Asian Journal of

Agriculture

| Asian J Agric | vol. 3 | no. 2 | December 2019 |
| E-ISSN: 2580-4537 |

Red onion buds photo by Maurice Mingay

Asian Journal of Agriculture

| Asian J Agric | vol. 3 | no. 2 | December 2019 | E-ISSN: 2580-4537 |

Published semiannually **PRINTED IN INDONESIA**

E-ISSN: 2580-4537

Asian Journal of Agriculture

| Asian J Agric | vol. 3 | no. 1 | June 2019 |

ONLINE
http://smujo.id/aja

e-ISSN
2580-4537

PUBLISHER
Society for Indonesian Biodiversity

CO-PUBLISHER
Universitas Mulawarman, Samarinda, Indonesia

OFFICE ADDRESS
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PERIOD OF ISSUANCE
June, December

Society for Indonesian Biodiversity

Universitas Mulawarman Samarinda, Indonesia

Published by Smujo International for The Society for Indonesian Biodiversity and Universitas Mulawarman Samarinda, Indonesia

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Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta*. 50th Annual Symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

Alikodra HS. 2000. Biodiversity for development of local autonomous government. In: Setyawan AD, Sutarno (eds.) Toward Mount Lawu National Park; Proceeding of National Seminary and Workshop on Biodiversity Conservation to Protect and Save Germplasm in Java Island. Universitas Sebelas Maret, Surakarta, 17-20 July 2000. [Indonesian]

Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com. DOI:10.1038/msb.2008.24

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ASIAN JOURNAL OF AGRICULTURE
Volume 3, Number 2, December 2019 E-ISSN: 2580-4537
Pages: 33-40 DOI: 10.13057/asianjagric/g030201

Distribution of major diseases of shallot in South Kalimantan, Indonesia

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Manuscript received: 9 December 2018. Revision accepted: 4 June 2019.

Abstract. *Safitri YA, Hasanah U, Salamiah, Samharinto, Pramudi MI. 2019. Distribution of major diseases of shallot in South Kalimantan, Indonesia. Asian J Agric 3*: *33-40.* Shallot is a vegetable crop with high economic value, but its productivity in Indonesia is still relatively low. One of the causes is due to diseases. The research was conducted from November 2017 to April 2018, with the purpose of studying the distribution of major diseases of shallots in South Kalimantan. It consisted of field, laboratory, and greenhouse research. Field research included the survey of shallots' extensive planting in eight villages of six districts in South Kalimantan, disease symptoms, the broad of attack, and the collection of secondary data. Laboratory research included the isolation and identification of the pathogen causing diseases. The greenhouse research was conducted to perform the Postulate Koch test. The research results showed that there were two major diseases of shallots, namely Moler and Anthracnose. Pathogen causing Moler disease (*Fusarium oxysporum*) attacked shallot plants in six districts (Tabalong, Balangan, Tanah Laut, Kotabaru, Tapin, and Banjarbaru) and pathogen causing Anthracnose disease (*Colletotrichum* sp) attacked shallot plants in five districts (Tabalong, Balangan, Tanah Laut, Kotabaru, and Tapin).

Keywords: Anthracnose, distribution of disease, Moler, shallots

INTRODUCTION

Shallot (*Allium ascalonicum*) is one of the vegetable commodities that has a high economic value with a diverse utility from the household to industrial scale. This commodity is needed year-round. Therefore, when the stock is low but the demand is high, the prices increase and sometimes can affect national inflation. This can happen outside of the growing season, especially at the beginning of the rainy season. To solve this problem, the government has the policy to expand production by planting outside the planting season, not only centralized in Java and lowland, but also in South Kalimantan, Indonesia. The obstacle of planting shallots outside the planting season, especially in rainy season, is the risk of disease.

Shallot has been developed in South Kalimantan since 2013 and was initially developed in only two districts. The two districts were Tapin District (5 ha) and Tanah Laut (3 ha). Then in 2015, it was further developed in 8 districts, i.e., Tanah Laut, Tapin, Hulu Sungai Selatan, Hulu Sungai Tengah, Tanah Bumbu, Balangan, Tabalong, and Banjarbaru with a total land area of 148 ha. Whereas in 2017, shallot planting was expanded to 10 districts from 13 districts in South Kalimantan with an area of 422 ha. But Hulu Sungai Tengah did not take part in this program, and was replaced with Hulu Sungai Utara, Kotabaru and Barito Kuala. The average shallot productivity in South Kalimantan is between 5-12 $t.ha^{-1}$. Based on the annual report in 2014-2018 by Dinas Pertanian South Kalimantan report that the main types of pathogens that attack shallot in South Kalimantan are *Fusarium*, *Anthracnose* and Purple blotch.

According to Udiarto et al. (2005), the loss resulting from diseased shallots could reach 24-100 %. The effectiveness in controlling plant disease is determined by the information accuracy of infecting pathogen types, factors affecting reproduction, and factors helping the spread of diseases. *Fusarium* wilt disease is a disease attacking the onion plants either in the growing season or out of season (the rainy season) and can reduce crop yields to 27-75% (Adiyoga et al. 2000). According to Bambang and Khusnul (2014), anthracnose can reduce crop yields ranging from 21-63% even to 100% if conditions support especially when wet, heavy rain, and humid. Porch blotch of onion caused by *Alternaria porri* is an important disease of onion worldwide (Glande and Simon 2019). The disease yields a loss of 30% (Everts and Lacy 1990).

