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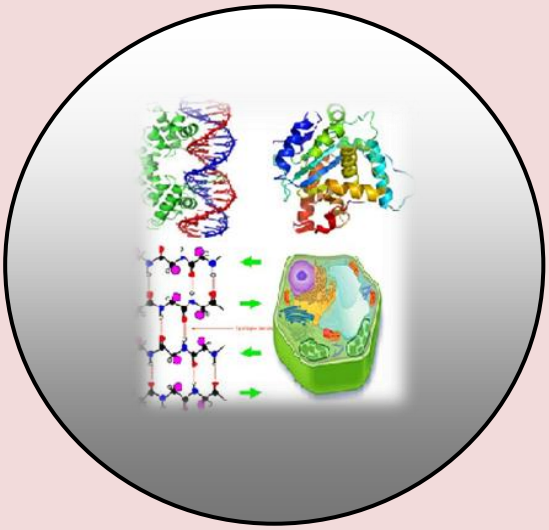

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Antibiosis Profile Compound of *Trichoderma* sp. in Attempt of Controlling *Aspergillus flavus* in Balinese Lontar

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ABSTRACT

Lontar is an important inheritance to be preserved. The writing in the Lontar contains an ancient Balinese script. Poor environmental conditions can cause the Lontar to be damaged. One of the cause of the damage is Aspergillus flavus fungi which is able to produce selulase enzyme. The enzyme may damage the basic component of the Lontar. An attempt to achieve an antibiosis having a fungicidal feature is needed to be done, which is by using Trichoderma. Trichoderma is a group of fungi that has the ability to inhibit the growth of other organism. Based on this fact, therefore a research was done in order to understand the antibiosis compound profile produced by Trichoderma sp. in controlling A. flavus in Balinese lontar. The isolation of A. flavus and Trichoderma sp. was done by using the plating method in sequential dilution. The identification of the Trichoderma sp. was done conventionally according to the book of identification by Pit and Hocking (1997). To test the inhibition strenght of Trichoderma sp. against the A.flavus, the dual culture methode was used, while the diffusion well methode was used to find test the inhibitory strenght of the Trichoderma sp. filtrate extract against the tested fungi. Antibiosis compound profile of the Trichoderma sp. was known by using the colomn dan GCMS (Gas Chromatography Mass Spectrometry) technic. The in vivo test of the inhibitory strenght of the Trichoderma sp. filtrate extract against the A.flavus was designed by using 6 treatment and 4 repetition. The result of the study shows the identified A. flavus isolated from Balinese Lontar and Trichoderma sp. identified from isolation result of rhizosphere of pea plant Trichoderma sp. has the strenght to inhibit the A. flavus in the percentage of 69,5±1,56 % and the obtained filtrate extract can form inhibitory zone in the percentage of 15,32±0,277 mm. The 15 compound identified in filtrate extract is allegedly act as an antibiosis agent namely Benzene,1,3,5-trimethyl-(CAS) 1,3,5-trimethyl; 2-Azabicylol(3,2,1)octan-3-one; 5,6-Dihydro-4-methyl-2H-Pyran-2-C; 2-Butenoic acid, 2-methyl-(CAS)2-methyl-2; 2H-Pyran-2-one,tetrahydro-4-hydroxy-4-methyl; 2-Bromo dodecane; Lyxitol,1-O-nonyl; Pentadecane,3-methyl-(CAS)3-methylpentax; Trichloroacetic acid, tridecyl ester; Heptadecane,3-methyl-(CAS)3-methylhepta; n-Hexadecanoic acid; Nonadecane,3-methyl-(CAS) 3-Methylnonad; Octadecanoic acid; 3-Methylheneiccosane and Heptadecane,3-methyl. The filtrate extract of the Trichoderma sp. with the concentration of 1,56/20µL based on the in vivo test (Balinese Lontar) shows a significant different (P≤0,05) in compare with the treatment without Trichoderma sp. filtrate extract.

Key word : Antibiosis, Trichoderma harzianum, Aspergillus flavus , Ekstrak and Filtrat.

