Diversity of Arbuscular Mycorrhizal Fungi (AMF) in Rhizosphere Plants at the West Bali National Park (TNBB)

Meitini Wahyuni Proborini1*, D. S. Yusup
Biology Dept, Basic Science Faculty Univ.of Udayana Kampus Bukit Jimbaran Bali
*Corresponding author: pmeitini@unud.ac.id

Abstract. The West Bali National Park (TNBB) is a source of various organisms such as plants, animals and microorganisms. The soil characteristics are dried temporally and spatially. Natural resources that has not been explored is Abuscular Mycorrhizal Fungi (AMF) that can be used as bio-fertilizers especially in nutrient-poor regions. Based on this background this research is to explored and identified AMF in the West Bali National Park. The sampling exploration, isolation screening and identification were carried out from April up to September 2019. The results of the study identified 5 genera and 8 species which were spread in the five rhizosphere zones of Lantana camara Plant. The eight species AMF are five species belong to Glomus genus i.e G. etunicatum, G. mossaeae, G. Aggregatum, G.intraradices, G.etunicatum two species of the Gigaspora genus; G. Margarita and G. Albida, one genus Acaulospora one genus Scutelospora and one genus Entropospora. The present of AMF species, number of spores and colonization of AMF shows spatially distribution at these areas.

1. Introduction
The Arbuscular Mycorrhizal Fungi (AMF) is natural fungal symbiosis with plant of roots. The fungi link plant and soil by transporting mineral nutrients to the plant and carbon compounds to the fungi (1, 2). Their associations showed unique structure and physiological relationship with symbionts which produce arbuscules, hyphae, and vesicles within host plants in the root cortical cells (2).

About 93% of flowering plant families and more or less 92% of land plant families are estimated to have mycorrhizal associations (4, 2). The potential AMF to enhance Plant growth and production in the arid soil is well recognized (5). Phosphorus is generally considered to be the most important plant growing factor, which can be supplied by AMF associations because of the many abiotic and biotic factors, which restrict its mobility in soils (2).

Natural soil communities in North-East Bali such as cashew Plantation in Sukadana district as an example of the arid soil contain a wide variety of AMF species (6, 7). There are many natural resources that has not been explored the micro-organism that have very important value such as Abuscular Mycorrhizal Fungi (AMF) (6, 7). These finding can be used as germ-plasms as a source of natural bio-fertilizers especially in nutrient-poor regions (7). Based on this background this research is to explored and identified AMF in the West Bali National Park. It is important to explore AMF species composition on other natural plantation as a source of germ-plasms and evenness may be varied among these plant rhizosphere.of the West Bali National Park.
2. Material and Methods

2.1 Collection of soil sample to identify species of AMF
The soil samples 10 g was took from the rhizosphere of plants by digging these soil up to a depth of 20 – 30 cm and put into plastic bags, labeled and stored at 4ºC until the process were done.

2.2 Preparation of root samples to examine the AMF Colonization
For each plant specimen, 100 feeder root pieces were thoroughly washed under tap then water and soaked in 10% KOH at room temperature for 24 hours. The root segments were washed in distilled water, acidified with 1(N) HCL and stained in 0.05% trypan blue. Root segments were observed under stereo microscope. The percentage of mycorrhizal association as formulation below

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\text{% Mycorrhizal association} = \frac{\text{No. of mycorrhiza associated segments}}{\text{Total no. of segments scored}} \times 100
\]

2.3 Counting of AMF spores from soil samples
The 250 gram soil sample was taken and dissolved in 1000 ml distilled water in a flask, then flask was shaken for 30 min and it was kept in undisturbed condition for 30 min. So that the soil particles can precipitate and the spores were floated on the surface of the liquid. AMF spores were obtained by wet sieving and decanting. The solution was passed through 250, 150, 53 and 45 μm sieve and the spores were collected from the residue present on the sieves. Spores of large size were considered. This residue was dissolved in distilled water and filtered. The residue present in the filter paper was taken and mounted on a slide in lacto-phenol and cotton blue and were examined under stereo microscope.

