

The following are details of the results of research in terms of the level of income and feasibility of rice mina farming conducted in Subak Umalayu, Penatih Village, East Denpasar District, Denpasar City.

Table 1. Income and Business Feasibility of Mina Padi Farmers

No	Description	Ciherang Paddy (Rp)	Parrot Fish (Rp)	Total (Rp)
1	Land area	0,53 are	0,05 are	
2	Rice and fish seeds	432.000	1.364.500	1.796.500
3	Tillage	1.925.000		1.918.000
4	Making ponds		2.150.000	2.150.000
5	Nursery	535.000		535.000
6	Farmer's wages (irrigation, planting, weeding, fertilizer, spraying	2.325.000		2.325.000
7	Fish feed (pellets)		1.445.250	1.445.250
8	Inorganic fertilizer	655.000		655.000
9	Organic fertilizer	945.000		945.000
10	Labor's Wage in Maintenance of rice fields		375.000	375.000
11	Harvest Cost	1.798.000		1.798.000
	Total Cost	**8.615.000**	**5.334.750**	**13.949.750**
	Production Revenue	**28.920.000**	**12.500.000**	**41.420.000**
	Net Profit	**20.304.400**	**7.165.250**	**27.469.650**
	R/C Ratio			**2,97**

Source: Primary Data Processing Research, 2019

In rice fish farming for rice using ciherang varieties on an average irrigated paddy area of 0.53 ha. The technological innovations applied in rice farming are integrated crop management, among others: ciherang variety, jajar legowo planting system, fertilizing according to Minister of Agriculture Regulation (Permentan No 40 of 2007), the use of organic fertilizer, intermittent irrigation, Control of plant-disturbing organisms refers to integrated pest control. In controlling plant-disturbing organisms as early as possible, as soon as symptoms of an attack appear and approaching the economic threshold scale is immediately controlled. Whereas in the Parrot fish business by utilizing technical irrigated rice fields by making a pond near the rice field dike as needed. Fish seeds are sown by 5.000 parrot fishseeds or with a density per m^2 of 20-25 parrot fishseeds. From the results of tilapia farming when harvested an average of 5-7 heads per kg. In this rice mina farming business, it can receive an income of Rp.41.420.000. While the net profit gained Rp. 27.469.650. The R/C ratio is 2,97. Value added the results of the analysis of rice mina farming show that it can provide added value, namely additional revenue of Rp 7.165.250, -. Previously, only in rice farming, the net profit received was Rp. 20.304.400. So with the additional benefits from the tilapia business, the total net profit received will be Rp. 27.469.650. Presented in Table 1.

3.2 Mina Padi Cultivation Development Strategies as Efforts to Increase Farmer's Income and Sustainability of Sustainable Agriculture

Based on the results of the research, the strategy of developing mina-paddy cultivation as an effort to increase farmers' income and preservation of sustainable agriculture at the research sites of Subak Umalayu, Subak Umalayu, Penatih Village, East Denpasar District, Denpasar City, is described as follows.

-

IFAS / EFAS	Strength	Weakness
	a. Rice cultivation is usually done intensively	a. Lack of distribution of irrigation water for fish maintenance due to infrastructure network improvements
	b.Fish food can be made by farmers	b. Many farmers are still controlling crop pest organisms with chemicals that have an impact on the death of tilapia
	c. Parrot fishcan be harvested together with rice plants (double income)	c. Nutrition has not been maximized that affects the weight of tilapia
	d.Parrot fishcontain high protein, low fat, and safe for consumption	d. Treatment of sorting and grading has not been maximized so that the price sold by farmers is below the market price
Opportunity	**S-O Strategy**	**W-O Strategy**
a. Hard is a basic need that is much needed by the community.	a. Increase production and quality according to market demand	a. Implement environmentally friendly agricultural cultivation
b. Fish seeds are easy to get c. Market and restaurant requests for lots of tilapia d.Price for selling fish is quite good	b. Establish market institutions	b. Improve post-harvest handling of product yields
Threat	**S-T Strategy**	**W-T Strategy**
a.Organism plant pests in rice plants always develop	a. Control of plant-disturbing organisms and predators as early as possible according to the economic threshold	a. Development of human resources skills in the management of production, post-harvest, marketing of environmentally friendly rice products
b. Predatory organisms (snakes, pestle) often prey on fish		

Table 2. SWOT Matrix Analysis Research In Subak Umalayu, Penatih Village, Denpasar City.

Source: Primary Data Processing Research, 2019

In detail, the development strategy of fish rice cultivation as an effort to increase farmers' income and the preservation of sustainable agriculture in Denpasar City, is described as follows:

S-O Strategy

1. Increase production and quality according to market demand. Rice food is a basic need so that it is needed by many people, farmers are used to rice cultivation. Likewise, fish is a household consumption need to improve family nutrition, so that consumers / markets always need it. Therefore, production and product quality must be maintained.
2. Establish market institutions. The market is the spearhead for marketing products, therefore it is necessary to establish a good and maintained relationship. Product continuity needs to be maintained to meet market and restaurant demands. This will be mutually beneficial for both parties and for the continuation of mina rice farming.

W-O Strategy

1. Implement environmentally friendly cultivation and harvesting of products. For the use of production facilities, especially drugs, medicines must be chosen that are not harmful to fish. Need types of drugs that are environmentally friendly or organic, and its application needs attention.
2. Improving the handling of post-harvest products through the implementation of grading and care, namely after the fish is harvested it needs to be sorted and treated before being

taken to the market and restaurants. Therefore, for novice farmers is needed adequate skills.

S-T Strategy

1. Pest control as early as possible according to the economic threshold. Plant-disturbing organisms (OPT) are a threat in rice and fish cultivation if not controlled seriously will hamper and even frustrate the business. Therefore this pest control must be non-negotiable.

W-T Strategy

1. Development of Human Resources skills, so that rice mina farming can be more successful, farmers still need to be fostered so that they are more involved in controlling what is threatening and maintaining and handling product yields before being marketed to support sustainable rice mina business.

CHAPTER IV. CONCLUSIONS AND SUGGESTIONS

4.1. Conclusions

1. Net income from rice mina is Rp. 7.165.250 (35,29%), with an R/C ratio of 2,97.
2. The alternative strategy developed is the SO (Strength Opportunities) Strategy by: (a) Increasing production and quality according to market demand, (b) Establishing market institutions, (c). Implement environmentally friendly cultivation and handling product yields, (d) Organism Control Integrated Control as early as possible in accordance with economic threshold, and (e) Development

of quality skills in Human Resources.

4.2. Suggestion

1. For farmers, the development of rice fish to be more intensive is carried out at each planting season as an effort to increase income and preserve sustainable agriculture.
2. For the government, through agricultural extension services, agriculture and fisheries services to be more optimal in the application of organic-based urban agriculture, as well as partnerships in accessing capital and marketing of agricultural products.

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BIOACCUMATION OF LEAD (Pb) AND CADMIUM (Cd) IN THE BLOOD AND TISSUES OF DUCKS REARED IN THE RICE FIELDS AROUND OF PETANU RIVER

I Ketut Berata[1], Ni Nyoman Werdi Susari[2], I Made Kardena[1], I Wayan Sudira[3], Ida Bagus Oka Winaya[1]

[1]Pathology Laboratory of Faculty of Veterinry Medicine, Udayana University
[2]Anatomy Laboratory of Faculty of Veterinary Medicine of Udayana University
[3]Pharmacology Laboratory of Faculty of Veterinry Medicine, Udayana University
Jl.PB. Sudirman Denpasar Bali 80234
Corresponding author : berata_iketut@unud.ac.id

Abstract

Lead (Pb) and cadmium (Cd) heavy metals can accumulate in the body of animals and humans, if they consume through food, drinks or inhalation. Ducks as a source of food, especially those that are reared in rice fields around the Petanu river, have the potential to be polluted by Pb and Cd, due to the many objects and tourism facilities. This study aims to determine the presence of Pb and Cd contamination in the blood and tissues of ducks reared in the rice field around of the Petanu river. Samples were taken from 10 ducks, then its were necropsied for blood, liver, kidneys, and ovaries tissues. The blood and tissue samples were examined for Pb and Cd levels using atomic absorption spectrophotometry (AAS) method at the Analytical Laboratory. The examination of Pb levels were obtained results : blood =

0.295 ±, 052 ppm, liver = 0.235 ± 0.039 ppm, kidney = 0.201 ± 0.050 ppm, ovary =0.180 ± 0.031 ppm, respectively. This Pb levels are still lower than the maximum standard that is suitable for consumption, which is 2.00 ppm. Examination of Cd levels was only found in the blood and ovary of a duck was 0.222 ppm and = 0.393 ppm respectively, and 2 ducks in the liver were 0.278 ppm and 0.373 ppm, respectively. For Cd examination more samples are needed to obtain higher validity. It can be concluded that there are bioaccumulation of Pb in the blood, liver, kidneys and ovaries tissues of ducks reared in the rice field around of Petanu river, but its are still safe for consumption.

Keywords: bioaccumulation, lead, cadmium, blood, tissue

Background

Environmental pollution, especially by lead (Pb) and cadmium (Cd), threatens the health of animals and humans. These heavy metals enter the body through food, drinking water or inhalation of air in a polluted environment. Ducks as a source of food are vulnerable to exposure to heavy metals (Ferreyra et al., 2015), both in meat and innards (Muselin et al, 2010). Duck meat in several types of processed highly favored by domestic and foreign tourists in Bali. To maintain the quality of duck meat, it must be free from lead or cadmium contamination.

The lead accumulation in tissues can cause oxidative stress, so that it impacts on the disruption of various tissues or

organs, including disorders of the hemopoietic, cardiovascular, reproductive and nervous systems (Brochin et al., 2008). Pb^{++} ions in circulation in the nervous system can substitute Ca^{++} ions so that it will disrupt brain function, especially in children (Jaishankar et al., 2014). Lead contamination in bone marrow in ducks is closely related to immune system disorders characterized by decreased spleen (Ferreyra et al 2015). Similarly, Cd contamination is very vulnerable to damage the blood filtration system in the kidneys and can be a trigger for carcinogenic (Sharma et al., 2014).

Increased developing of tourism facilities along the Petanu river, environmental pollution has also increased so that waterfowl including ducks are suspected of being heavily contaminated with lead and cadmium. These two heavy metals are widely studied because they are the most common environmental pollutants and are easy to accumulate in the bodies of waterfowl including ducks (Mateo et al, 2007; Kalisińska and Salicki, 2010). Meat and innards of ducks is widely used as a favorite menu in restaurants in Bali. How much prevalence of ducks that are raised around the Petanu river are exposed to lead and cadmium, and in whatever tissue accumulates, it is important to be periodically examined.

Material and Method

Sample

Samples in the form of ducks are kept in rice fields around the Petanu river, as many as 10 ducks. Parts that are examined for lead and cadmium levels include: blood, liver, kidneys, lungs and ovariy, respectively..

Measurement of heavy metal content

The tissue samples, such as: bloods, liver, kidney, lungs and ovary, were processed for the measurement level of lead and cadmium heavy metals by using atomic absorption spectrophotometry (AAS) method (Sikiric, et al., 2003). The samples were divided into two parts, 0.5 ml for positive control and 0.5 ml as sample to be evaluated. Standard solution 0.25 ml of 1 mg/l was added as positive control. The control was evaporated on a hot plate at a temperature of 100ºC until it dried. Then, the spike and the samples were inserted into a furnace and covered half of their surface. In the process, the temperature furnace was raised gradually 100ºC every 30 minutes up to 450ºC and maintained for 18 hours. After that the spike was removed from the furnace and chilled at room temperature. Next, 1 ml HNO3 65% was added, before they were shaken carefully so that all the ash dissolved in acid and then they were evaporated on a hot plate at a temperature of 100ºC until they were dried. The samples and spike put them back into the ash furnace. Its temperature was raised gradually 100ºC every 30 minutes up to 450ºC and maintained for 3 hours.

After they were formed white ash, the spike and samples were cooled at room temperature. A 5 ml of HCl 6 M solution was added to each sample and spike then shaken carefully so that all the ashes were dissolved by acid. Then they were evaporated on a hot plate at a temperature of 100ºC until dried. A 10 ml of 0.1 M HNO3 was added and cooled at room temperature for 1 hour, the solution was transferred into a 50 ml flask poly propylene before they were added with matrix modifier solution, then added with 0.1 M HNO3 until it reached to the mark limit. Lead and cadmium heavy metals working standard solution was prepared at least five points concentration. Working standard solution, samples, and spike were read on graphite fumace atomic absorption spectrophotometry at a wavelength of 288.3 nm for both lead and cadmium heavy metals. Data of research analized by qualitative descriptive

Result

Result of measurements of lead (Pb) and cadmium (Cd) heavy metals in the blood, liver, kidney and ovary, as presented in Table 1.