INTRODUCTION

Lontar is an important inheritance to be preserved. The writing in the Lontar contains an ancient Balinese script. Manusript in lontar is a writing cultural tradition in Bali which has a religious tendency (Geriani, 2010, Sedana et al., 2013). The inappropriate preservation of Lontar can cause damage. One of the damage cause is *Aspergillus flavus*. This fungi can grow well in tropical region and has the ability to produce cellulase enzyme that can degrade cellulose component. Based on Yosmar et al. (2015) cellulose is a carbohydrate that is a part of the plant cell wall which is very susceptible to be damaged by cellulase enzyme. The Lontar comes from Lontar leaf may become a good medium for the *A. flavus* growth, therefore this fungi may trigger the damage of the Lontar. This is in accordance to the Setiani (2018) research that said the fungi that was able to be isolated as one of the Lontar damage cause was the *A. flavus*.

The control of *A. flavus* is necessary by using the materials coming from the other living organism, because it is believed that the utility of those materials may minimize the side effect caused by the use of the synthetic materials. Trichoderma is one the fungi group which is believed to have the competency to inhibit the growth of other organism. This fungi is a saprophyte fungi known of having a antagonist biocontrol agent yang which is very effective against phytopathogen fungi (Ainy et al., 2015). Based on Gusnawaty et al. (2014), *Trichoderma* sp. is a parasite fungi that can attact and taking nutrients form other fungi because of the deadly antagonist feature or inhibiting the growth of the other fungi. According to Mendosa et al. (2015), the antagonist mechanism done by the *Trichoderma* sp. fungi is microparasite and antibiosis, in addition to that the *Trichoderma* sp. has some advantages namely easy to be isolated due to its the wide spreading feature, it is able to grow easily in the media and non pathogenic for the plants. The research that has been done by Darmayasa (2015) reported that *T. asperellum* TKD can inhibit the growth of the *A. flavus* FNCC6109 in vitro as well as in vivo. In the dual culture test, the inhibition rate of the *T. asperellum* TKD against *A. flavus* FNCC6109 reached 98,849±1,100%. While in vivo test in feed concentrate the inhibition rate was 74,93%. Based on this result, there is a possibility that a Trichoderma from another kind also has the same potential in inhibiting the *A. flavus* growth. Therefore, in this research an isolate will be searched which has the potential in inhibiting *A.flavus* in the Lontar and observing the antibiosis compound produced by this fungi.

MATERIALS AND METHOD

Trichoderma sp. and *Aspergillus flavus* isolation

Trichoderma sp. was isolated from the rhizosphere of pea nut plant using *plating method* through serial dilution (Nester *et al*., 2007). The steps that have been done were, scaling 10 g soil sample then it was put into 90 mL a sterile physiological salt to get 10^{-1} of dilution factor, followed by homogenisation using vortex. 5 test tubes were prepared filled with 9 mL physiological salt and 6 steril Petri dish. To get 10^{-2} of dilution factor, 1 mL from the 10^{-1} of the dilution factor was taken, then it was put into 9 mL physiological solution, and this method was repeated untul 10^{-4} dilution factor was achieved. 1 mL from each of the dilution factor was taken then it was put into each sterile petri dish labelled from 10^{-1} until 10^{-4} then it was poured into a PDA media and incubated the temperature of 28^{o}C for 4 days. The macroscopic and the microscopic characteristic of the *Trichoderma* sp. were defined according to the book by Pit and Hocking (1997). By using the same way, an isolation of the *A. flavus* was done as well, in Balinese Lontar, however the sample was taken by swab technique.

Inhibition Test of the *Trichoderma* sp.

The inhibition property test of *Trichoderma* sp. against *A. flavus* was done by using *dual culture* method (Noveriza et al.,1999), namely by preparing sterile petri dish filled with 15 mL PDA media. 1 colony of *Trichoderma* sp. and 1 colony *A. flavus* each with a diameter of 5 mm. Both of the colony were inoculated within the distance of 2 cm in the same Petri dish. Next, the Petri dish was incubated in the temperature of 28^{o}C in 4 – 10 days. The inhibition percentage can be determined by measuring the fungi colony area of the *A. flavus* and the colony area of the *Trichoderma* sp. The next step is by using the following mathematical formula (Noveriza et al., 1999).

$$P = \frac{L2 - L1}{L2} \times 100\%$$

The production of *Trichoderma* sp. filtrate extract maserized with chloroform:methanol