3. Result and Discussion
Result of the study identified 5 genera and 8 species of AMF which were spread in the five rhizosphere of the Tembelekan plant (Lantana camara L). The eight species were five species of the Glomus genera namely G. etunicatum, G. mosseae, G. Aggregatum, G.intraradices, G.etunicatum two species of the Gigaspora genera; G. Margarita and G. Albida one genus Acaulospora one genus Scutelospora and one genus Entropospora. Detail data of the AMF species can be seen on Table 1 below.

| Species               | I   | II  | III  | IV  | V   |
|-----------------------|-----|-----|------|-----|-----|
| G. etunicatum         | v   | v   | v    | v   | v   |
| G. mosseae            | vv  | vv  | vv   | v   | vv  |
| G. Aggregatum,        | v   | v   | v    | v   | v   |
| G.intraradices        | vv  | vv  | vv   | v   | v   |
| G. margarita          | vv  | v   | v    | v   | v   |
| G. albida             | v   | -   | vv   | v   | -   |
| Acaulospora sp        | vv  | v   | vv   | v   | v   |
| Scutelospora sp       | v   | -   | v    | v   | v   |
| Entropospora sp       | v   | v   | v    | -   | v   |

Note : (v) = present  (-) = not present  (vv) = abundant

According to the result on the colonization of AMF in the Lantana camara plant roots during April to September 2019 is as follow
Based on the graphic showed that the AMF hyphae colonization in all samples of root plants species were having AMF colonization. The colonization presented as vesicle, urbuscle, internal-external hyphae and spore forms. Result of the AMF spores obtained from five station of TNBB from April to September 2019 can be seen on the graphic 2 is as follow

The graph showed that the number of AMF spores number was not related to the season however depend on sampling locations (station). The number of spores relatively low on station II especially during August and September 2019

**Discussion**

Result in accordance with Table 1 showed that there were eight species of AMF found the five location of TNBB in which the species belonging to the Glomeraceae family, the order of Glomerales phylum Glomeromycota (8- Schüßler et al., 2001). The AMF identification results in the TNBB showed a smaller number of species than the number of AMF species in the cashew rhizosphere in Karang asem and in Buleleng, which identified 13 species of AMF (9-Proborini et al., 2013). The AMF G. intraradices can be found more abundant in all station sampling compared to other AMF species (see table 1). This finding seem that the G. intraradices and G. moseae have small size compared to Gigaspora and Entrosporosa as the consequence these species is more easily spread widely in its environmental. This is argument is supported with finding result by Proborini et. al. (9-2013). The species of based on soil analysis shows that in the cashew plantation area of Sukadana Karang asem the
area shows that the texture of the soil tends to be sandy, whereas in the West Bali National Park (TNBB) the condition of the soil texture is similar to the soil texture in the Gerokgak Buleleng area which is sandy clay (9-Proborini et.al. 2013). This is probably due to the edaphic factor in the West Bali National Park making one of the distinguishing factors that cause the number of different AMF species (10-Delvian, 2008)

According to Graphic 1 seem that the pattern of AMF colonization in the root plant which occurred in April to September in TNBB areas indicated that there was no significantly different pattern of AMF colonization. The result shows a interesting trend on the percentage colonization increase gradually from August to September. The trend of the percentage of colonization dynamic fit to the beginning of rainy season on August and September. The result agree with (10) and (1) indicated that AMF hypha colonization is season related. That is colonization increase during rainy season and decrease during dry season. Such AMF–host plant interrelationship gives benefit to each other. The AMF takes a benefit by obtaining nutrient from the host plant (7). The host plant gets some benefits from AMF colonization i.e. the AMF takes a role on re-mineralization of Phosphorous and providing Phosphate (P) in soil (1), on water and mineral uptake (11, 12) on producing plant growth hormone-like similar to auxin, cytokine (13) and Gyberelin (14)

The Graphic 2 indicated that the number of AMF spores relatively stable temporal and spatially in five location of TNBB. This finding indicated that the five location of research in TNBB is positive signal as a source of AMF. Moreover, according to data that the number of AMF spores in the five sampling locations of the West Bali National Park shows that the five sampling locations areas are still natural ecosystem. This is in accordance with (11, 12) and (14) that in non-polluted ecosystem, there will be as a source of natural microorganisms.

4. Conclusion
There were 5 genera and 8 species of AMF which were spread in the five location of Lantana camara plant rhizosphere of TNBB. The present of AMF species and number of spores shows spatially distribution at these areas

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