Table 1. Result of the lead and cadmium measurement on the sample duck tissue

No Sample	Level of Lead (ppm)				Level of cadmium (ppm)			
	Blood	**Liver**	**Kidney**	**Ovary**	**Blood**	**Liver**	**Kidney**	**Ovary**
1	0,249	0,266	0,124	0,259	-	0,278	-	-
2	0,241	0,288	0,245	,0166	0.222	0,373	-	0.393
3	0,206	0,203	0,219	0,149	-	-	-	-
4	0,341	0,226	0,218	0,188	-	-	-	-
5	0,284	0,228	0,123	0,197	-	-	-	-
6	0,291	0,169	0,184	0,177	-	-	-	-
7	0,304	0,249	0,262	0,168	-	-	-	-
8	0,344	0,214	0,172	0,164	-	-	-	-
9	0,315	0,293	0,209	0,155	-	-	-	-
10	0,375	0,218	0,258	0,179	-	-	-	-
Mean ±SE	0,295 ±0,052	0,235 ±0,039	0,201 ±0,050	0,180 ±0,031	0.222	0,326	-	0,393

(-) = not detected

The average levels of lead in the blood = 0.295 ± 0.052 ppm, liver = 0.235 ± 0.039 ppm, kidney = 0.201 ± 0.050 ppm

and ovary = 0.180 ± 0.031 ppm. Comparison of lead levels among in the blood, liver, kidney and ovary tissues graphically is presented in Figure 1. While cadmium heavy metal was found in one sample, that is in the blood = 0.222 ppm, and ovary = 0.393 ppm and two liver samples respectively 0.278 ppm and 0.373 ppm, respectively.

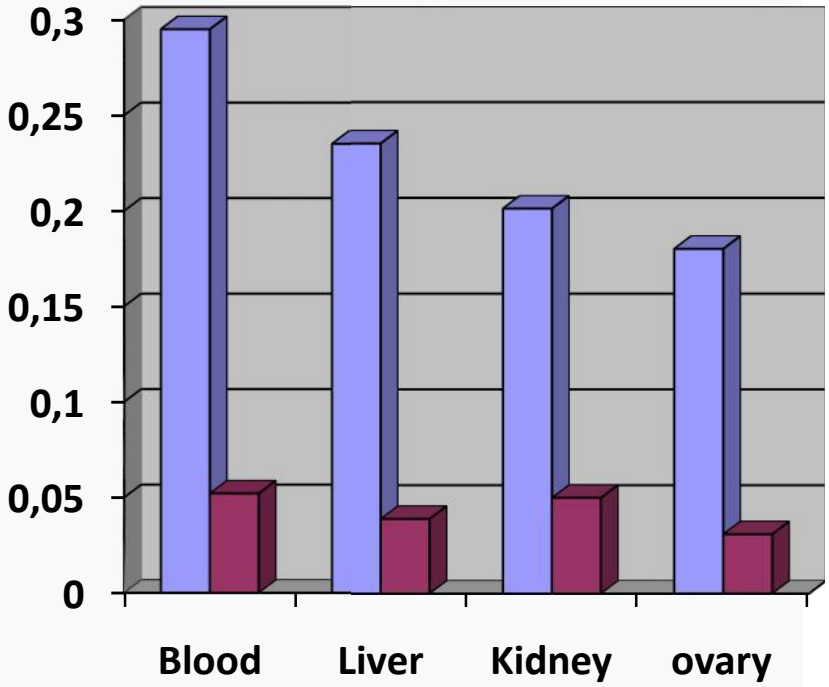

Figure 1. Comparison of lead level among in the blood, liver, kidneys and ovaries of the ducks. The highest level of lead appear in the blood and lowest in the ovary

Discussion

Bioaccumulation of lead in ducks was highest in blood (0.295 ± 0.052 ppm) compared to liver, kidneys and ovaries. These results indicate that the portion of blood is the most risky part if consumed by humans or animal feed ingredients will pollute the body and cause pathological disorders. The same thing happened in cattle that are kept in Suwung landfills found the highest lead contamination in the blood compared to other tissues (Berata, et al 2017). Thus ducks can be used as indicators of the presence of lead contamination in their environment, as studies of bio-indicators of environmental pollution that use waterfowl (Ferreyra, et al 2015; Kalisińska and Salicki, 2010) and wild birds (Mateo et al. ., 2010). Based on the results of research on heavy metal contamination in the body of animals and humans, the most accurate for lead examination is in the blood (Sharma, et al., 2014).

The liver is an organ as a metabolic center for various nutrients and detoxification against dangerous and toxic substances, including heavy metals (Brochin et al., 2008). This liver function causes high accumulation of heavy metals, so that it will have chronic pathological effects. Laboratory studies with the administration of lead acetate cause to liver pathological disorders in the form of centrolobular vein swelling, congestion

and necrosis (Muselin et al., 2010). High levels of lead in the liver also occur in fish (Alina et al., 2012). Impact of heavy metal intoxication on various animals show similarly in their lession (Akan et al., 2010).

Kidney as an organ that plays a role in blood filtration and excretion of waste material from the body, is an organ that is equally vulnerable to liver in terms of intoxication. Within the chronic dose limit, the kidneys experience lower contamination than the liver, according to the results of this study. If the lead is toxic, the kidneys to become necrosis of the tubule of the constortus and glomerular amyloidosis (Muselin et al., 2010).

Lead accumulation in ovary is an indication that this heavy metals can transmit to their fetus, so why babies or newborn animals have been exposed by lead heavy metal. Heavy metal contamination in infants in humans and animals can cause a decrease in cognitive ability (Brochin et al 2008). The mechanism begin with the substitution of Ca^{++} ions by Pb^{++} so that lead can penetrate through the endothelial barrier in the blood vessels of the brain (Toscano and Guilarte, 2005). Heavy metal accumulation in chicken eggs proves that heavy metals in the parent's body can move to it’s chicks by intrauterine (Abdulhaliq et al 2012)

The limited found cadmium in this study shows the possibility of ducks is not a good bio-indicator for the cadmium.

For that, cadmium examination research is needed with a larger number of samples, so that more valid results can be obtained.

Conclusion

There is bioaccumulation of lead in the blood and tissues of duck maintained around the Petanu river. Ducks can be used as bio-indicators of environmental contamination of lead heavy metal.

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COMPARISON OF EXAMINATION METHODS FOR IDENTIFICATION GASTROINTESTINAL PARASITES IN PIGS

Ida Ayu Pasti Apsari1; Nyoman Sadra Dharmawan2 ; I Made Dwinata1;

Ida Bagus Ngurah Swacita3

1. Parasitology Laboratory, 2. Clinical Pathology Laboratory, 3. Veterinary Public Health Laboratory, Veterinary Studies Program, Faculty of Veterinary Medicine, Udayana University, Denpasar Jln.PB.Sudirman - 80234

Email: pastiapsari.45@unud.ac.id.

Abstract

Research has been carried out on pigs at Sanggaran Slaughterhouses. The accuracy of the method used is one of the successful identifications carried out in the examination of faecal samples. The aim of the study was to compare the two examination methods used to identify gastrointestinal parasites in pigs. Samples of 100 pig feces were obtained from Sanggaran Slaughterhouses. Examination of pig feces using two methods, namely floatation with sugar sheater and saturated salt. Results of examination of pig faecal examination obtained 44% Coccidia oocysts and 21% Ascaris worm eggs through examination of the floatation method with sugar sheater. Examination by the method of saturated salt float obtained 23% Coccidia oocysts and 23% Ascaris suum. Analysis with chi square sugar sheater floatation method was more significantly used to identify Coccidia oocysts, while the saturated salt floatation method to identify the two is no different. The conclusion of examination to identify protozoa (Coccidia) sugar sheater method is more effective than the floatation method saturated salt, but the examination for identification parasitic worms (Ascaris suum) the use of the floatation method between the two methods is not different.

Keywords: sugar sheater, saturated salt, pigs, Coccidia, Ascaris suum

Background

Pigs in Bali have a very important role, because besides being a provider of animal protein for the community it also plays a role in socio-cultural customs. Based on statistical data on pig population by province, Bali occupies the fifth place after East Nusa Tenggara, North Sumatra, Papua and South Sulawesi (Badan Pusat Statistik, 2018). There are three systems for raising pigs in Bali, namely traditional, semi-intensive and intensive (Agustina, 2013). The maintenance system will affect the health of the livestock itself, in addition to environmental conditions, feed and germs. In pigs the percentage of infected by endoparasites reaches 30% - 96% (Yasa et al., 2010; Lai et al., 2011; Dey et al., 2014; Tolistiawaty et al., 2016). Endoparasitic infections result in losses that also affect the farmer's economy.

Pigs are susceptible to infection with ectoparasites and endoparasites. Endoparasites which often infect pigs are protozoa such as Coccidia, Balantidium, Entamoeba and Giardia and nematode worm parasites including Ascaris, Trichuris, Strongyl type, Strongyloides. Endoparasitic infections in pigs result in losses for farmers as well as for their own animals that suffer from parasitic diseases. More importantly, pigs are reservoirs for various diseases including parasitic diseases (Schuster and Ramirez, 2008), let alone parasites that are zoonotic.

The diagnosis of gastrointestinal disease in pigs is done based on stool examination to establish the diagnosis as identification of the parasitic agent. Stool examination includes qualitative and quantitative examinations. Qualitative inspection with native and concentration methods (sedimentation and floatation methods). The floating concentration method was used to identify gastrointestinal parasites from pig faeces in this study. The accuracy of the method will affect the results of parasitic identification. The floating concentration method is more appropriate for identification of protozoan and

nematode parasites from examination of gastrointestinal parasites. Some of floating solution that can be used for identification methods include saturated sugar, saturated salt, magnesium sulfate, zinc sulfate (Garcia et al. 2018).

The use of faecal examination methods that have a high level of sensitivity and specificity is very important to get an accurate parasitosis (parasitic infection) status (Regina et al., 2018). Gastrointestinal parasitosis status in the host (human / animal) can be ascertained by finding worm eggs or cysts or tropozoites from protozoa on stool sample. Gastrointestinal nematode worm parasites in pigs are dominated by Ascaris suum 50.9% (Dey et al., 2014), 40% (Suryastini et al., 2012), 27.27% (Tolistiawaty et al., 2016) and 14.7 % (Tiwari et al., 2009). Coccidia protozoan parasites (Eimeria spp. And Isospora spp.) 68.2% (Yuliari et al., 2013), 60% (Yasa et al., 2010), 56.4% (Dey et al., 2014) and 40, 3% (Tiwari et al., 2009).

To compare the two examination methods, the floating concentration method was used in this study by comparing two different float solutions, namely sugar sheater and saturated salt. Detection of the parasite was chosen by finding Ascaris suum nematode worm eggs and oocysts from Coccidia protozoa.

Materials and Methods

The research material was pig feces obtained from Sanggaran Slaughterhouse (RPH) for 100 faecal samples. Saturated salt and sugar sheater (500 g of sugar + 6.5 g of phenol crystals and 320 ml of distilled water) as a floating solution used in this study.

The floating concentration method use two different floating solutions, namely sugar sheater and saturated salt according to the method of Garcia et al. (2018). Stool examination is carried out according to the method used to find Ascaris suum worm eggs and oocyst Coccidia protozoa. One hundred stool samples examined recorded the number of positive or negative checks from the two methods (floatation with saturated salt and sugar sheater) used.

The data obtained in the form of a percentage of Ascaris suum or oocyst Coccidia were found from each examination with saturated salt and sugar sheater. Data were then analyzed using chi-square to compare the two examination methods. The sensitivity and specificity of the discovery of Ascaris suum worm eggs and Coccidia oocysts can be calculated by the percentage of positive and negative results of the examination.

Results

The results of the examination of 100 samples pig feces obtained from the Sanggaran Slaughterhouse are presented in Table 1. Based on these results, they are divided into worm parasites (Ascaris suum, Trichuris sp. And Strongyl type) and protozoa (Coccidia, Entamoeba and Giardia).Text (Time New Romans, 12 font size, justified)

Table 1. The Results Examination of Pig Faecal Samples

Parasites	Sugar Sheater Floatation Methods (%)	Saturated Salt Floatation Methods (%)
Coccidia	44	23
Ascaris suum	21	23
Trichuris	3	5
Tipe Strongyl	6	9
Entamoeba	1	0
Giardia	4	0

Thus the results of the overall stool examination (Table 1), then for the comparative test the method was tested for the results of Ascaris suum and Coccidia oocyst. Pig fecal samples with saturated salt floatation method obtained Ascaris suum 23% and Coccidia 23%, while using sugar sheater floatation method obtained Ascaris suum 21% and Coccidia 44%. After being tested with chi-square sugar sheater floatation method was more significantly used to identify Coccidia oocysts, while the saturated salt floatation method was no different to identify both (Table 2)

Table 2. Chi-square Test of the Sheater Sugar and Saturated Salt Floatation Method

Examination	Coccidia		significansi	Ascaris suum		significansi
Methods	Positif	negatif		positif	negatif	
Sugar sheater	56%		0,003**	21%		0,5ns
44%				79%		
Saturated Salt	77%			23%		
23%				77%		

Note : ** very significan

ns : non significan

The sensitivity and specificity tests of Coccidia and Ascaris suum are presented in Table 3 and Table 4, respectively.

