Characterization of endophytic fungi in bali grapevine (*Vitis vinifera* l. var alphonso lavalle) in Buleleng, Bali

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**Abstract.** Bali grapevine is a local fruit cultivated in banjar district, but the productivity and quality of the grapes has decreased each year because of disease, consequently farmers have to use chemical pesticides to deal with disease attacks that actually cause adverse effects. So a solution is needed to solve this problem by using endophytic fungi as a natural biological agent. This research was aimed to determine the (1) total number of fungi colonies and (2) genus of endophytic fungi on Bali grapevine in a vineyard at Banjar village. The observation using descriptive research. Based on the observation that had been done, the result of this research were(1) There was a variation of total number of endophytic fungi colonies in the roots part 6.5 x 10^4 CFU/gram, in the stems2.9 x 10^4 CFU/gram, and in the leaves2.6 x 10^4 CFU/gram, so it was known that the largest total number of endophytic fungi colonies found in the root. (2) Genus of endophytic fungi found on the root, stem, and leaf, were Trichoderma, Fusarium, Scytalidium, Aspergillus, Scedosporium, Alternaria, dan Microsphaeropsis.

1. Introduction

Bali is one of the grape producing regions of Indonesia, the cultivated wine is a type of black wine or commonly referred to as Balinese wine (*Vitis vinifera* L. var Alphonso Lavelle), Balinese grape is a local grape with a slightly round oval round fruit morphology, 2-13 cm long, green, purple, blackish, and has a sweet taste of sweet fruit. Bali's grape cultivation is conducted in the area of North Bali in Buleleng District, located in several sub-districts of Gerokgak, Banjar and Seririt. Vineyards in Bali, especially in Buleleng Regency still have enormous potential to be developed with 1,077,77 hectares of land that can not be optimized for Bali vineyard (Agriculture and Livestock Service of Buleleng Regency, 2017).

Based on data of grape population in Buleleng Regency obtained from Agriculture and Animal Husbandry Office of Buleleng Regency (2018) that in 2008 to 2017 it is known that the number of Bali's vineyard population each year continues to decline, recorded in 2008 the number of Bali's vineyard population reached 592,668 trees and in 2017 reduced to 349,840 trees alone this decline also directly impacted the production of grapes of Bali, in 2008 recorded total production of 22,125 tons while in the year 2017 only amounted to 8,369 tons only. This indicates a serious problem that
resulted in a large decrease in both the population of Bali's vine and from the harvests obtained each year. Vineyards are the most vulnerable species for disease caused either by the environment or from living things that can cause Bali's grape crops to decline in crops, this has a direct impact on Bali wine growers who are stung dependent on the grape harvest Bali. The diseases that attack Bali vine plants caused by microorganisms are downy mildew, powder mildew, rust of leaves, dry rot and rotten fruit. Vulnerable diseases of wine farmers generally use chemical fungicides that are harmful to the environment, if continuous and unsuitable use of dosage other than killing disease-causing microorganisms can also kill microorganisms that can support grape growing. So that required a way out to overcome the use of chemicals by replacing with the use of biological agents one of which is using endophytic fungi that have the potential in dealing with disease attacks on grape Bali.

Endophytic fungus is a fungus that performs mutualism symbiotic relationships that occur between plants and microscopic fungi that all or part of its life cycle is in live plant tissue that can help the growth and endurance of plants, generally endophytic fungi can be found in the roots, stems, leaves, and fruit (Gandjar, 2006). Based on research conducted by Intan (2014) found that endophytic fungus from banana plant able to overcome sigatako yellow spray disease, this is in line with Octaviani (2015) using Trichoderma harzianum and Gliocladium sp. found in the root part is able to cope with dead shoots on plants jabon. Based on this, it is important to do research on the genus of endophytic fungi and the number of endophytic fungal colonies in other plants, one of them in Bali vineyards (Vitisvinifera L. varAlphonsoLavalle).

2. Method

2.1. Types of research
The type of this research is descriptive research using Purposive Sampling method.

2.2. Location
This research was conducted in Microbiology Laboratory of Biology Department Faculty of Mathematics and Natural Sciences Ganesha University of Education.

2.3. Subject and Object
The subjects of this study were all endophytic fungi isolated from Balinese vineyards (Vitisvinifera L. varAlphonsoLavalle) and the object of this study were total colonies of endophytic fungi isolated from Balinese roots, stems and leaves of vineyards (Vitisvinifera L. varAlphonsoLavalle).