Before producing the *Trichoderma* sp. filtrate, the YES (Yeast Extract Sucrose) media was prepared containing yeast extract 20 g/L, 150 g/L sucrose and 1 Liter of distillated water. The homogenized media was then sterilized. As much as 1 Liter sterilized YES media was added with 20 mL spora *Trichoderma* sp. suspension. The YES Media previously was divided into 10 bottles, in such that each bottle was filled with 100 mL media added with 2 µL spora suspension. Then, the media which has been added with spora suspension was incubated for 21 days in the temperature of 28^{o}C and was filtered using filter paper. The filtrate resulted from the filtration process was then evaporated to get a concentrated filtrate. The generated concentrated filtrate was then added with 300 mL comparation of chlorofom and methanol (2:1.v/v) (Darmayasa, 2015). After 24 hours, the mixture separated and formed 2 phases, namely the chloroform:methanol phase (clear) and water phase (concentrated). Then the choloform:methanol phase was evaporated using vacum rotary evaporator (*IKA.HB10 Basic*) to get the concentrated extract of the chlorofom:methanol phase. Finally, the inhibition potential against the *A. flavus* was ready to be tested.

Inhibition test of the Extract of the chloroform and the methanol Phase

The procedure of the extract inhibition strenght was done by using diffusion well method as followed, 200 µL of *A. flavus* spore suspension was taken, then it was placed into petri dish suspension then it was added with 15 mL PDA media. Next, it was homogenized by shaking to the left and right simultaneously so that the growth of the fungi spread evenly. After the media hardened, in the center of the petri dish, a well was made using 5 mm *cork borer*. As much as 20 µL filtrate extract of the chloroform and methanol phase were deposited into the well, made in the petri dish. The petri dish, was then incubated in the temperature of 28^{o}C for 4 days. The inhibitory strenght was shown by measuring the inhibiton zone diameter around the diffusion well.

Table 1. Treatment of The Aplication of The *Trichoderma* sp. Filtrate Extract to The Lontar.

Treatment	Information
A	Lontar without *A. flavus* spora suspension and without *Trichoderma* sp. filtrate extract
B	Lontar with *A. flavus* spora suspension and without *Trichoderma* sp. filtrate extract
C	Lontar with *A. flavus* spora suspension and *Nystatin* antibiotic, concentration 2,2 mg/20µL
D	Lontar with *A. flavus* spora suspension and *Trichoderma* sp. filtrate extract, concentration 1,2mg/ 20µL
E	Lontar with *A. flavus* spora suspension and *Trichoderma* sp. filtrate extract. concentration 1,4 mg/ 20µL
F	Lontar with *A. flavus* spora suspension and *Trichoderma* sp. filtrate extract. concentration 1,56/20µL (initial concentration)

The determination of the *Trichoderma* sp. antibiosis profile

As much as 2 mL chloroform:methanol phase as the result of the evaporation in the temperature of 40^{o}C, was then fractionated using chromatography column (1,5 cm diameter and 50 cm long), which is filled with silica gel (100g) inside of it, where it was activated previously by storing it in the oven at the temperature of 80 ^{o}C for 3 minutes. The column was passed by 500 mL eluent chloroform and methanol (2:1 v/v) and then was collected as much as 25 mL as fraction 1. Next, by using the same way, the next 25 mL was collected so that a few fractions were achieved (Darmayasa,2015). All of the achieved fractions were evaporated using vacuum rotary evaporator at the temperature of 40^{o}C, then a separation process was done using Thin Layer Chromatography (TLC) Keisel Del 60 F_{254} using an expander of N-butanol:acetid acid:water (4:1:5). The compound group that showed the same separation sign were grouped in one fraction and the inhibition activity was tested against the *A. flavus*.

The compound of the grouped fraction that showed an inhibition activity was then purified using TLC preparative method. After that, each stain located on TLC preparative was purified and the inhibition activity was tested. The stain showing a positive result by inhibiting the *A. flavus*, was analyzed using Gas Chromatography Mass Spectrometry (GCMS) type QP2010 Ultra Shimadzu, in order to know the antibiosis profile existing in the *Trichoderma.* sp filtrate.

In vivo Inhibition test of the filtrate extract of the *Trichoderma* sp. against the *A. flavus*

The in vivo inhibition test of the filtrate extract of the *Trichoderma* sp. was done using 6 treatment and 4 repetition. The Lontar was prepared with the size of 20 cm long and 3,5 cm wide as much as 24 sheet. The treatment given is shown in Table 1.

Next, each treatment of the Lontar, was placed in a plastic container. The part of the Lontar which was given a treatment had a length of 5 cm and 3.5 cm wide. After that, the lontar which had been given a treatment. Furthermore, the Lontar that has been given treatment is stored at room temperature for 30 days. *A. flavus* spores that grow on the Lontar leaves then rinsed with sterile water were then calculated indirectly through a dilution method.