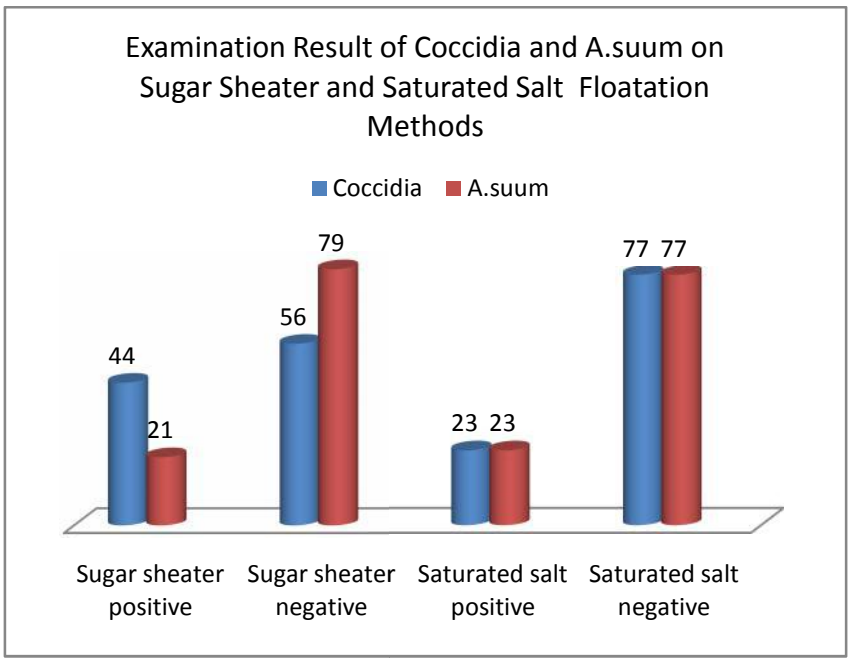

Figure 1. Histogram the results of Coccidia and A.suum examination using sugar sheater and saturated salt method.

Table 3. Test Sensitivity and Specificity of Coccidia

Sugar Sheater Methods	Saturated Salt Methods		Sum
	Positive	Negative	
Positive	23	21	44
Negative	0	56	56
Sum	23	77	100

The result of sensitivity Coccidia are 100% and specificity 27,27%.

Table 4. Test Sensitivity and Specificity of Ascaris suum

Sugar Sheater Methods	Saturated Salt Methods		Sum
	Positive	Negative	
Positive	17	4	21
Negative	6	73	79
Sum	23	77	100

The result of sensitivity Ascaris suum are 73,9% and specificity 94,8%

Discussions

The floatation method with the floating solution used will float the parasites (worm eggs and protozoan oocysts) to the surface of the tube, which will later be examined on a glas object looking clean making it easier to identify (Garcia et al., 2018). The principle of using a floating solution in the floation concentration method is the difference in specific gravity of each float solution. BJ 1.2 saturated salt while BJ saturated sugar 1.12 - 1.30. The floating solution with BJ is higher, so it can float more heavier worm eggs as well as for Coccidia protozoa. The use of a solution that has a higher osmotic, can lysis the protozoan oocysts and distortion if more than 15 minutes are examined (Baxby et al., 1984). The existence of such a statement, thus answers the situation that with saturated salt is significantly different in identifying Coccidia compared to Ascaris suum. Meanwhile, Ascaris suum is known to have a thicker wall of worm eggs so that it is more resistant to lysis if there is a long time in a solution that has a higher osmotic.

The use of faecal inspection methods that have a high level of sensitivity and specificity is very important to get an accurate parasitosis (parasitic infection) status (Regina et al., 2018). Coccidia with a sugar sheater floatation examination method gives a very high right positive result compared to saturated salt, so it has 100% sensitivity. In the otherwise Ascaris suum with saturated salt floatation methods give right negative results are very high compared to sugar sheaters, so it has a specificity of 94.8%. In contrast to the ordinary sedimentation method used to diagnose gastrointestinal parasites, such as the results of the study of Bayayibign et al. (2019) that ordinary sedimentation method is better than native methods. While, Ritchie's sedimentation method is better than native and ordinary sedimentation methods.

Conclusions

The identification of protozoa (Coccidia) using the sugar sheater floatation method is more effective than saturated salt, but the examination for identification of parasitic worms (Ascaris suum) using the floatation method between the two is not different.

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ABSTRACT

MECHANISM OF MOLD PROSPERITY OF Aspergillus aculeatus ON GROWTH OF FUNGUS Colletotricum gloeosporioides FUNGUS CAUSES OF ANTRACNOSA DISEASE IN ORANGE OF KINTAMANI CITRUS (CITRUS NOBILIS L.)

By: Ni Nyoman Darsini.

Biology Department, Faculty of Mathematics and Natural Sciences, Udayana University

Siam orange is one of the superior fruits from the agricultural sector in Indonesia besides bananas and mangoes. Siam fruit is also a superior fruit in the province of Bali. Based on information from the Bangli Regency Agriculture Office and the results of a field survey in the last two years (2013 and 2014) citrus plants in Kintamani are threatened by anthracnose disease. This disease is characterized by symptoms of round brown spots on the fruit.

Darsini (2018) has conducted research on identifying fungi that cause anthracnose disease in Kintamani conjoined citrus fruits as well as exploring potential fungi as antagonistic fungi that can inhibit the growth of pathogenic fungi that cause anthracnose disease in Kintamani conjoined oranges. Based on the results of these studies have identified fungi that cause anthracnose and antagonistic fungi that inhibit the growth of pathogenic fungi in Kintamani conjoined oranges. These mushrooms in a row: Collettoricum gloeosporioides and Aspergillus aculeatus.

This research was conducted to find out the mechanism of inhibition of growth of C. gloeosporioides by A. aculeatus. The method used in this study is the SEM method, namely by observing ultrastructures taken from the SEM (Scaning Electron Mychroskophy) electron microscope.

This research was conducted to find out the mechanism of inhibition of growth of C. gloeosporioides by A. aculeatus. The method used in this study is the SEM method, namely by observing ultrastructures taken from the SEM (Scaning Electron Mychroskophy) electron microscope.

Based on the images obtained from the SEM method, it can be seen the mechanism of inhibition of the growth of pathogenic fungi by antagonist A. aculeatus fungi is by the process of colonization and melysis of pathogenic fungal hyphae C. gloeosporioides.

Keywords: Pathogenic Fungus, antagonist, inhibitory mechanism

CHAPTER I

PRELIMINARY

1.1 Background

B uah orange is one of the leading commodity of sec tors of agriculture in Indonesia, in Bali, especially in addition to bananas and mangoes. Some types of oranges that have been cultivated in Indonesia are: tangerines (Citrus reticulat a) , large oranges (C. maxima) , limes (C. aurantifolia) , and lemons (C. lemons) . Tangerine (Citrus nobilis L .) Is the orange yes ng most cultivated (60 %), and citrus dominate the national market (Se Mangun, 2000).

Production and quality of citrus fruits Indonesia is still low caused by the presence of various growth disorders citrus plants, among other things CPVD disease, belendok , anthracnose, defi ciency of nutrients , and others (Ministry of Agriculture, 2013).

A khir these days with the occurrence of a disturbance anthracnose , production and quality of citrus fruit production dropped significantly kintamani s . Initially fruit production j

meet the needs of consumers and farmers still get optimal income. Citrus plants in Kintamani are expected to remain conserved so that the effects of antacnosis are not a big problem for citrus farmers in Kintamani. Citrus disease which has become a big problem for farmers is CVPD (Citrus Vein Phloem Degeneration) which attacks citrus plants in Singaraja. (Bangli Regency Agriculture Office , 2013).

1.2 Research Objectives

To determine the mechanism of inhibition of Aspergillus aculeatus on the growth of Colletotricum gloeosporioides fungi in anthracnose in conjoined citrus fruits Kintaman i (Citrus nobilis L.)

1.3 Research Benefits

The results of this study are expected to be used as a reference or alternative in developing an environmentally friendly anthracnotic disease control strategy, especially for farmers and entrepreneurs of conjoined citrus plants

CHAPTER II
RESEARCH METHODS

The study was conducted using the field survey method and *in vitro* research in the laboratory

CHAPTER III
RESULTS AND DISCUSSION

3. The first symptoms of the disease a ntraknosa on orange Kintamani

Symptoms of anthracnose disease can be found in citrus fruit organs . Symptoms of citrus fruit with anthracnose symptoms are brown patches , if the weather supports infection, it will get worse and the fruits will fall. The attacked fruit is eradicated by farmers to avoid transmission of healthy fruit. Ningsih *et at.* , 2012) .

The condition of infected Kintamani conjoined oranges while still being in a tree was destroyed . P Felling trees trimming logs orange siam kin Tamani complain because very dir ugikan by anthracnose. Condition Citrus fruits that are almost harvested fall because they are heavily infected by phatogenic fungi that cause anthracnose disease (figure 2.1)

Figure 3 . 1

The condition of the Kintamani conjoined orange that falls after being infected with the fungus pathogens that cause disease a ntraknosa (personal collection)

Pathogen causes anthracnose causes farmers no longer able to sell fruit oranges outside Bali, such as Jakarta, Bali and Lombok.

Causes and cycles of anthrax

P enyakit anthracnose of citrus caused by a fungus *Colletotricum gloiosporioides* are classified into Class *Deuteromycetes,* order *Melanconiales* and family *Melanconiaceae* . (Semangun, 2000).

Cycle Life mushrooms *Colletotricum* sp . begins with the formation of white cabbage mycelium which turns gray with mycelium arising on the surface. Slowly turns black and finally aservulus, conidia are oval shaped with rounded edges (Rusli *et al.*, 1997).

The initial stage of infection with *Colletotricum* sp. originated from spore germination on the surface of plant organs and then produced a sprout tube. The next stage is penetration marked by the formation of hyphal tissue, intra hyphal and intercellular hyphae that spreads through plant tissue. Spore *Colletotricum* sp. d apat spread by rain and on a suitable host will develop well (Dickman, 2000).

The infection process occurs after apresoria is formed, hyphae penetrate the cuticle then grow and destroy the plant cell walls , so that adjacent cells die. When the tissue is damaged, it enters the Xylem and *Colletotricum* sp. Generally *Colletroticum* sp. survive by colonizing intracellular tissue. *Colletotricum* sp. tend to produce latent infections in raw fruit, this is because the raw fruit is rich in organic acids and phenol compounds but poor

in carbohydrates. In contrast, ripe fruits are rich in carbohydrates but poor in organic acids and phenol compounds. Therefore during ripening the sprouts can begin to develop from apresoria and penetrate the cuticle and epidermis. Hifa invades fruit tissue further and causes necrotic spots (Agrios, 2005).

Anthracnose disease control

Anthracnose disease control in citrus plants can be done in the following ways : (1) keep the plant always in optimum condition because the disease is mainly determined by the condition of the plant. For example with adequate maintenance, fertilizing, and irrigation. (2) The graft should be replaced with a patch (grafting) because the paste has stronger roots so that the orange plants avoid drought and are more resistant to poor maintenance. (3) To reduce the source of infection of dead branches as a source of infection cut and burned. (4) washing contaminated fruit at harvest to prevent the source of the inoculum in the skin of the fruit and transmitted to healthy fruit during post-harvest (Rukmana, 1997).

Control with synthetic pesticides can indeed produce results, but farmers should also carry out plant maintenance optimally . B erdasarkan consideration that p engendalian with pesticides synthetic suli t implemented by farmers citrus umumn so weak economy. Because the source of infection is always there, in the humid period (rainy season) plants need to be treated continuously, which in itself requires a lot of costs. The fungus *C. gloeosporioides* is a polyphagous fungus that can infect a variety of plants, so that the source of infection is always around the citrus plants . In the affected part in humid and shady weather the fungus forms large amounts of spores (conidium), which are bound in a pink mucus mass. Spores are

mainly scattered by splashes of rainwater and insects. Anthravenous disease control can be done with garden sanitation. Diseased branches are cut so that they do not become a source of infection for the fruits. Reducing garden moisture, for example by pruning or planting that is not too tight (Semangun, 2000 and Agrios, 2005).