2.4. Implementation Procedures

2.4.1. Isolation of Endophytic Fungus. Isolation of endophytic fungi on the roots, stems, and leaves was carried out according to the method described by Samson et al. (2010). Samples of roots, stems, and leaves are washed with running water until clean, then dried. The sample was muffled in 2% NaOCl for 1 minute, then in the alcohol 70% for 1 minute, then in the sterile aquadest for 1 minute and the process was repeated 2 times, the sample was dried over sterile tissue until completely dry. The section is cut to 1x1 cm in aspetis condition and planted in a petri dish containing the PDA. The stem and root part are cut longitudinally then inside the PDA medium.

2.4.2. Dilution. The initial technique of dilution is to prepare a sterile reaction tube, the sample weighed 10 grams and then poured into a beaker glass containing 90 ml of sterile aquades then shaken to homogeneous, then from the solution is taken 1 ml and put in a reaction tube containing 9 ml of aquadest sterile to get 10-1 dilution then this stage continues to be repeated until 10-5 dilution rate. The dilution suspension result is then taken 1 ml to be poured into a petri dish containing the PDA that
is still liquid. Next shaken briefly to form a number eight so that the suspension and media mixed evenly and wait for the media solid and incubated for 3-7 days in room temperature.

2.4.3. Stage Counting Fungi Colony. After incubation for 5 days, the calculation of fungal colonies grow on PDA (Potato Dextrosa Agar) medium. The method used is direct attack using the counting method of the cup (HC) or also known as Total Plate Count. Formulas Used in calculating the number of these fungal colonies as follows (Maturin & Peeler, 2001).

\[
N = \frac{\sum C}{[(1 \times n_1) + (0.1 \times n_2) \times (d)]}
\]

N = Number of colonies per gram (CFU / g) or ml (CFU / gram)
\(\sum C\) = Total colony in first and second sample culture spoons
\(n_1\) = Number of colonies in first sample bowl
\(n_2\) = The number of colonies in the second sample cup
d = First sample dilution rate

2.4.4. Purification Stage. Purification (purification) is performed on every fungal colony that grows on PDA media to new PDA media in aseptic state. Purification is done based on the appearance of macroscopic morphology that includes the color and shape of the fungal colony. Each of these fungi are taken by using ose needle, then re-grown on petri dish containing PDA media for 3 x 24 hours. If after purification there are other fungi then repeated purification until obtained pure fungi.

2.4.5. Identification. Isolates of purified endophytic fungi were then subjected to macroscopic and microscopic observations which were further identified using Barnett identification guidelines (1969). Macroscopic observations include colony color, colony shape, colony texture, and colony growth. While microscopic observations include sealed hyphae or not insulated, hyphae growth, hyphae color, conidia color, presence or absence of conidia and color and conidia form.

2.5. Data analysis method

In this study, data analysis was conducted descriptively, ie exploring endophytic fungi from root tissues, stems, and leaves on Balinese vineyards (Vitisvinifera L. varAlphonsoLavalle) and macroscopic and microscopic morphological observations were then matched with identification books of Kapang Morphology Tropical General daiGandjar (1999), Microfungi on Land Plants from Martin and J.Pamela (1985), Description of Medical Fungi from Kidd et al., (2016) and on the Mycobank database available online.

3. Results and Discussion

3.1. Number of Endofit Fungus Colonies on Balinese Vineyard (Vitisvinifera L. varAlphonsoLavalle)

Based on the research that has been done on the calculation of the number of endophytic fungi colonies in Bali's grape, the result of counting the number of endophytic fungal colonies found in the root, stems, and leaves as follows (table 1) Comparison of the number of endophytic fungal colonies in Balinese vineyards (Vitisvinifera L. varAlphonsoLavalle) can be seen in the following diagrams:
3.2. Characterization of Genus Morphology of Endophytic Fungus Isolated from Balinese Vineyard (Vitis vinifera L. var Alphonso Lavalle)

The result of macroscopic and microscopic observation on endophytic fungi isolates found in the root, stem, and leaf, the result of identification of endophytic fungi obtained data as follows (table 2)

Table 1. Number of Endofit Fungus Colonies in Bali's Vineyard (Vitis vinifera L. var Alphonso Lavalle).