Data analysis

The data obtained is descriptive data that is displayed in the form of tables and images and quantitative data which are analyzed statistically (ANOVA).

RESULT AND DISCUSSION

Characteristics of *Aspergillus flavus* isolated from Balinese Lontar

The results of isolation of *A. flavus* isolated from Balinese Lontar showed the following characteristics: on macroscopic observations as shown in Figure 1.A. the incubation period of 4 days the diameter of the fungi colony reached 5.1 cm, and this diameter would increase linearly with the addition of the incubation period. The fungi colonies are yellowish green with slightly whitish colored edges which showed that this area was still dominated by hypa growth. On the surface of the colony, hypa-aerial appeaed and was filled by conidiophores that support conidia as one of the breeding tools. Microscopically by using a microscope at 400x magnification, there are visible parts of *A. flavus*, namely conidia, sterigma, vesicles and conidiophores, all of which are presented in Figure 1.B. When the observation probe is increased to 100x, there is a hypa variation in the form of foot cells in Figure 1. C.

Aspergillus flavus is a contaminant fungus that often causes damage to organic ingredients such as wood, leaves, cotton, compost, insects, dead meat, carcasses, and grain storage (Yu et al., 2005). Bhatnagar et al. (2000), mention that this fungus is believed to have a very strong resistance to extreme conditions and is very easy to compete with other organisms. The results of isolation of the fungi that have been carried out on Balinese Lontar showed that the fungus isolated, identified as the fungus *A. flavus* based on macroscopic and microscopic features. The same study conducted by Sancana (2014) stated that a group of fungi that were successfully isolated as one of the causes of Lontar damage were Fusarium, Penicellium and Aspergillus. Setiani (2018) also identified the types of fungi from the Aspergillus group that had been isolated from Balinese Lontar Gede Siwa Manggis Manuaba, Nyanglan Village Klungkung Regency Bali was *A. flavus*, *A. niger* and *A. fumigatus*.

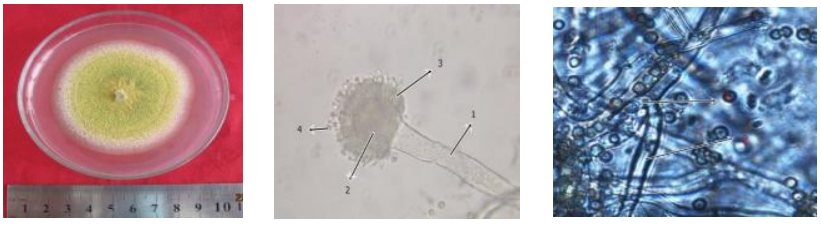
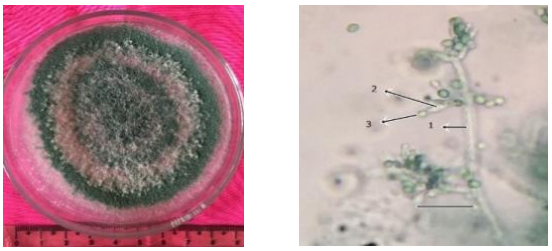


Figure 1. A. Morphology of *A. flavus* colonies on PDA media at 4 days incubation period at 28 ^{0}C B. Microscopic structure of A. flavus under a binocular microscope at 400x magnification (Arrow 1 = Konidiophore; 2. = Vesicles; 3 = Sterigma; 4 = Conidia S) C. Microscopic structure (arrow 1 = Foot Cell; 2 = Konidiophores).

Characteristics of *Trichoderma* sp. isolated in the rhizosphere of peanuts

The macroscopic characteristics of *Trichoderma* sp. isolated from pea plant rhizosphere showed dark green colonies which were interspersed with white color and usually ended at the edge of the white colony with a colony diameter of 8.4 cm in a 4 day incubation period on PDA media (Figure 2 .A). While the microscopic characteristics in figure 2.B. with 400x enlargement, conidia is seen, the phialid is a cushion of branched conidia and conidiophores. Based on the above characteristics it can be ascertained that this fungus belongs to *Trichoderma* sp. which is in accordance with the guidebook of Pitt and Hocking (1997).