Biopesticides

Biopesticides are divided into two namely bio-pesticides and bio-pesticides. Biopesticides vegetable is a bi o pesticides based on extracts of organs plant that is able to g endalikan OPT (Plant Pest Organisms). Biopesisida Biopesisida is a biopesticide based on microorganisms such as bacteria , viruses and fungi that are biologically capable of controlling pests. Vegetable biopesticides are sourced from leaves, fruit, seeds, tubers and roots that contain secondary metabolites and have toxic properties against certain pests and diseases. Vegetable pesticides are generally used to control pests (insecticidal) and disease (bactericidal). Biological pesticides are formulations that contain certain microbes in the form of fungi, bacteria, and viruses that are antagonistic to other microbes (causing plant diseases) or produce certain compounds that are toxic to fungal pathogens, insects (pests) and nematodes (causes of plant diseases) (Andra, 2013 and Suprapta, 2014).

Estopicidal biop can be classified into various based on their function and origin, the classification is as follows:

(1) Biological Fungicide (b iofungisida) comes from the Latin word fungus or the Greek word spongos which means mushroom, functions to kill fungus. Some fungicides that have been used are: Spore *Trichoderma sp* . used to control white root disease in rubber plants and fusarium wilt in chilies.

Gliocladium species *G . roseum* and *G. virens* . to control root rot in chilies due to the attack of the *Sclerotium Rolfsii* fungus . *Bacillus subtilis* which is a saprophytic bacteria is able to control the fungus attack *Fusarium sp* . in tomato plants. (2) Herbicides biology (b ioherbisida) , p estisida derived from microbes that are used to control weeds, for example *Phytophthora palmivora* used to control *Morrenia odorata* , weeds in citrus. (3) Insecticides biology (b ioinsektisida) b erasal of microbes used as an insecticide. Microorganisms that cause disease in insects cannot cause interference with other animals or plants. The types of microbes that will be used as insecticides must have specific characteristics, which means they must attack the targeted insect and not other species. Pathogenic microbes that have been successful and have potential as biological insecticides, one of which is *Bacillus thuringiensis* . Other types of biological insecticides are those derived from protozoa, *Nosema locustae*, which have been developed to eradicate grasshoppers and crickets. nematode was first diunakan as an insecticide is *Neoplectana carpocapsae* . This insecticide is used to kill all forms of termites. (4) Biological nematicide (b ionematicide), derived from the Latin word nematode or Greek nema meaning thread, functions to kill nematodes (a kind of worm that lives at the root) (Andra, 2013)

Observation Results of Pathogenic Hyphae Fever Damage by Antagonistic Fungus

Observation of pathogenic hyphae fungal damage by antagonistic candidate fungus through SEM method was carried out to determine the surface damage of pathogenic fungal hyphae caused by the treatment of candidate antagonistic fungus treatment. Samples were prepared from the results of a *dual*

culture between pathogenic fungi with antagonistic fungi. The part taken was part of the meeting between the pathogenic fungal meselia and antagonistic fungi / parts circled in green (figure 5.24).

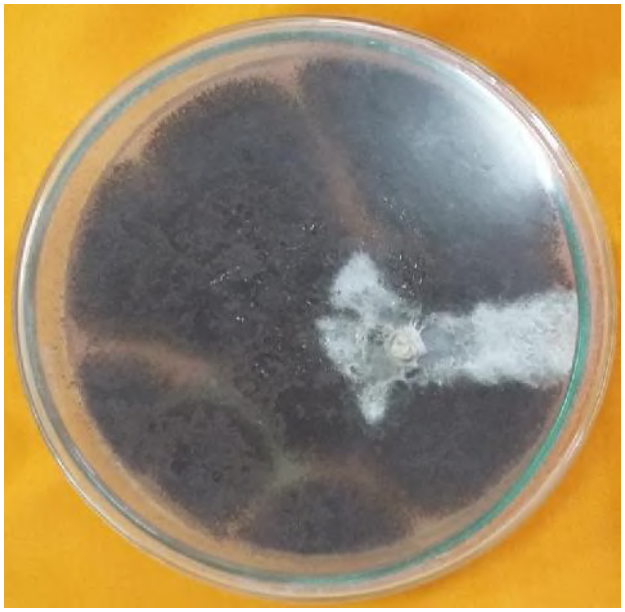

Figure 3.2
Parts taken as ingredients prepared for the SEM (ling karan green) method of Aspergillu a (black) mushroom and Colletotricu g mushroom (white)

Based on the picture obtained by the SEM method (Figure 3.1), it can be observed that the hypogenic fungal cell hypha cell walls that cause anthracnose disease appear to be torn, shriveled and perforated and fungal patho gene spores are not formed (Figure 3.1 B) due to antagonistic fungal activity in damaging the pathogenic fungus (arrow). Based on SEM images, it can also be observed that antagonistic fungal spores

are developing rapidly whereas p- phthogenous fungal spores are absent (Figure 3.1 A)

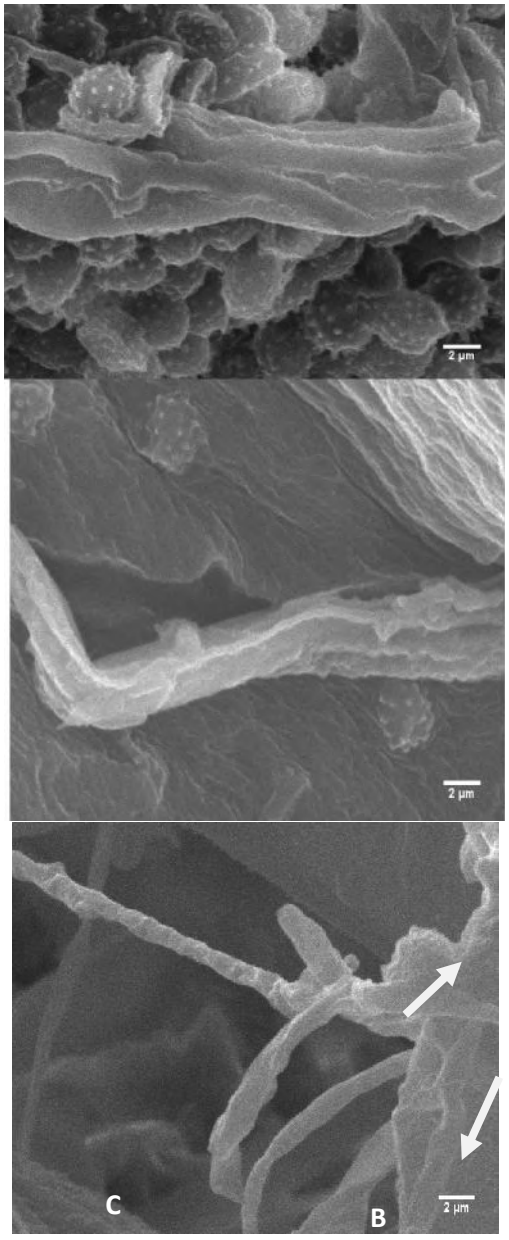

Figure 3.1
Image of the meeting between antagonistic fungi and pathogenic fungi obtained through the SEM method. A. Antagonistic fungal spores surround the pathogenic fungal hyphae. B. Pat hogen fungal hyphae torn and hollow. C. Pathogenic hyphae fungus that is still sufficient intact visible branching

Pathogenic fungal hyphae that are quite normal and colored can be seen in Figure 3.1. C derived from pure culture of the pathogenic fungus *C. gloeosporioides* . A amur antagonists inhibit the growth of fungal pathogens causing anthracnose disease by mastering the growing medium and lysis of the cell wall of fungal hyphae pathogens (fig 3 .1 B). Hifa mushroom pathogen looks torn and hollow (arrow).

Inhibition of growth carried out by antagonistic fungi in the results of this study of pathogenic fungi is a space competition characterized by antagonistic fungi controlling the media area per fungal plant. In picture 3 . 1 A can be seen that the pathogenic fungus does not grow properly which is marked by the formation of pathogenic fungal spores, in the picture only visible antagonistic fungal spores . H ifa fungal pathogen Mengke rut and perforated (figure 3.1 B) indicates that fungal antagonists inhibit the growth of fungal pathogens with meli sis hyphae cell wall of fungal pathogens, suspected fungal antagonist capable memp roduksi compound anti- fungal against fungal pathogens.

General Discussion

B erdasarkan the result of isolation of the fungal pathogen, the conventional identification and identification by molecular can be stated that the fungus causes anthracnose on citrus plants and fruit Kintamani is a fungus *Colletotri ch um gloeosporioides.*

The fungus *Aspergillus aculeatus* can be used as a reference ingredient in making biofungicides as an alternative to reducing the use of synthetic pesticides.

This study is similar to Rumayomi (2010) who conducted research on fungi that are antagonistic to *Gloesporium piperatum* (sexual form of the fungus *Colletotricum acutatum*) that causes anthracnose disease in large chilies. Based on the results of his research, it is known that there are three antagonistic fungi that can be used to control *G. piperatum* namely: *Aspergilus niger, A. flavus* and *Mucor* sp.

Pengetahu an on isolates of fungal antagonist with high potential as a biological control , and comes da ri same ecosystem with p at h Ogen be controlled very important . The use of agents of biological those will be easel min success of her in control of plant diseases biologically . Such knowledge is the first step that needs to be done in the use of antagonistic fungi as agents of disease control. Disease control anthracnose on citrus plants Kintamani is environmentally friendly and support sustainable agriculture can be achieved using biological agents such as fungi *A. aculeatus* (findings in pene Litian this) . Biological control aims to reduce the use of synthetic pesticides which have a negative impact on the environment and food safety . The use of microbes or microbial products to control plant diseases is an integrated part of sustainable agriculture (Suprapta and Sudarma, 2016)

M eka nisme antagonist divided into four groups, namely : colonization, ko mpetisi space , lysis and antibio sis. Growth suppression mechanism fungus *Aspergillus aculeatus* pitch toward the growth of the fungus *Colletotrichum* g. the cause of anthracnosas in Kintamani conjoined oranges is space and lysis competition . It is on the basis of i images obtained through SEM (figure 3 . 1 A) . The competition mechanism occurs with competition in the mastery of media growing. The lysis mechanism is suspected because the fungus *A. aculeatus is* able to produce antifungal compounds that are capable of lysis (tearing and perforating) the walls of the pathogenic fungal hyphae cells as shown in the SEM results (Figure 3.1B) .

A spergillus is one of the endophytic fungi, the mechanism of inhibition of endophytic fungi against pathogens can be directly with the antagonistic mechanism and indirectly with an induced resistance mechanism. Protection of plants with induced resistance is based on the stimulation of the resistance mechanism by the existence of metabolic changes that allow plants to more effectively endurance. It is estimated that induced resistance can develop if plant cells are able to produce new enzymes that activate plant genes responsible for plant resistance mechanisms . A amur endophyte potential as biological control agents, among others because of the presence of fungal endophyte is very diverse and plentiful, can be found both on land, crops and in grasses . A amur endophyte in plants diketahu i can reduce damage to the cells or tissues of the plant, increased kan ability of plants to air fo tosintesis . Colonization fungal endophyte in plants causes terinduksinya secondary metabolites that are antagonistic to the organism e pat h Ogen . In addition, colonization of endophytic fungi can also increase phenol compounds and possibly other compounds such as

pathogenesis-related protein (PR-Protein) in the host. Phenol compounds can inhibit the growth of pat h Ogen directly or with oxidation products and also by improving the complex metabolic changes such as seny awa who menebabkan plants are able to survive (Agrios 1997).

Fungus *Aspergillus* is a fungus phosphate solvent from sources soluble. The *Aspergillus aculeatus* isolate has been used to produce a number of important industrial enzymes (cellulases, hemicellulases, proteases) that are used commercially in the food industry. *Aspergillus aculeatus* is a member of a microbial community that is very easily found and abundant in nature, capable of producing a variety of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocellulose. *Aspergillus aculeatus* is a species that is often isolated from the soil (Petersen, LM *et al* . 2014).

Examples of studies similar to the results of this study are those of Ting *et al.,* 2010 which stated. *Aspergillus nidulans* can be antagonistic to *Colletotrichum gloeosporioides that* causes anthracnose disease in vanilla plants. Fungus *Aspergillus nidulans* can produce compounds that dioxomorpholine, okaramine, aflavinine , aculenes AC , calbistrins and secaloni acid k. These compounds can kill pathogenic fungi, in this case *Colletotricum gloeosporioides.* The mechanism of antibiosis occurs due to secondary metabolites produced by microbes in the form of antibiotics . *Aspergillus, Penicilli m* , and *Rhizobium* also act as natural enemies of the *S. rolfsii* fungus . Inhibition of the growth of *S. rolfsii* by *Aspergillus,* in *vitro* can reach 46-57%. (Balitkabi, 2012).