| Bali Grapevine Plant (Sample) | Fungal Colony Microscopy (CFU/gram) |
|-------------------------------|-------------------------------------|
|                              | Root  | Stem  | Leaf  |
| 1                             |       |       |       |
| I                             | 1.3 x 10^4 | 0.7 x 10^4 | 0.6 x 10^4 |
| II                            | 2.0 x 10^4 | 0.6 x 10^4 | 0.7 x 10^4 |
| Colony Total in Sample 1      | 2.3 x 10^4 | 1.4 x 10^4 | 1.3 x 10^4 |
| 2                             |       |       |       |
| I                             | 2.0 x 10^4 | 0.9 x 10^4 | 0.7 x 10^4 |
| II                            | 2.2 x 10^4 | 0.6 x 10^4 | 0.4 x 10^4 |
| Colony Total In Sample 2      | 4.2 x 10^4 | 1.6 x 10^4 | 1.1 x 10^4 |
| Total Colony                  | 6.5 x 10^4 | 2.9 x 10^4 | 2.6 x 10^4 |
| Average Number of Colonies    | 3.2 x 10^4 | 1.4 x 10^4 | 1.3 x 10^4 |

Table 2. Genus of Endophytic Fungus Colonies on Balinese Vineyard (Vitis vinifera L. var Alphonso Lavalle)

| Code | Endophytic Genus | Location | Information |
|------|------------------|----------|-------------|
| P1   | Trichoderma      | Root     | Endophytic (Rizosfer) |
| P2   | Fusarium         | Root     | Endoparasit |
|      | Leaves           |          | Endoparasit |
| P3   | Scytalidium (1)  | Root     | Endophytic |
|      | Stem             |          | Endophytic |
|      | Leaf             |          | Endophytic |
| P4   | Aspergillus      | Root     | Endophytic |
|      | Stem             |          | Endophytic |
| P5   | Scedosporium     | Root     | Endoparasit (Rizosfer) |
| P7   | Alternaria       | Stem     | Endoparasit |
| P9   | Microsphaeropsis | leaf     | Endoparasit |
Based on data on table 1 on the number of endophytic fungal colonies in Bali vineyards (Vitisvinifera L. varAlphonsoLavalle) in the root, stems, and leaves, it is known that the number of colonies between samples 1 and sample 2 is varied number of endophytic fungal colonies. In the root part of sample 1 has fewer colony counts when compared with the roots of sample 2, the stem also found that the number of fungi in sample 1 had fewer amounts than the sample 2, and the leaf part of sample 2 had the number of colonies mushroom more than the sample 1. Variation of the number of colonies in each part of Bali’s vine plants can be influenced by several factors, one of which is the environmental factor when viewed from the location of Bali’s wineries, the location of the plantation is in accordance with the conditions required for Bali’s vineyards can grow well, so other factors that greatly affect the occurrence of the difference between the number of fungal colonies in samples 1 and sample 2 is the state of the plant where the sample is taken.

Endophytic fungal colonies at 1 sample root were 2.3 x 104 CFU / gram and in sample 2 of 4.2 x 104 CFU / gram, the difference in colony count occurred due to the soil condition in which the sample was taken in sample 1 soil given urea fertilizer while in sample 2 the soil has been supplied with natural fertilizer from cow dung, it is possible to differentiate the number of endophytic fungi colonies obtained because the use of inorganic fertilizers can indeed help in the growth of plants but cause the soil to be poor nutrients. This is in line with Sarjiaya and Dwi (2011) that the provision of organic fertilizer will increase microbial activity in the soil when compared with inorganic fertilizer provision this occurs because the inorganic fertilizer will cause the composition of nutrients in the soil to be disturbed, decreasing the activity of microbes in in the soil, it will also have an impact on the endophytic fungus that grows in the roots because the fungus is in need of enough nutrients to grow.

In the stem part the number of fungal colonies in sample 2 is 1.5 x 104 CFU / gram whereas in sample 1 that is 1.4 x 104 CFU / gram, the number of different colonies between the two stems is due to the stem condition used as the sample, in sample 1 the condition of the stem has aged and has been attacked by disease in some parts as well as on the skin has begun to peel it is different when compared with the condition of sample 2 where the stem condition is still fresh and there is no disease and the skin condition is still intact so this causes the difference in the number of colonies in part stems, this is in line with Kusumawati (2014) the growth of fungi in miana plants found that the number of fungi on the stem is influenced by the useful stem function to transport photosynresearch results through the phloem vessels that make the stem part is the right location for the growth of fungal colonies. The older the plant, the function of these vessels will decrease and the decrease in the function of the phloem vessels directly affect the decrease in the number of endophytic fungal colonies found in the stem. So the difference between stem samples 1 and sample 2 is due to the stem conditions of different Balinese vineyards.