Efforts to control *A. flavus* on Balinese Lontar must be carried out. In this study, *Trichoderma* sp.from rhizozper of peanut plants has been isolated. This fungus is expected to be one of the mushroom candidates explored for its antibiosis to be used to control contaminant fungi and Balinese Lontar destroyers. Gusnawaty et al. (2014) reported that *Trichoderma* sp.was successfully identified from 11 *Trichoderma* spp. isolates. indigenous Southeast Sulawesi. Then Rahman et al. (2011) obtained *T. harzianum* in samples on soil, topsoil, kitchen waste and compost to be used as bioconversion of solid waste. From peanut rhizosphere *T. harzianum* were also isolated and identified by Seedevi at al. (2011) which is used to control root rot.



A **B**

Figure 2. A. Morphology of the *Trichoderma* sp. colonies on PDA media with an incubation period of 4 days at 28°C. B. Microscopic structure of *Trchoderma* sp. under a binocular microscope at 400 x magnification (Arrow 1 = Konidiofor; 2 = sterigma (phialed); 3 = Conidia).

The inhibition of *Trichoderma* sp. against *Aspergillus flavus* on PDA media

Trichoderma sp. obtained in this study was able to inhibit *A. flavus* by 69.5 ± 1.56% in vitro on PDA media with a 4 day incubation period (Table 2. and Figure 3). Its inhibition continues to increase with increasing incubation period. The results of the statistical analysis showed that there was significant difference (P<05) in the percentage of *Trichoderma* sp. inhibition against *A. flavus* during different incubation periods. The incubation period of 10 days showed the greatest inhibition of *Trichoderma* sp. with 80.9 ± 1.08%. Another type of Trichoderma which has been studied by Darmayasa (2015) namely *Trichoderma asperellum* TKD was able to inhibit *A. flavus* FNCC6109 by 98.849 ± 1.100% on PDA media with a 4 day incubation period. The same incubation period was conducted by Setiani (2018) that *A. flavus* isolated from Lontar was able to be inhibited by *Trichoderma* sp. in vitro at 76.4 ± 3.58. The inhibitory ability of *Trichoderma* sp. against *A. flavus*, there is a probability that it is caused by faster growth and its ability to produce metabolites that can suppress the growth of *A. flavus*. Bhatnagar et al. (2000), stated *Trichoderma* sp. have better life capabilty and high competition in various conditions. Furthermore, Chaube et al. (2003) said that in addition to having high competition for nutrients and space, *Trichoderma* sp. can produce antibiotiosis which can inhibit or kill other microbes.

Table 2. Average Percentage of Inhibition *Trichoderma* sp to *A. flavus* on PDA Media With Different Incubation Periode.

Incubation Period (days)	Inhibitory Strenght (%)
0	0,0 ± 0,00[a]
4	69,5±1,56[b]
6	72,0±1,00[c]
8	78.1±1,09[d]
10	80,9±1,08[e]

The average value of the percentage of inhibition with different letter notations in the same column shows that the average value is significantly different (P≤0.05), based on the Duncan test after analysis of variance (ANOVA).

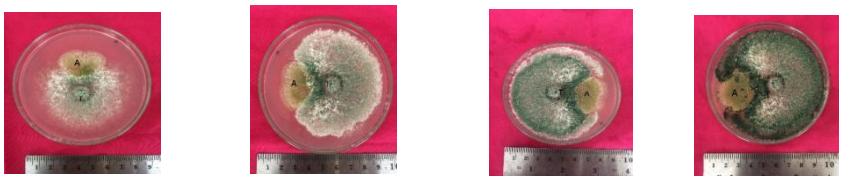


Figure 3. The inhibition strenght of *Trichoderma* sp.(T) against *A. flavus* on PDA media with an incubation period (1) 4 days; (2) 6 days; (3) 8 days and (4) 10 days.

Fractionation results and profiles of active compounds in *Trichoderma* sp. filtrate extract by GCMS (Gas Chromatography Mass Spectrometry)

The initial weight of *Trichoderma* sp. filtrate extract using chloroform: methanol (2:1) solvent after evaporation was obtained 0.078 g. Furthermore, in the test of the inhibition of *A. flavus* by using the diffusion well method at a concentration of 15.6 µg / 20µL, the inhibitory strenght was 15.32 ± 0.277 mm. The inhibition zone formed can be seen in Figure 4.

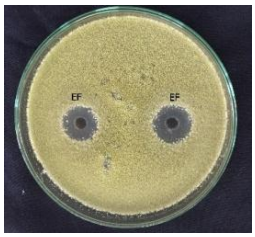


Figure 4. Inhibition zone *Trichoderma* sp. filtrate (EF) extract against *A. flavus* on PDA media with 4-day incubation period.