Noveriza (2008) states that mycotoxins are secondary metabolites produced by fungal metabolism and are cytotoxic, damaging cell structures such as membranes, and damaging cell-

forming processes such as proteins. This has been proven in this study that the antagonist fungus *Aspergillus aculeatus is* able to perforate with the lyse of the pathogenic fungal hyphal cell wall. It is suspected that the fungus *A. aculeatus is* also able to produce compounds that can damage the fungal hyphal cell walls of the pathogen. Naturally a number of fungi produce mycotoxins during the process of metabolism. *Aspergilus* sp . produce okratoxin A, *gliotoksin, verrukolagen, fumitremorgin* and *tryptoquivalent* (Samson *et al* ., 1995).

Based h acyl pen elitian Sudarma (2011) it is known that *Aspergillus* sp. potential as a microbial antagonist to the fungus *Fusarium oxysporum* in banana plants. Food competition takes place in terms of utilizing growing media as a food source. This is similar to the results of this study, macroscopically in antagonistic tests which show that the edge of the pathogenic colony of the *Colletotricum gloeosporioides* that is in contact with the antagonistic fungus *Aspergillus aculeatus, its* growth continues to be depressed due to competition for growing space. Antagonistic fungi and pathogens both need nutrients from food to grow.

Fungus *Aspergillus* is a fungus phosphate solvent from sources soluble. The *Aspergillus aculeatus* isolate has been used to produce a number of important industrial enzymes (cellulases, hemicellulases, proteases) that are used commercially in the food industry. *Aspergillus aculeatus* is a member of a microbial community that is very easily found and abundant in nature, capable of producing a variety of hydrolytic and oxidative enzymes involved in the breakdown of plant lignocellulose. *Aspergillus aculeatus* is a species that is often isolated from the soil (Petersen, *et al* . 2014).

Aspegillus fungi *that* survive in drought areas can also live in soils with high salinity, besides that the fungus *Aspergillus* also has the ability to break down cellulose compounds into simple carbon compounds needed by soil microbes as a carbon source (C). This fungus can also dissolve phosphate rock into organic phosphate compounds and be able to produce IAA hormones that can increase plant growth. It has been proven in this study that the application of *A. aculeatus* antagonist fungus in *this vivo* can increase the height and number of leaves of Kintamani conjoined orange seedlings (tables 5.9 and 5.10) . It is suspected that the fungus *A. aculeatus* mam pu produces the hormone IAA (auxin) which functions to improve the growth process of the Kintamani siam citrus seedlings. It is also proven that the addition of biological fertilizers containing *Aspergillus* mushrooms can increase the growth of Ciherang variety rice plants planted on land with 0.5% salinity. Increased growth occurs in plant height, number of tillers (*tiller*), total dry weight of biomass (straw) . (Subowo, 2009).

Ting *et al.*, (2010) stated that *Aspergillus nidulans* can be antagonistic to *Colletotrichum gloeosporioides that* causes anthracnose disease in vanilla plants because it emits volatile compounds such as: α- phellandrene, acetic acid pentyl ester and 2-acetyl-5-methylfuran. His research results also state that *Aspergillus terreus* can inhibit the growth of the pathogenic fungus *Botrytis cinerea*

Based on the results of research by Fakhrunnisa *et al.* (2006) it can be seen that *A. niger, A. flavus, A. terreus and A. versicolor* can inhibit the growth of *Fusarium* spp. Bosah *et al.* (2010) also found that *Aspergillus* sp. Can inhibit the growth of *Sclerotium rolfsii* pathogenic fungi with inhibition power up to

88.35%. The obstacle process is caused by *Aspergillus* sp. produce enzymes chitinase and β -1,3 glucanase Laminarinase) which have the ability to break down the cell wall components of pathogenic fungal cells such as chitin and β -1, 3 glucan.

Control of plant diseases that are environmentally friendly and that support sustainable agriculture can be achieved by using biological agents. Control of the liver aims to reduce the use of synthetic pesticides that have an impact on the environment and food safety . The use of microbes or microbial products to control plant diseases and to increase crop production is an integrated part of sustainable agriculture (Haggag and Muhamed, 2007).

The advantages of biopesticides include: safe impact on the environment because it does not kill non-target organisms, healthy agricultural products are safe because it is free of pesticide residues, low *phytotoxicity* , ie does not poison and damage plants , does not cause immunity to organisms e pathogens, does not kill non-target organisms, k ompatibel when combined premises n how to control the other, b ahan raw slag to t abundant and available in nature . (Djafaruddin, 2004).

Based on studies of various types of *Aspergillus* mushrooms above, it can be stated that the fungus genus *Aspergillus* in general has been proven by many researchers , functioning as an antagonistic fungus against various plant diseases . In this study it was found that *Aspergillus aculeatus* as fungal antagonists fungal pathogens *Colletotroticum gloesporioides* cause anthracnose p no citrus plants allegedly able m enghasilkan type of antibiotics that kill the fungus p ato h gene. Mechanisms antagonistic fungi *A aculeatus* apart in lysis hours ur is also capable of mendesa k or suppress the growth of fungi p at h Ogen *C. gloeosporioides* . The fungus *A. aculeatus*

is thought to also produce enzymes chitinase and β -1, 3 glucanase , Laminarinase which have the ability to break down the components of pathogenic fungal cell walls such as: chitin and β -1 , 3 glucan.

CHAPTER IV
CONCLUSIONS AND SUGGESTIONS

6.1. Conclusion

Based on the results of research that has been done, the following conclusions can be drawn: the inhibitory mechanism of *A. aculeatus* antagonist fungi against pathogenic *C gloeosporioides* is by lysis of the pathogenic cell wall walls so that the cell walls are torn and hollow.

6.2. Suggestion

1. Further research is needed to develop the biological pesticide formula based on the fungus *Aspergillus aculeatus* in controlling anthracnose disease, especially in the Kintamani conjoined orange plant in particular and other plants in general.
2. Further research is needed to find out the active compound produced by the fungus *Aspergillus aculeatus* which has the most role in controlling anthracnose disease.

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THE ROLES PARASITOID EGGS FOR CONTROLLING OF YELLOW RICE STEM BORERS*Scirpophagaincertulas*WALKER (LEPIDOPTERA: PYRALIDAE)

ABSTRACT

By

I Nyoman Wijaya, I G P. Wirawan, Adiartayasa Wayan and Made Sritamin

Rice stem borers are the main pest and endemic for the rice plants. The intensity of the attacks paneled fluctuating until it Reaches 90% and causes loss and it is requisite to get to pay serious attention. One of efforts to overcome it is the implementation of integrated pest control (IPM) through biological control by natural competitors. Research objectives is to know the role of an egg parasitoids in controlling rice stem borer. Research conducted at Subak Teba, Ship village, sub-district Mengwi, Badung regency since May to July 2019. Research is done with the survey by truncate leaf rice containing eggs of rice stem borers at random. The sampling done every weeks since the plants were two weeks old in Ciherang rice plants. Identification of eggs parasitoids observed under microscope used the key determination insects (Kalshoven, 1981) and the percentage of eggs parasitoids is calculated by a formula Nishida and Torri (1970). The result Showed that found three species of parasitoid eggs roomates played the role to suppress the development of yellow rice stem borer namely *Tetrastichusschoenobii* Ferr., *Telenomusrowani* Gahan and *Trichogrammajaponicum* Ashm with parasitation capacity of

38%. The highest capacity parasitation played by *T. schoenobii* Followed by *T. japonicum* and *T. rowani.*

Keywords: eggs parasitoid, parasitation capacity

I. Introduction

1.1 Background

Rice stem borer is a major pest of rice crops. The intensity of the attack can reach 90% and yield losses caused by the pest reaches 125,000 tons per cropping season (Soejitno, 1984). In Bali at broad 2001-2014 consecutive attacks reached 1,105; 1672.2; 1689.5; 1,872; 1724.5; 2673.5; 1265.15; 823.55; 1223.25; 763.55; 639.4; 904.15 and 612.40 ha with an attack of mild to severe intensity (BPTPH Bali, 2014).

Until now, control efforts by the farmers still rely on insecticides, for efficient time and energy. However, improper handling can cause the target to become resistant pests, natural enemies were killed so that the rate of growth of pest populations increase and cause poisoning and environmental pollution (Kartohardjono 2011; Makarim*et al*., 2003). Another alternative that can be selected to address the pest problem is to apply the concept of integrated pest management (IPM), which controls an integrated and environmentally friendly. Biological control with the use of natural enemies (parasitoids, predators and pathogens) are the main components. Natural enemies can be used in biological control programs for natural enemies already available in nature,

One of the natural enemies used in biological control are egg parasitoids. Egg parasitoid has the best potential to be developed because of the death of the pests in the egg stage will

greatly reduce the damage caused when compared with mortality in the larval stages and pupae. Parasitoid eggs over easy were bred especially when it can be cultured in the host egg substitute and have the nature of a relatively immobile compared to the larvae so as to facilitate and provide greater opportunities for the parasitism parasitoids (Widyarti, 2003).

Egg parasitoid most developed to control pests before damage crops. Rice stem borer egg parasitoid is *Trichogrammajaponicum* Ashmead (Hymenoptera: Trichogrammatidae), *Telenomusrowani* (Gahan) (Hymenoptera: Scelionidae), and *Tetrastichusschoenobii* Ferriere (Hymenoptera: Eulopidae) (Soejitno, 1991; Rauf 2000). The third parasitism parasite approximately 37% (Untung, 1983). Meanwhile, according Soehardjan (1976) ranged between 23% - 57%.

1.2 Objectives

This research aims to :

1. Knowing the species of egg parasitoids of rice stem borer
2. Analyzing parasitoid most instrumental in suppressing the development of the rice stem borer

1.3. Significance of the Study

Expected by this research can provide information about the role of egg parasitoids in controlling rice stem borer, thereby reducing the use of synthetic chemical pesticides that are harmful to the environment and to create an environmentally friendly control

II. RESEARCH METHODS

2.1 Time and Place of Research

This study was conducted in April 2019 until the month of August 2019 at farmer's paddy crop in Subak Teba, Boat Village, Mengwi, Badung, Bali Province.

Identification of the egg parasitoid conducted at the Laboratory of Plant Pests, Faculty of Agriculture and Genetic Resources Laboratory Udayana. Laboratorium University Faculty of Animal Feed Udayana University to record the progress of the rice stem borer egg parasitoids.

2.2 Equipment and Materials

The tools used in this study is an altimeter, a dissecting microscope, the glass tube (test tube), a stake with a length of 100 cm, knife, magnifying glass, paper labels, petridisc, cotton and gauze. Materials used are, rice stem borer eggs and 90% alcohol.

2.3 Implementation of Research

The study was conducted by survey on rice plants Ciherang was two weeks after planting until eleven weeks after planting, so do ten times observation. Information how farmers and local farming are often attacked rice stem borer obtained from the Department of Agriculture and Horticulture Badung. Then held a preliminary survey in the affected areas of the rice stem borer. From the survey results are set rice fields is used as a research location.

2.4 The abundance of parasitoid eggs Rice Stem Borer

Observations abundance of rice stem borer egg parasitoid done by collecting eggs of rice stem borers as many as 20 groups each observation in the respective different heights. The group collected eggs were each put into a glass tube and

maintained, then after hatching species and populations parasitoidnya observed. Identification of parasitoid done under mikroskup using insect determination key by Kalshoven (1981). The percentage of the rice stem borer egg parasitoids are calculated in a way that suggested by Nishida and Torri (1970). Identification and development carried out in the laboratory of parasitoid Genetic Resources Udayana University. In this study the parasitoid calculated in percent of the original number of eggs on the basis of the number of larvae of rice stem borer and parasitoid hatch. Therefore a *Tetrastichus* sp. may spend an average of three eggs rice stem borer and two *Trichogramma*sp. hatched from one egg rice stem borer, the percentage of parasitoid can be calculated as follows:

$$\frac{3a}{3a + (1/2)\,b + c + d} \times 100\%$$ to calculate a

where :a : *Tetrastichus* sp. b :*Trichogramma* sp.
c :*Telenomus* sp. d : rice stem borer larva

The formula is an example of a group when the rice stem borer eggs hatch and the larvae of three species of parasitoid rice stem borer

2.5 Analysis of Data

Data were analyzed descriptively. Data analysis results are presented in tables and images.

III. RESULTS AND DISCUSSION

3.1 The presence of egg parasitoid Rice Stem Borer

The results showed three types of egg parasitoid memarasit rice stem borers in Subak Teba. Three of the egg parasitoid

species that *T. japonicumrowani T.rowani* and *T. schoenobii* (Figure 3.1, Figure 3.2 and Figure 3.3). The results of the same research also found by Wijaya (1992) in Badung. It turned out that for 27 years to three species of parasitoid eggs still play a role.