In the leaf part of sample 2 that is 1.2 x 104 CFU / gram has more colony of fungi when compared with the number of fungal colonies in leaf of sample 1 that is 1.1 x 104 CFU / gram, this condition happened because of excessive use of pesticide this is observed immediately when taking the sample of which leaves sample 1 has a stronger pesticide aroma when compared to the leaves of sample 2, so this affects the number of colonies in the fungus that are found differently and indeed the growth of fungal colonies on the part will be less according to Enny (2013) the growth of microbes in the leaves especially mushrooms are strongly affected by nutrient conditions less when compared to other parts of the plant.

The results shown on the graph illustrate the total amount of the entire colony on the roots, stems, and leaves of Bali's vine. Based on the graph, it can be known that the largest number of colonies are in the root position of 6.5 x 104 CFU / gram, if viewed from the environmental conditions with pH 6.3 and temperature 24oC it is known that the environmental conditions are in accordance with the habitat of Bali's vineyards grow. Rao (2010) states that most fungi are found in parts of the soil, especially those with ideal environmental conditions, affecting the roots because this part is the closest part to the soil and contains many nutrients so that the root has the largest number of endophytic fungal colonies compared with stem and leaf part of this matter due to the use of chemicals so that the number of colonies found different according to Ariyono (2014) the diversity of endophytic fungi on the ground
kangkung plant on organic farms more when compared with the number of endophytic fungi on conventional land. So that the use of chemicals that will have a direct impact on the number of endophytic fungal colonies in Bali's vineyards.

Table 2 shows the mushroom genus that has been identified from Balinese grape plants isolated from root, stem and leaves. Of the total genus that is found there are four genus of fungus endoparasit namely Fusarium, Scedosporium, Alternaria, and Microsphaeropsis. As well as three genera of endophytic fungi Trichoderma, Scytalidium, and Aspergillus.

Trichoderma is found in the root part with typical macroscopic features of irregular colonies, rectangular side-shaped colonies, undulate colonies. The form of rough colonies such as velvet, with the color of the surface of young green colonies to old hijua and pigmentation that occurs in the colony of this fungus yellowish green and if the colony has opacity that is translucent. As well as microscopic characteristics of this isolate has hyphae that is insulated and has no pigmenmtasi. The conidiofor can branch, the shape at the base will form lateral branches while at the end of the branching will become shorter, conidia semibulate to oval and short with size ranging from 1.5 to 3.2 μm, and has a smooth wall of this genus is a genus that can found in parts of plants, especially on the root, Soesanto (2013) found Trichoderma found in the ginger rhizome.

Fusarium fungus is found in the root and leaves with macroscopic features of the form of filamentous colonies, the structure of the colonies from the raised side, with a filiform shaped edge. Colony surface shape such as carpet, colony surface color is yellowish brown while colony pigmentation is yellowish brown and if colony is directed towards light then opacity is not opaque, there is radial line but concentric line not found. The microscopic characteristic of the Fusarium Isolate has a microscopic structure, in the hyphae there is a divider, having a branched conidiofor and carrying a monofialid, macroconidia having 2-4 septa. No microcidia found. Makrokonidai will bend on the dorsal so it looks like a sickle, macrokonidia size 20-30 μm. Khlamidospores are present in mycelia or in conidia. This fungus belongs to the type of endoparasit based on Istiqomah (2015) Fusarium attacking tomato plants so that tomato plants have a development that is not maximum.

Scytalidium genus is found in the roots, stems, and leaves. Its macroscopic feature is the form of a circular colony, with nmbonate side colonies, curled colony edges. If viewed the shape of the surface of the colony looks like cotton, if it is explained to light then opacity that is not translucent, and the color of colony pigmentation that is blackish green with dark surface color. Microscopic characteristics Scytalidium isolates have a microscopic structure as follows: hyphae have bulkhead with thick hyphae wall thickness especially on the part of the swelling. These isolates form the arthroconidia. Konidia is smooth-walled, and generally it is not a cigarette. Konidia in this isolate has pigmentasi and has a size of 4-5 μm. The genus of this fungus belongs to the endophytic fungus, Lisdayani (2015) on gaharu stems found the genus Scytalidium as endophytic fungus species and kusumawardani (2015) found also on the leaves of the pepper plant.