The results of fractionation and TLC (Thin Layer Chromatography) extract of *Trichoderma* sp. filtrate obtained 5 fractions with Rf values listed in Table 3. All fractions obtained after the inhibitory test on *A. flavus* all showed negative results in the sense of not forming a inhibitory zone around diffusion wells (Figure 5).

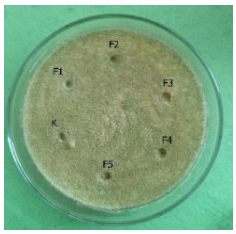


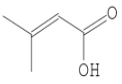
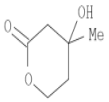
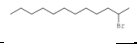
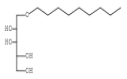
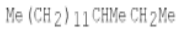
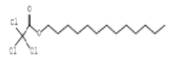
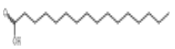
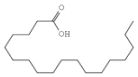
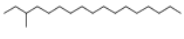
Figure 5. Control inhibition and fraction 1-5 extract of *Trichoderma* sp. filtrate against *A. flavus* on PDA media with a 4 day incubation period.

Table 3. Rf. Values Inhibitory Strength of Each Extract of *Trichoderma* sp. Filtrate Extract Against *A. flavus* on PDA Media.

Fraction	Rf value	Inhibitory strength
F 1	0,90	-
F 2	0,85	-
F 3	0,75;0,51; 0,21	-
F 4	0,23	-
F 5	0,9 ; 0;15	-

Description: Sign - (negative)

Table 4.Type of Seconary Matabolites Contained in Extract of *Trichoderma* sp. Filtrate.

No	Types of Compounds	*R. time*	*Mol. Weight*	*Molecular Structure*
1	Benzene,1,3,5-trimethyl-(CAS) 1,3,5-trimethyl	5.19	120	
2	2-Azabicylol(3,2,1)octan-3-one	6.919	125	
3	5,6-Dihydro-4-methyl-2H-Pyran-2-C	7.735	112	
4	2-Butenoic acid, 2-methyl-(CAS)2-methyl-2	8.834	100	
5	2H-Pyran-2-one,tetrahydro-4-hydroxy-4-methyl	9.267	130	
6	2-Bromo dodecane	10.30	248	
7	Lyxitol,1-O-nonyl	13.230	278	
8	Pentadecane,3-methyl-(CAS)3-methylpentax	13.306	226	
9	Trichloroacetic acid, tridecyl ester	15.546	344	
10	Heptadecane,3-methyl-(CAS)3-methylhepta	15.615	254	
11	n-Hexadecanoic acid	17.556	256	
12	Nonadecane,3-methyl-(CAS) 3-Methylnonad	17.704	282	
13	Octadecanoic acid	19.480	284	
14	3-Methylheneiccosane	19.611	310	
15	Heptadecane,3-methyl	21.362	254	

Description: R = Retention BM = Molecule weight

After combining the five fractions, the in vitro inhibitory test of *A. flavus* showed positive results in the sense that the combined fraction was able to inhibit the growth of *A. flavus* with a diameter inhibitory zone of 14.2 mm. The results of combining fraction analysis using GCMS (Gas Chromatography Mass Spectrometry type QP2010 ultra Shimadzu) 15 different types of secondary metabolites were identified as presented in Table 4.

This means that there are antibiosis compounds contained in the extract of the filtrate. A similar study conducted by Darmayasa (2015) reported 10 compounds in the extract of *T. asperellum* TKD filtrate which were thought to have potential as fungicides. The compounds obtained are as follows: *dl-Mevalonic acid lactone; 3-Hexadecene,(Z)-(CAS); Tetradecane; Phenol, 3,5-bis(1,1-dimethylethyl); Dodecanoicanoic acid, methyl ester(CAS)methyl laurate; 3-Eicosene(E)-(CAS), Hexadecane; 2-Undecene, 3-methyl-, (Z)-; 3-Eicosine,(E)- dan Hexadecanoic acid, methyl ester (CAS)methyl palmitate*. While *Trichoderma* sp. filtrate extract in this study 15 compounds were obtained, namely*: 5,6-Dihydro-4-methyl-2H-Pyran-2-C; 2-Butenoic acid, 2-methyl-(CAS)2-methyl-2; 2H-Pyran-2-one,tetrahydro-4-hydroxy-4-methyl; 2-Bromo dodecane; Lyxitol,1-O-nonyl; Pentadecane,3-methyl-(CAS)3-methylpentax; Trichloroacetic acid, tridecyl ester; Heptadecane,3-methyl-(CAS)3-methylhepta; n-Hexadecanoic acid; Nonadecane,3-methyl-(CAS)3-Methylnonad; Octadecanoic acid; 3-Methylheneiccosane* and *Heptadecane,3-methyl*.