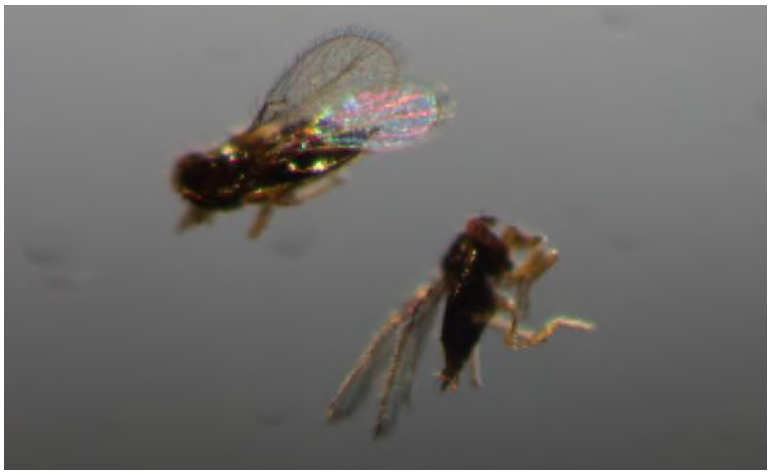

Figure 3.1

The egg parasitoid *Trichogrammajaponicum*

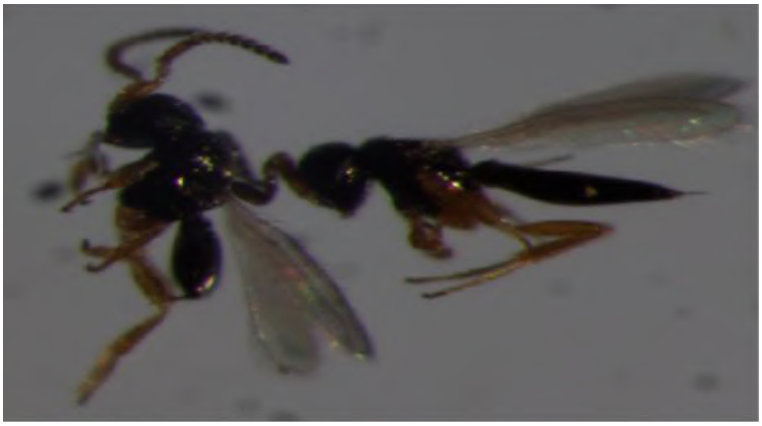

Figure 3.2

Egg parasitoids *Telenomusrowani*

Figure 3.3

Egg parasitoids *Tetrastichusrowani*

3.2 The role of each parasitoid Eggs In Progress Suppressing Rice Stem Borer

Average parasitation power of rice stem borer egg parasitoid shows a parasitoid *T. schoenobii* dominating than others. The role of parasitoid *T. schoenobii* highest in two weeks old plants after planting and the lowest was nine weeks after planting. While *T.rowani* and T. *japonicum* role tends to increase with increasing age of the plant (Figure 3.4).

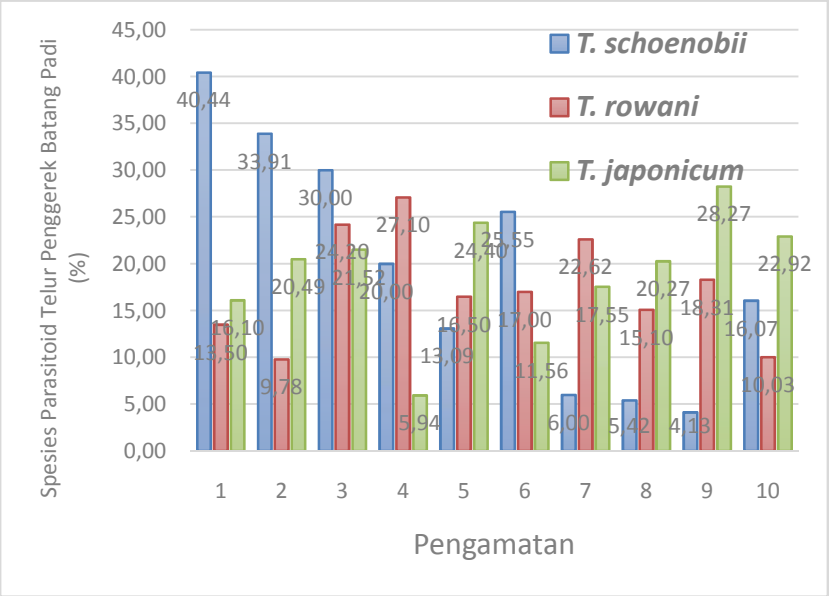

Figure 3.4
The role of each parasitoid Eggs In Progress Suppressing Rice Stem Borer

IV. CONCLUSIONS AND SUGGESTIONS

4.1 Conclusion

1. There were three species of parasitoid eggs of rice stem borer in Subak Teba was found all the three These egg parasitioids play a role in suppressing the development of rice stem borers, namely: *Tetrastichusschoenobii, Telenomusrowani* and *Trichogrammajaponicum.*
2. *T. schoenobii* is the most dominant species suppress the growth of the rice stem borer population.

4.2 Suggestions

The role of egg parasitoids in suppressing the development of the rice stem borer more than 50%, so that its presence must be maintained by not using pesticides to control pests. If necessary role further enhanced by planting flowering plants as feed for adult parasitoids

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BIOLOGICAL CHARACTERISTICS AND VIRULENCY OF *Cucumber Mozaic Virus*(CMV) ISOLATES BALIHORTICULTURE PLANT

Ni Putu Pandawani[a], Cokorda Javandira[a], Farida Hanum[a]

[a]Agrotecnology Department, Faculty of Agriculture, Mahasaraswati University Denpasar, Jl.Kamboja 11A Denpasar,

*Corresponding author:***pandawaniputu@hotmail.com**.

ABSTRACT

Information regarding the biological character and virulence of CMV isolates from some horticultural plants from Bali is still limited. The results of research on this information are very much needed in further research and development of science and technology, because it is known that CMV infects several hoticulture plants and can cause various symptoms of infection or specific symptoms in certain plants. Based on these problems, this research was carried out through CMV biological testing and the virulence of CMV isolates found in a number of horticultural plants in Bali. So that the biological characteristics and virulence level of each isolate is known. The biological test results of CMV isolates from several Bali horticultural plants namely, cucumbers (CMV-MB isolates), eggplants (CMV-TRB isolates) watermelon (CMV-SB isolates), chilies (CMV-CB isolates),pumpkin (CMV-LB isolates), and tomatoes (CMV-TB isolates), showed mosaic symptoms occurred in the indicator plants *Nicotianaglutinosa,Capsicum annuum* (big chili), *Cucumissativus* (cucumber), *Lycopersumesculentum* (tomato), and *Cucurbita moschata* (pumpkin). Severe mosaic symptoms only occurred in *Cucumissativus* (cucumber) plants. In *Vignasinensis* (long bean) infection CMV-Bali isolates only

cause symptoms of malformation and even cause no symptoms. Virulence test of these isolates in chilli plants showed results, the incubation period varied from 8.67 days in Pumpkin isolates (CMV-LB isolates) to 12.40 days in watermelon isolates (CMV-SB isolates). The highest percentage and intensity of disease occurred in Pumpkin isolates (CMV-LB isolates), with disease percentage reaching 80% and disease intensity 65%. Likewise in cucumber isolates (CMV-MB isolates), the percentage of disease reached 80% and the intensity of disease 62%. Pumpkin isolates (CMV-LB isolates) and cucumber isolates (CMV-MB isolates) are more virulent isolates compared to eggplant isolates (CMV-TRB isolates), watermelons (CMV-SB isolates), chilies (CMV-CB isolates), and tomatoes (CMV-TB isolates).

Keywords: Cucumber mosaic virus; Cucumissativus;Cucurbita moschata;

Lycopersumesculentum;Vignasinensis

Background

Cucumber Mosaic Virus (CMV) is a virus species from the genus *Cucumovirus,* family *Bromoviridae* (Mochizuki and Ohki, 2012). *Cucumber Mosaic Virus* (CMV) is also one of the viruses that cause mosaic disease which is commonly found in *Cucurbitaceae*plants.Symptoms of the disease due to the *Cucumber Mosaic Virus* (CMV) infection vary depending on the host species or CMV strain, which include: mosaic, chlorosis, dwarf, malformed leaves and necrosis. Differences in host species or CMV strains cause different symptoms and this happens is certainly a reaction from the host to a viral infection, so it appears the character of the host reaction to CMV infection that can be pointed out by the molecular character of CMV isolates and CMV isolates can isolated from various host plants.

The severity of symptoms that arise due to viral infection depends on the resistance of each host plant so that there will be a different virulence from each molecular character of CMV isolates.

Information regarding the biological characteristics and virulence of CMV isolates from some horticultural plants from Bali is still limited. The results of research on this information are needed in further research and development of science and technology, because it is known that CMV infects several hoticulture plants and can cause various symptoms of infection or specific symptoms in certain plants. Based on these problems, this research was carried out through CMV biological testing and virulence of CMV isolates found in a number of horticultural plants in Bali. So that the biological characteristics and virulence level of each isolate is known.

Materials and Methods

CMV isolates collection through the biological test on indicator plants

Indicator plants used to study the symptoms of infection by all isolates obtained were plants: *C.annuum* (big chili), *Cucumissativus* (cucumber), *L.esculentum* (tomato), *Solanummelongena* (green eggplant), *Vignasinensis* (longbeans), and *Cucurbitamoschata* (pumpkin). All CMV positive isolates were inoculated on several indicator plants. The differences in isolates are expected to be identified based on differences in symptoms in indicator plants so that CMV isolates collection from several hosts was obtained.

Virulence test of CMV isolates in chilli plants

The virulence test of CMV isolates used chili as an indicator. Inoculation was carried out mechanically with sap plant sources of CMV inoculums when the chili plants were 2 weeks after planting. Test chili plants were maintained and observed parameters for incubation period, type of symptoms that appeared, intensity of disease and percentage of disease events in chilli plants as a result of inoculation of each CMV isolate. Test chili plants that showed symptoms and showed no symptoms after inoculation in the study were detected by ELISA test using CMV antiserum to confirm the presence of CMV in the chili indicator plants.

Results

In indicator plants, *Nicotianaglutinosaisolates* CMV-MB, CMV-SB, CMV-CB, and CMV-TB cause mosaic symptoms, whereas other isolates cause symptoms of malformation. In *Capsicumannuum* (bigchili) isolates CMV-MB,CMV-TRB,CMV-CBandCMV-LB cause symptomatic mosaics, whereas CMV-S Band CMV-TB isolate cause symptoms of malformation. In *Cucumissativus* (cucumber) plants,CMV-MB isolates and CMV-SB showed severe mosaic symptoms, CMV-TRB, CMV-LB, and CMV-TB isolates caused mosaic symptoms while CMV-CB isolates showed no symptoms. In *Lycopersumesculentum* plants (tomato), CMV-S Band CMV-C Bisolate because of mosaic symptoms while CMV-MB, CMV-TRB, CMV-LB, and CMV-TB isolates cause symptoms of malformation. In indicator plants of *Vignasinensis* (longbean) isolates CMV-M Band CMV-TB cause symptoms of malformation while CMV-TRB isolates, CMV-SB, CMV-CB, and CMV-LB do not cause symptoms. In

the *Cucurbita moschata* plant (pumpkin), CMV-MB, CMV-CB, and CMV-LB isolate caused mosaic symptoms, CMV- S Band CMV-TB isolates caused symptoms of malformation while CMV-TR Bisolates showed no symptoms. In *Chenopodiumamaranticolor* plants all isolates cause local necrotic symptoms.