The genus Aspergillus is found in the roots and stems, the macroscopic feature is that the colony has a filamentous form, when viewed from the side of a raised-shaped colony, with curled edge shape. The shape of the colony surface looks like cotton and its opacity is translucent. Colony color from the surface is yellowish brown and pigmenmtasi yellowish white. The colony appears to have a radial line and a concentric line. The microscopic features of hyphae have no insulation. The heads of conidia are round and tend to be columns of old colonies. Konidia is round to semi-round, 3-5 μm in size, round to semi-rounded vesicles, fialids formed in metula. Metules are generally hialin to brown. This fungus belongs to the endophytic fungus, as does the study by Yadav et al. (2014) found spergillus fungus in leaf tissue, petiolus, and stem tissue proving these fungi including fungi that could potentially be antimicrobial.

Mushroom Scedosporium found in the root, macroscopic characteristics of the colony has an irregular shape, when viewed from the side of the colony shaped nmbonate. With curled edge. Rough colony surface shapes such as velvet and opacity can not be penetrated by light. The surface color of the colony is whitish green and the pigmentation is whitish green. Colonies appear to have radial lines and concentric lines. Microscopic characteristics Isolate Scedosporium has the following microscopic
structure, hyphae has a bulkhead, hyaline-shaped conidia that is 3-5 μm in size, generally conidia has a smooth wall. This fungus belongs to a type of endoparasitic fungus, Harun et al. (2010) Scedosporium is a type of pathogenic fungus that can live there is a network of plants and animals and soil that suffered severe contamination.

Alternaria fungus is a type of fungus found in the stem, macroscopic characteristics of this fungus is colonies have a circular shape, when viewed from the side of a raised-shaped colony, with curled colony-shaped edges, with the shape of the entire colony edge. The surface looks like velvet with opacity impenetrable to light. Colors of black-green colony surface with black pigmentation. This colony has a concentric line while a radial line is not found. The microscopic characteristic as follows, 1 to 3 berseptaconidiofor, looks simple and there are several branching, straight or swollen parts, sometimes geniculate with one or several pores 3-6 μm in edges. Chocolate and smooth walled. Konidia brown, walled shy or slightly rough, forming a chain that is often branched, generally sized 7-18 μm. This fungus belongs to the type of endoparasitic fungus, according to Muksin et al. (2013) found that the Alternaria fungus attacked many onions causing onion plants to have purple rickshaws, and Kurnia et al. (2014) that the Alternaria fungus causes tomato plants to suffer from yellow spots causing farmers to experience crop failure.

The genus of the last fungus is found from the genus Microsphaeropsis, the fungus is found in the leaf, this fungus has macroscopic features as follows colonies have a circular shape, when viewed from the side then the form of raised colonies, with curled colony edge form. The surface of the colony resembles velvet with opacity that is not translucent. Colony surface color is blackish brown with black pigmentation. This colony has a radial line and no concentric lines are found, with microscopic features as follows: Microsphaeropsis isolates have the following microscopic structure as hyphae having a bulkhead, having a brown colored pigmentation, and having a not-so-perfect shape. Each swelling region has a size of about 4 μm. Pyecnia has a diameter of about 250-350 μm. Has a brown konidia with a size ranging from 3.4 to 4.5 μm. This fungus belongs to the type of endoparasite fungus, according to Milicevic (2014) found that isolation from plants in the area in crops found that the genus Microsphaeropsis causes some types of plants to experience disease, as well as according to Ashahina (2015) found that the isolation of parts of plants in the area tropical forests of genus fungi derived from the genus Microsphaeropsis is a pathogen in plants and can also cause infection of human skin.

Based on the results of research on the genus of endophytic fungus found in the grape of Bali (Vitisvinifera L. varAlphonsoLavalle) found that from 7 genus are found there are 4 genus belonging to the type of endoparasitic fungi and 3 genus included in the endophytic fungus, so it can be known population endophytic fungi in Bali's grape growing shrinking due to unsuitable environmental conditions caused by excessive use of chemicals on Bali's vineyard.

4. Conclusion
The conclusions of the research that has been done, as follows:

a. There are variations of endophytic fungal colonies on Balinese vine with an average number of colonies at the root of 3.2 x 104 CFU / gram, on stem 1.4 x 104 CFU / gram, and at 1.3 x 104 CFU / gram leaf. As well as the total number of colonies of endophytic fungi most found in the root of the colony of 6.5 x 104 CFU / gram.

b. Identified genus of endophytic fungi are 7 genus, Scytalidium, Fusarium, Aspergillus, Trchoderma, Scedosporium, Alternaria, and Microsphaeropsis.

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