There are allegations that the different species of Trichoderma seen by the antibiosis profile are one of the causes of the different types of compounds that they produce. Although there are groups of compounds obtained from the above research, they show similarities such as the presence of methyl groups and organic acids.

Vianele at al.(2006) isolated and characterized the secondary metabolites contained in the filtrate *T. harzianum* strain (T22 and T39) were azaphilone compounds from the T22 strain and the T39 strain found in butenolide compounds; *harzianolide*; *dehydro harzianolide*; *harzianopyridone*; *6-pentyl-a-pyrone*; *1-hydroxy-3-methyl-anthraquinone*; *1,8-dihydroxy-3-methyl anthraquinone*; *Harziandione*; *Koninginin* A; *Heptelidic acid*; *Trichoviridin*; *Harzianic acid*; *Gliotoxin*; *Gliovirin*; *Viridin; viridiol* dan *Trichorzianines*. Zeilinger et al. (2016) mentions a group of secondary metabolites produced by *Trichoderma* spp. are terpenoids and 6-Penthyl pyrone which can inhibit the growth of other microorganisms. This is also confirmed by the statement of Jelen et al (2013) that many volatile compounds produced by *Trichoderma* sp. like 6-n-penthyl-2H-pyran-2-one (6-PAP) can degrade the cell walls of phatogenous microorganisms. It is known that there are several secondary metabolites produced by *Trichoderma* sp. namely volatile and non-volatile antibiotic compounds and peptaibols which play a role in inhibiting pathogens Steindorffl et al. (2015). Furthermore, most of the derivatives of isocyanide and water-soluble compounds, Heptelidic acid or koningic acid, are conveyed.

Youssef and Hasan (2015) reported that essensial oil of *Perkinsonia aculeate* plant was analysed by GC/MS contains Heptadecane (35.4%). This compound has antimicrobial activity from several bacteria and fungi such as *Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 12228 , *Staphylococcus epidermidis* ATCC 12228), *Pseudomonas aeruginosa* ATCC 27853, *Escherichia. coli* ATCC 10536 and *Escherichia. coli* ATCC25922) as well as *Aspragillus niger* ATCC 16404. Furthermore Igwaranm et al. (2017) showed essensial oil of *Tagetes minuta* flower which is analysed by GC/MS contain Butanoic acid, 2-methyl-, ethyl ester and it has antibacterial activities against *S.aureus*, *S. uberisat*, *L. ivanoli*, *Vibrio* spp, *E. cloacae* and *E.coli*

Extract of Iranian propolis and analysed by GC/MS showed the presence of fatty acids, flavonoids, terpenes, aromatic-aliphatic acids, and esters. Those compound have antimicrobial activities against *C. albicans*, *E. coli*, and *S. aureus* (Afrouzan et al., 2018). *Anisopus mannii* hexane extract contains hexadecanoic acid, ethyl ester as major compound (34%) and has antibacterial activities *Bacillus cereus* (29 mm), *Streptococcus pyogenes* (28 mm), *Enterococcus faecalis* (27 mm), *Proteus vulgaris* (26 mm) and MRSA (25 mm) (Musa et al., 2015). *Tribulus terrestris* plant show three components viz. 2,2,4-Trimethyl-3-pentanol (23.58%), n-Hexadecanoic acid (9.77%) and Z-1,6-Tridecadiene (66.66%). 2,2,4-Trimethyl-3-pentanol analized by GC/MS. These compound have antibacterial activities against *E. coli* (Rao and Balasubramaniam, 2018).

The in vivo Inhibition of extract of *Trichoderma* sp. filtrate against *Aspergillus flavus*

The highest *A. flavus* population was in the Lontar which only added *A. flavus* by 28.5x103 CFU / mL and the lowest population was found in the Lontar which was added with extract of *Trichoderma* sp. filtrate with a concentration of 1.56 / 20µL of 0.00 CFU / mL (Table 5). In Table 5 it also showed the treatment without adding *A. flavus* and without the extract of *Trichoderma* sp. showed no growth of *A. flavus* fungi after 30 days of storage.