Table 1.Symptoms of indicator plants infected with CMV isolates from Bali

Indicator plant	CMV-Bali isolates					
	Symptoms					
	CMV-MB	CMV-TRB	CMV-SB	CMV-CB	CMV-LB	CMV-TB
Nicotianaglutinosa	mosaic	malformation	mosaic	mosaic	malformation	mosaic
Capsicumannuum	mosaic	mosaic	malformation	mosaic	mosaic	malformation
Cucumissativus	weight mosaic	mosaic	weight mosaic	asymptomatic	mosaic	mosaic

Lycopersum. esculentum	malfor matio n	malfor matio n	mosaic	mosaic	malfor matio n	malfor mation
Vignasinensis	malfor matio n	asymp tomatic	asym ptom atic	asympt omatic	asymp tomatic	malfor mation
Cucurbitam oschata	mosaic	asymp tomatic	malfo rmati on	mosaic	mosaic	malfor mation
Chenopodium amarantico lor	necro tic	nckro tic	necro tic	necro tic	necro tic	necro tic

Severe mosaic symptoms only occurin *Cucumissativus* (cucumber) plants due to infection with CMV-MB isolates and CMV-SB. Mosaic symptoms occur in indicator plants *Nicotianaglutinosa* (due to infection with CMV- MB,CMV-SB, CMV-CB, and CMV-TB isolates), in *Capsicumannuum* plants (bigchili) (due to infection with CMV-MB isolates, CMV-TRB), CMV-CB and CMV-LB, in *Cucumissativus* (cucumber) plants, (due to CMV- TRB, CMV-LB and CMV-T Bisolates), in *Lycopersumesculentum* (tomato) plants (due to CMV-SB and CMV isolates)-CB), in *Cucurbitamoschata* (pumpkin) plants (due to infection with CMV-MB,CMV-CB and CMV-LB isolates) In *Vignasinensis* (longbean) infection

CMV-Bali isolates did not cause mosaic symptoms, only caused symptoms of malformations (due to infection with CMV-MB and CMV-TB isolates) and even CMV isolates from Bali, namely CMV-TRB isolates, CMV-SB,CMV-CB, and CMV-LB did not cause symptoms.

A. Pumpkin Isolate B.Tomato Isolate C.Egg plant Isolate

D. Cucumber Isolate E.Watermelon Isolate F.Chili Isolate

Figure 1.CMV-Bali isolates

Table 2.Virulence of CMV Isolates from Bali

No.	Name isolate	Source of isolate	Incubation period (dai)	Percentage of disease (%)	Disease intensity (%)	PositiveELISA
1	CMV-MB	Cucumber	9,13	80	62,00	70 %
2	CMV-TRB	Eggplant	12,20	50	37,00	50 %
3	CMV-SB	Water melon	12,40	50	33,00	40 %
4	CMV-CB	Chili	10,83	60	47,00	60 %
5	CMV-LB	Pumpkin	8,67	80	65,00	70 %
6	CMV-TB	Tomato	10,50	60	48,50	60 %

Discussions

The symptoms result from infection with six CMV isolates from Bali on several important indicator plants are highly varied and some are asymptomatic. It appears varying from 8 days after inoculation to 12 days after inoculation with severe mosaic, mosaic, local malformations, and necrotic symptoms. The incubation period of the six isolates in the virulence test with chilli plants showed mixed results, with an incubation period ranging from 8.67 days after inoculation in Pumpkin isolates (CMV-LB isolates) to 12.40 days after inoculation in watermelon isolates (CMV

isolates) -SB). Percentage and intensity of disease in virulence tests with chili plants showed various results, which were also highest in Pumpkin isolate (CMV-LB isolates) with disease percentage reaching 80% and disease intensity 65%. Furthermore, also in cucumber isolates (CMV-MB isolates) the percentage of disease reaches 80% and the intensity of disease 62%. ELISA from chilli plants in virulence test from Pumpkin isolate (CMV-LB isolate) and cucumber isolate (CMV-MB isolate) obtained that 70% positive CMV. The higher the intensity and percentage of disease caused by inoculation of one of the CMV isolates, the higher the virulence of the isolate can be estimated, while paying attention to the incubation period.

Conclusions

1. From this study found CMV isolates namely isolates derived from cucumber plants (CMV-MB isolates), eggplant (CMV-TRB isolates) watermelon (CMV-SB isolate), chili (CMV-CB isolate), pumpkin (CMV-LB isolate) and tomatoes (CMV-TB isolates).
2. Biological test of CMV isolates from Bali, mosaic symptoms occur in the indicator plant *Nicotianaglutinosa* included *Capsicumannuum* (bigchili), *Cucumissativus* (cucumber), *Lycopersumesculentum* (tomato), and *Cucurbitamoschata* (punpkin). Heavy mosaic symptoms only occuron *Cucumissativus* (cucumber) plant. In *Vignasinensis* (longbean) plant

infection, CMV-Bali isolates only cause symptoms of malformation and do not even cause symptoms.

3. Pumpkin isolates (CMV-LB isolates) and cucumber isolates (CMV-MB isolates) are quite virulent isolates compared to eggplant isolates (CMV-TRB isolates), watermelons (CMV-SB isolates), chillies (CMV-CB isolates) , and tomatoes (CMV-TB isolates).

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ANALYSIS OF LIMONENE AND OTHER ANTIOXIDANTS IN COMMERCIAL ESSENTIAL OIL PRODUCTS COMPARED TO HOMEMADE VIRGIN COCONUT OIL

Ni Made Suaniti[1*], I Wayan Bandem Adnyana[2], Manuntun Manurung[1], Oka Ratnayani[1], Raisyah Anjani[1]

[1]Chemistry Study Program, Mathematic and Natural Sciences Faculty, Udayana University, Bukit Jimbaran, Badung, Bali, Indonesia 80361

[2]Mechanical Engineering Study Program, Engineering Faculty, Udayana University, Bukit Jimbaran, Badung, Bali, Indonesia 80361

*Corresponding author: madesuaniti@unud.ac.id

Abstract

Essential oil production in the market is very diverse with various brands labeled as antioxidants that have gained more attention in the society recently. The purpose of this study was to analyze limonene and other antioxidants content of the essential oils in the market compared to homemade virgin coconut oil. The method used was Gas Chromatography - Mass Spectrometry (GC-MS) and Fourier-transform infrared (FTIR) Spectroscopy. The infrared spectrogram showed the presence of alkanes and esters respectively at wave numbers of 3000-2850 and 1750-1730 cm^{-1}. Chromatogram results showed the presence of limonene compounds and some antioxidants with a

fairly good separation in the several types of oils, which was not detected in the homemade virgin coconut oil.

Keywords: antioxidants, limonene, product oil, virgin coconut oil

Background

Refresher oil with brand "Fresh" that has been used by the society recently continues to raise attention and allows it to be developed following the development of science and technology, which is also supported by the current availability with a variety of scents. Meanwhile, virgin coconut oil is also very potential and beneficial to be used as refresher oil.

Therefore, it is necessary to develop more attracted scents as well as conduct chemically qualitative and quantitative analysis for homemade virgin coconut oil compared to the commercial product of essential oils. The qualitative analysis can be done by suspecting the functional groups that appear by using infrared spectroscopy, and also the appearance of the compounds can be determined by gas chromatography through retention time and mass spectrometry as ion fragments (m/z). The oil content in the essential oils which are volatile compounds, namely antioxidants, varies greatly both in the chemical structure and amount. The percentage of volatile compound such as limonene in the essential oils from plants is determined by GC-MS (Derwich et al., 2010; Ibrahimi et al., 2013). Pinene compounds in the essential oils are also detected in small amounts as volatile compounds through extraction methods (Tomasz et al., 2013). Active compounds in essential oils are also found qualitatively and quantitatively using GC-MS (Kaluzna-Czaplinska, 2007).

The quality of oil is very dependent on the method of preparation and analysis. The amount and variety of chemical compounds of oil from plants requires an easy and fast analysis effort such as GC-MS. This research was developed to analyze the limonene compounds and other antioxidants in the commercial essential oils product compared to homemade virgin coconut oil.

Materials and Methods

The materials used were homemade virgin coconut oil, commercial essential oil products, ethanol pro analysis, and the equipments were volumetric flask, glass beaker, Fourier-transform infrared spectroscopy (FTIR) Prestige 21, and Gas Chromatography-Mass Spectrometry (GCMS) Shimadzu 210 Ultra.

Results

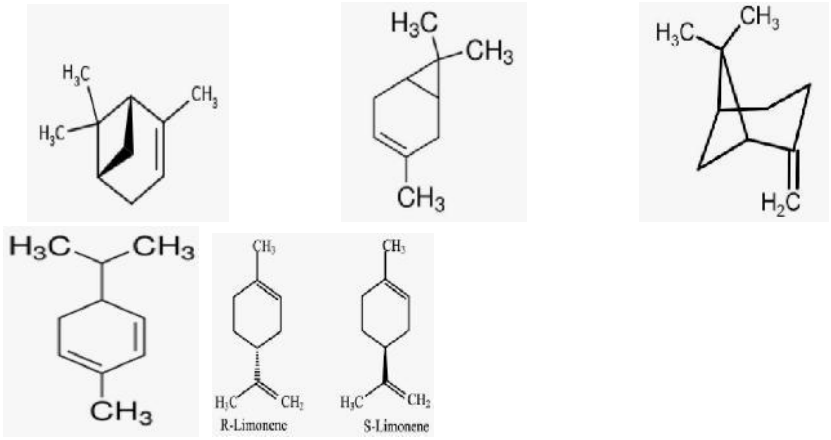
GC-MS analysis of compounds contained in fresh oils in the market marked with the codes Fc, F, A, P, and MA resulted in limonene compound with ion fragments (m/z) of 68 and the alleged antioxidant compounds such as alpha pinene, delta 3 carene, beta pinene, and alpha phelandrene with m/z of 93 as shown in Table 1. The structure of the compound is in accordance with the literature as can be seen in Figure 1.

Table 1. The presence of limonene and some antioxidants in fresh oil in GC-MS

Compounds	Area x 1000000

	Fc	F	A	P	MA
Alpha pinene (m/z 93)	n.d	6.1	0	26.6	5.2
Delta-3-carene (m/z 93)	n.d	n.d	0	1.1	n.d
Beta pinene (m/z 93)	6.7	12.4	15.1	2.7	n.d
Alpha phelandrene (m/z 93)	n.d	0.8	n.d	4.1	15.8
dl Limonene (m/z 68)	n.d	n.d	46.9	6.1	9.7

n.d= no detection



Alpha Pinene Delta-3- Carene Beta pinene Alpha Phelandrene Limonene

Figure 1. Structure limonene and other antioxidants in product fresh

Similar infrared spectrograms are shown as in Figure 2, alleged alkanes, alkenes and esters were detected. Alleged group alkanes were indicated by sharp absorption at wave numbers 3000-2850 cm^{-1}, alkenes at 1680-1600 cm^{-1} and carbonyl between 1820-1600 cm^{-1} supported by ester groups at 1750-1730cm^{-1} very weak. The Fc sample had the strongest absorption and sharp band shape indicating the highest concentration of the compound content of the other two samples (A and Sc) as well as homemade virgin coconut oil.

Separation by gas chromatography followed by mass spectrometry fragments obtained was shown in Figure 3. The figure showed the ion fragments (m/z) of the sample of commercial fresh oil (a); virgin coconut oil and traditional cooking oil (b).

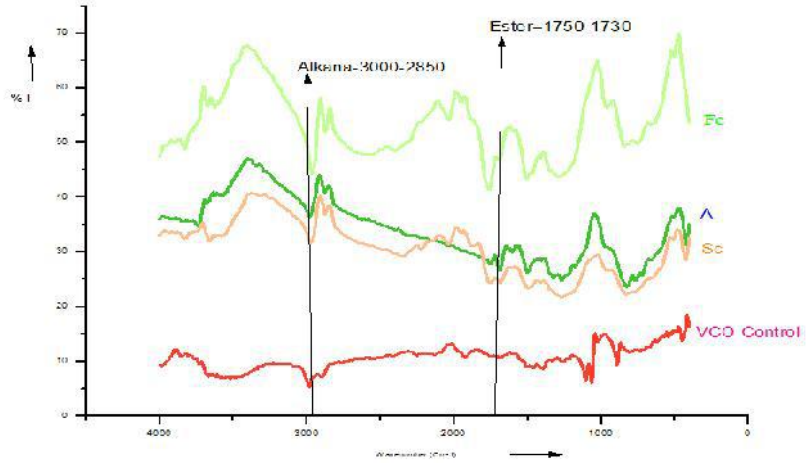
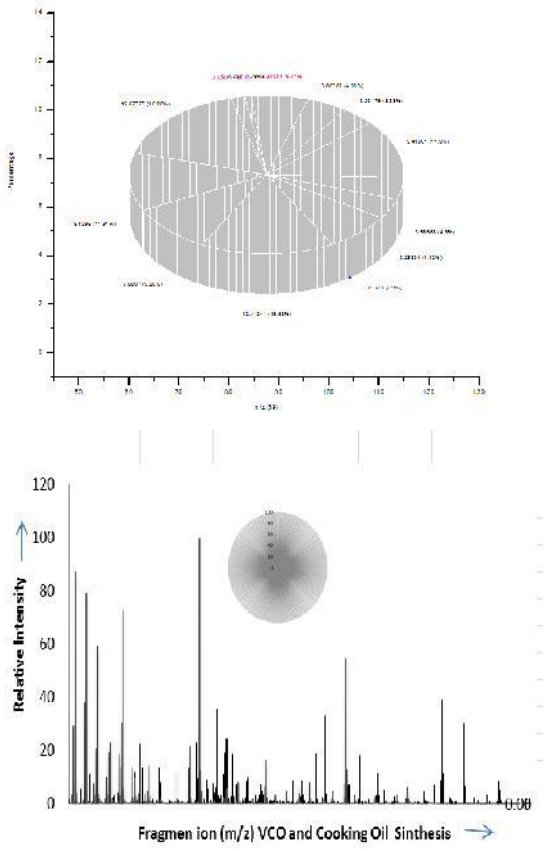


Figure 2 Spectogram of FTIR some fresh oil and VCO synthesis (control)



(a) (b)

Figure 3. Ion fragment ion limonene and other antioxidant in commercial fresh oil (a) and ion fragment from ester in homemade virgin coconut oil and traditional cooking oil.