The extract of *Trichoderma* sp. filtrate with a concentration of 1.56 / 20µL in in vivo testing (Balinese Lontar) showed significantly different result (P≤0.05) when compared with the treatment without being given *Trichoderma* sp. filtrate extract. The average population of *A. flavus* in Balinese Lontar after being stored for 30 days without filtrate extract was 28.5 ± 0.056 CFU / m, whereas in the extract of filtrate with concentrations of 1.56 / 20µL on Balinese Lontar with the same storing period was 0.00 CFU / mL. This means that the administration of *Trichoderma* sp. filtrate can inhibit the growth of *A. flavus* in in vivo testing. The decline in population of *A. flavus* is caused by the content of secondary metabolites in the form of active compounds contained in extracts of *Trichoderma* sp. filtrate. In accordance with research conducted by [Yu et al., 2005], about the ability of *T. harzianum* filtrate against post-harvest disease caused by *R. stolonifer* showed a significant reduction in mean lesion diameter *R. stolonifer* in apples, pears, peaches and strawberries compared to control treatment, in injured apples with lesion diameter from 73.2 mm in control to 9.7 mm in treated fruit. Then by [Zeilinger, et al., 2016], reported the culture of *Trichoderma* sp. Filtrate. H921 strain is able to inhibit the germination of pathogenic fungal spores in pear and tomato plants.

Table 4. Average Population of *A. flavus* in Treated Lontar After 30 Days of Storage.

Treatment	Average population of *A. flavus* (CFU / mL)
A	$(0,00\pm0,000)10^{3a*}$
B	$(28,5\pm0,056)10^{3c}$
C	$(0,00\pm0,000)10^{3a}$
D	$(5,10\pm0,178)10^{3b}$
E	$(4,75\pm0,428)10^{3b}$
F	$(0,00\pm0,000)10^{3a}$

Description: *Numbers followed by different letter notations in the column show significantly different values (P≤0.05) based on the Duncan test at the level of 5%. A: Lontar without suspension of *A. flavus* spores and without extract of *Trichoderma* sp. filtrate

B: Lontar with suspension of *A. flavus* spores and without extract of *Trichoderma* sp. filtrate

C: Lontar with suspension of *A. flavus* spores and added antibiotics Nystatin with the concentration of 2.2 mg / 20µL.

D: Lontar with suspension of *A. flavus* spores and added extract of *Trichoderma* sp. filtrate with the concentration of 1.2mg / 20µL.

E: Lontar with suspension of *A. flavus* spores and added extract of *Trichoderma* sp. filtrate with 1.4 mg / 20µL concentration.

F: Lontar with suspension of *A. flavus* spores and added extract of *Trichoderma* sp. filtrate with the concentration of 1.56 mg / 20µL.

CONCLUSIONS

Based on the results and discussion, it can be concluded several things as follows:

1. One of the causes of lontar Bali damage which was successfully isolated from this study was *Aspergillus flavus*.
2. Identified types of fungi *Trichoderma* sp. which were isolated from peanut rizhosper capable of inhibiting *A. flavus* by 69.5 ± 1.56% (incubation period of 4 days) and 80.9 ± 1.08% (incubation period of 10 days.
3. In vitro *Trichoderma* sp. extractive extents were able to inhibit *A. flavus* by 15.32 ± 0.277 mm.

4. Identified 15 compounds that have potential as antibioses against *A. flavus* namely: 5,6-Dihydro-4-methyl-2H-Pyran-2-C; 2-Butenoic acid, 2-methyl- (CAS) 2-methyl-2; 2H-Pyran-2-one, tetrahydro-4-hydroxy-4-methyl; 2-Bromo dodecane; Lyxitol, 1-O-nonyl; Pentadecane, 3-methyl- (CAS) 3-methylpentax; Trichloroacetic acid, tridecyl ester; Heptadecane, 3-methyl- (CAS) 3-methylhepta; n-Hexadecanoic acid; Nonadecane, 3-methyl- (CAS) 3-SMethylnonad; Octadecanoic acid; 3-Methylheneiccosane and Heptadecane, 3-methyl.
5. The filtrate extract of the *Trichoderma* sp. with the concentration of 1,56/20µL based on the in vivo test (Balinese Lontar) shows a significant different (P≤0,05) in compare with the treatment without *Trichoderma* sp. filtrate extract.

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