Discussions

GC-MS as a modern analysis method has been developed for qualitative analysis with retention time markers adjusted to the NISH and Willey libraries, then the percentage results obtained were used for further analysis quantitatively. This is supported by published research by Harvey (2000); Silverstein et al., 1991; Kaluszna-Czaplinska (2007); Derwich et al. (2010), Ibrahimi et al. (2013) and Tomasz et al. (2013).

Conclusions

Limonene and other antioxidants such as pinene, careen, and phelandrene obtained quite good separation while was not detected in the homemade VCO characterized by GC-MS. The analysis using FTIR of VCO and commercial essential oil products showed the presence of alkanes and esters functional groups at the wave numbers of 3000-2850 and 1750-1730 cm^{-1} respectively.

Acknowledgement

This study supported by National Grant from Research, Technology and Higher Education Ministry of Indonesia 2019 and LPPM Udayana University with the contract No. 492.22/UN 14.4.A/LT/2019.

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RETURN COST RATIO AND VALUE ADDED AGRIBUSSINES OF SWEET CORN "F1" *PRIMA* "ORGANIC HYBRID

Ni Putu Sukanteri, , SP., M.Agb

Agribusiness Study Program, Faculty of agriculture,
Mahasaraswati Denpasar Universit
Corresponding email : putusukanteri@unmas.ac.id

Abstrac

Sweet corn is one of the commercially important groups of corn cultivars. Its specially is the high sugar content (especially sucrose) when harvested. The process of harvesting sweet corn is always done at a young age (the "cooking milk" stage). The purpose of this study was to determine the return cost ratio of sweet corn farming in paddy fields that have limited water, and the income of F1 "Prima" sweet corn farmers that are cultivated organically. In addition to knowing the added value obtained by farmers through post-harvest processing on F1 sweet corn "Prima. The results of research on sweet corn farming conducted in this study from April to July, the average income on corn farming in the village of Megati amounted to Rp 9,224,000 per hectare in one planting season. The average amount of R / C in sweet corn farming is 2.54. The results of the study show that sweet corn damaged in Megati village can provide benefits for farmers, if sweet corn is sold after post-harvest processing will provide added value to the selling price of sweet corn for 51.6% greater than the selling price of young harvested sweet corn

Key Word: Sweet Corn, Organic Corn.

Background

Sweet corn is one of the commercially important groups of corn cultivars. Its specialty is the high sugar content (especially sucrose) when harvested. The process of harvesting sweet corn is always done at a young age (the "cooking milk" stage). Sweet corn has a sweet taste at harvest time because this maize has mutations in one or several genes that regulate the formation of polysaccharide chains, so that the grains of corn fail to form starch in sufficient quantities. As a result of this failure, when the grain will dry out wrinkled. corn is an important food other than rice. Corn can be used as a substitute for rice and can be consumed as a staple food for the community. Moreover, from these conditions corn can be used as a substitute for rice in certain conditions. Sweet corn is usually not sold as animal feed, but rather as human consumption. This corn processing can be boiled, burned, or made porridge. Sweet corn in the trade classification is classified as vegetables even though field corn is classified as secondary crops. This is because sweet corn is sold fresh and perishable. Sweetness does not last long (only one to four days) so "shelf life" is an important determinant of quality. Corn plants are very useful for human or animal life, corn is the second staple food after rice, corn production can now be consumed by humans in the form of serving, corn is one food ingredient that contains charcoal hydrate, which can be used to replace (substitute) rice. Sweet corn has a high selling value so that sweet corn offers a better price so that the interest in sweet corn cultivation never recedes. Because of its nature that can be consumed directly such as roasted corn or boiled corn, the sweet corn market opens up to retail level based on this background so a study of Return Cost Ratio analysis was carried out on the "PRIMA" F1 Hybrid sweet corn farming organically. The purpose of this study was to determine the return cost ratio of sweet corn farming in paddy fields that have limited water, and the income of F1 "Prima" sweet corn farmers that are cultivated organically. In addition to knowing the added value obtained by farmers through post-harvest

processing on F1 sweet corn "*Prima*"
opens up to retail level based on this background so a study of Return Cost Ratio analysis was carried out on the "*PRIMA*" F1 Hybrid sweet corn farming organically. The purpose of this study was to determine the return cost ratio of sweet corn farming in paddy fields that have limited water, and the income of F1 "Prima" sweet corn farmers that are cultivated organically. In addition to knowing the added value obtained by farmers through post- harvest processing on F1 sweet corn "*Prima*"

Resesarch Method

The research was conducted at the Suka Mandiri Farmer Group in Megati Village, East Selemadeg District, Tabanan Regency. The study was conducted on groups of farmers who carried out organic sweet corn farms which were first carried out on lowland rice fields. In an effort to help farmers to be able to harvest outside the rainy season.

Data analysis method

The data obtained were analyzed using farm analysis. To analyze corn farming in Handapherang Village, Cijeungjing District, Ciamis Regency is used with the following formula: 1) Cost Analysis According to Suratiyah (2009) to calculate the total cost (Total Cost) obtained by adding up fixed costs (FC) with variable costs (Variable Cost) with the formula: TC = TFC +
TVC Where: TC = Total Cost TFC = Total Fixed Cost TVC = Total Variable Cost 2) Analysis of Revenue According to Suratiyah (2009) in general the calculation of total revenue (Total Revenue / TR) is the multiplication between the amount of production (Y) and the selling price (Py) and expressed by the formula as follows:
TR = Py. Y Where: TR = Total Revenue Py = Product price Y = Total production 3) Revenue
Analysis According to Suratiyah (2009) income is the

difference between revenue (TR) and total cost (TC) and expressed by the formula: Pd = TR - TC Where: Pd = Revenue TR = Total Revenue TC = Total Cost (Total Cost) Soekartawi, 2006.

Data analysis conducted in this study is the analysis of the return cost ratio on F1 "Prima" sweet corn farming with organic treatment through empowerment of the Sipadu farmer group in the province of Bali.

Return Cost Ratio (R / C) is the ratio between revenue and costs written as follows:

A = R / C

R-Py.Y

C = FC+ VC

A == {(Py.Y) / FC + VC)}

Where

R = Revenue

C = cost

Py = Price of output

Y = Output

FC = Fixed Cost

VC = Variable Cost

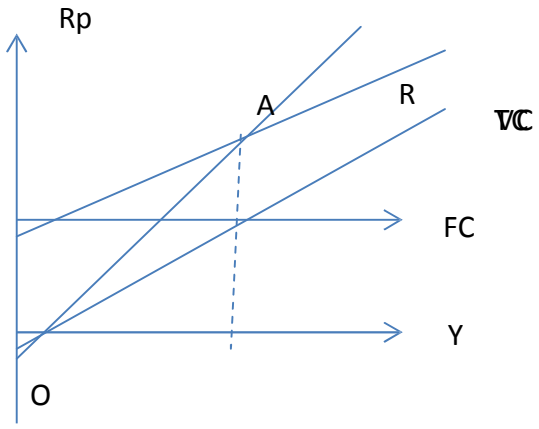
Criteria:

R / C =1 means that F1 "Prima" sweet corn farming does not provide benefits but also does not
suffer losses

R / C> 1 means that the "Prima" F1 sweet corn farm provides benefits

R / C <1 means that F1 sweet potato farming "Prima" suffered a loss

Tabel



Results and Discussion

Land conditions in the Farmer Group

Sweet corn is a common commodity found in the market, but sweet corn is a commodity that was first developed in the *Suka Mandiri* Farmer Group. For the last ten years or so, farmers have only been able to harvest once a year in their paddy fields, because their paddy fields only get water during the rainy season so they can only harvest once a year, that is only in the rainy season. In one year outside the rainy season farmers do not produce crops because the availability of water is very low. Land cannot be cultivated for farming. Farmers' production in one year is only rice.

Sweet Corn Farming F1 "*Prima*"

Production on *Suka Mandiri* Farmer Group land is classified as water shortage. To obtain the harvest more than once a year, the cultivation of sweet "F1" sweet corn. Efforts to sweet corn F1 is done with the aim of trying to harvest outside

the rainy season. Because corn plants do not need water like rice plants. This sweet F1 corn plant requires large amounts of nitrogen (N). With the application of fertilizer, attention must be paid to the balance between nitrogen, potassium (K) and phosphate (P) by using compost fertilizer processed from livestock managed by the Group. Sweet corn cultivation is planted directly in the former paddy fields or can be made into beds. If the land used by the former rice fields, try to keep the land from being flooded. Beds in corn plants function to regulate drainage channels. In *Suka Mandiri* Farmer Group land, sweet corn cultivation is only in the canopy without cultivating land in the form of beds so that it does not require much labor. Spacing with a size of 75 cm by 35 cm. Basic fertilization for the cultivation of organic sweet corn is carried out using fertilizer from fermentation of cow dung with an N content of 17%. Seedlings planted are F1 sweet "*Prima*" corn. After planting.

Revenue, production costs and income of Sweet Corn Farming"*Prima*" F1

Acceptance of sweet corn farming is obtained by counting the amount of corn obtained multiplied by the selling price of raw corn per unit of young corn. So that the acceptance of sweet corn produced is Rp. 15,225,000 ... in one harvest period from April to July 2019. The acceptance of sweet corn is based on sweet corn yields of 10,500 sweet corn cobs produced in one hectare of land with the selling price of young corn an average of Rp 1,450. During sweet corn farming, total farming costs are required including the price of seedlings, price of organic fertilizer, biourine and labor required during one production period of Rp. 6.001,000 per hectare of land incurred by members of farmer groups. Like Variable Costs Variable costs are calculated in research This includes, seeds, organic fertilizer, labor costs while fixed costs are not needed because as long as farming does not use agricultural tools such as machinery or agricultural tools that

are made from metal, only using crowns from logs.

Revenue Analysis Revenue is the difference between revenue and total costs incurred, while revenue is the result of multiplying the selling price of corn with the amount of corn produced. Based on the results of research the selling price of F1 sweet corn "Prima" which has been developed in the Farmers Group *Suka Mandiri* Rp. 9,224,000 in one planting period Analysis of R / C (Revenue Cost Ratio) is known by dividing the revenue between total costs. Revenues amounting to Rp. 15,225,000 and total costs incurred in the amount of Rp. 6,001,000. Based on the research, it is known that the average R / C is 2.54, meaning that each expenditure costs Rp 1.00, then sweet corn farmers will receive an income of Rp 2.54. In farming sweet corn "Prima" F1 farmers get a profit of Rp 1.54.

Value Added Sweet Corn F1"Prima" With Post Harvest Processing

Sweet corn that has been sold is still raw with an average selling price of Rp 1,450. to increase the selling price of farmers to process sweet corn into simple processed by burning or boiling. Roasted sweet corn if sold at a price of Rp 3,000 per fruit. So that each sweet corn with simple post-harvest processing can increase the selling value by 51.6% of the price of sweet corn sold directly after harvest. Research results obtained by calculating the added value of sweet corn products provide an increase in income for farmers by 50% of the price received by farmers.

CONCLUSIONS AND RECOMMENDATIONS

Conclusion

Based on the results of research on F1 sweet corn farming and discussions that have
been carried out in this study from April to July 2019, it can be concluded that: 1) The average cost of corn farming in Megati Village is Rp 6,001,000 per hectare in one season planting. While the revenue is Rp. 15,225,000 per hectare in one

planting season, obtained from the harvest of young sweet corn at a unit selling price. The average amount of income from corn farming in the village of Megati is Rp. 9,224,000 per hectare in one planting season. 3) The average size of R / C in sweet corn farming is 2.54. The results of the study show that sweet corn damaged in Megati village can provide benefits for farmers, if sweet corn is sold after post-harvest processing will provide added value to the selling price of corn sweet amounted to 51.6% greater than the selling price of young harvested sweet corn.

Suggestion

Based on the conclusions, it is recommended that cornfarming activities in Megati Village constitute a profitable agricultural prospect and can provide benefits for farmers.

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