Hence, the information about the spread of disease in South Kalimantan is needed as the starting data point. The spread of plant diseases will determine accurate control measures. Therefore, this research was conducted to gain information about the distribution of major diseases to create a distribution map.

MATERIALS AND METHODS

The research was conducted in districts of Tabalong, Balangan, Tapin, Tanah Laut, Kotabaru, and Banjarbaru of

South Kalimantan, Indonesia from November 2017 to April 2018. The sample of infected plants and the soil surrounding them were collected for disease isolation and identification in a laboratory. The laboratory research was in the form of the pathogen reproduction or the cultural reproduction of pathogen causing the disease. This consisted of stages such as isolation, purification, and identification of the pathogens. Furthermore, the postulate Koch test was performed in the greenhouse.

Major diseases isolation and identification

The media used for the disease isolation and identification were Potato Dextrose Agar (PDA) and Nutrient Agar (NA) (Tuite 1969). The infected shallots were isolated and purified in the reproduction media. Meanwhile, the soil taken from around the roots of infected plants was weighed as much as 10 g and put in the glass bottle containing sterile distilled water about 90 ml. Then, they were homogenized for 15 minutes in an orbital shaker with a speed of 150 rpm and were diluted to 10^{-6} to observe the shape of spores and hyphae of fungi needed in the Contrast phase microscope ML 2000. The identification of the diseases was based on key identification literature for pathogens (Barnett 1960; Booth 1971; Alexopoulos dan Mims 1978; Agrios 1996).

Koch Postulate test

The Koch Postulate test was conducted to gain an accurate result of disease identification. Isolated and purified culture of the pathogen was cultured in the reproduction media. The pure culture pathogen obtained from previous isolation in the laboratory were then inoculated to healthy shallots aged 15 days after planting. Inoculation was done by spraying and flushing the suspension of *Fusarium oxysporum* fungi and *Colletotrichum* sp. fungi as much as 10 ml with each spore density of 3x107 conidia.ml^{-1}. The observation was conducted every day until the symptoms appeared on shallots. The plants showing the symptoms were then isolated again to create a pure isolate. The pathogen isolate was observed under the microscope to find out whether the morphology of isolated pathogens was like the inoculated pathogens. The identified pathogens were clarified by comparison of similarity of both conditions.

Mapping of major diseases in South Kalimantan

Mapping preparation activity consisted of collecting toponym data and analyzing the major diseases of shallots in South Kalimantan. The supporting data collected included the maps of Tabalong, Balangan, Tapin, Tanah Laut, Kotabaru, and Banjarbaru districts. The map of Indonesian toponym or Rupa Bumi Indonesia (RBI) was taken from Ina-Geoportal, a web of Indonesian geospatial agencies owning geospatial data including RBI map. Secondary data was collected through a questionnaire by interviewing farmers about the area of onion planting, when the land was first planted with shallots, a disease that had attacked the plant, and how the control was carried out.

The field observation comprised the symptom elements of major diseases of shallots in chosen areas. The symptom observations were carried out in plant areas in each district in South Kalimantan. The coordinates were determined using GPS to find out the site coordinates where the major diseases attacked shallots. The observation parameters for observing the large of the major disease attack on shallots in South Kalimantan used the formula:

$$Percentage\ of\ the\ large\ of\ attack = \frac{Plant\ area\ under\ attack}{Total\ plant\ area} x\ 100\%$$

The map of research results referred to the data gained from the field. All the areas, whether spatial or attribute, were digitized into GIS application. In this case, ArcGIS was used for processing data spatially. With the help of GIS analysis, the distribution map of major diseases affecting shallots in South Kalimantan was made. Next, the field observations were inputted as coordinate points and the data analysis of major diseases was put in Global Mapper application to convert the file format from .gpx for GPS into .shp that could be read by ArcGIS application. The converted file was put in ArcGIS application. The results comprised of the map and description of major diseases distribution on shallots in districts/cities in South Kalimantan.

RESULTS AND DISCUSSION

According to the results of field observations from six districts in South Kalimantan, there were two types of symptoms that attacked shallots in South Kalimantan, namely the symptoms of Moler and Anthracnose.

Disease identification

Moler symptom disease caused by *Fusarium oxysporum* fungi and had isolate characteristics as presented in Table 1. The morphology of the colony and the shape of conidium is shown in Figure 1.

Table 1. The characteristics of *Fusarium oxysporum* fungi on shallot plants

Characteristics	Description
The part which the symptoms are visible (the part of shallots plant)	Whole parts of leaf (rolled)
The color of colony	Pale white-yellowish
The diameter of colony (4^{th} day) in PDA medium	±4.63 mm
The color of spores	Pale white
The shape of hyphae	Insulate
The shape of mycelium	Cotton-like
Microconidia	
- Shape	Oval
- The number of septa	0 septa
Macroconidia	
- Shape	Crescent moon-like
- The number of septa	3-5 septa

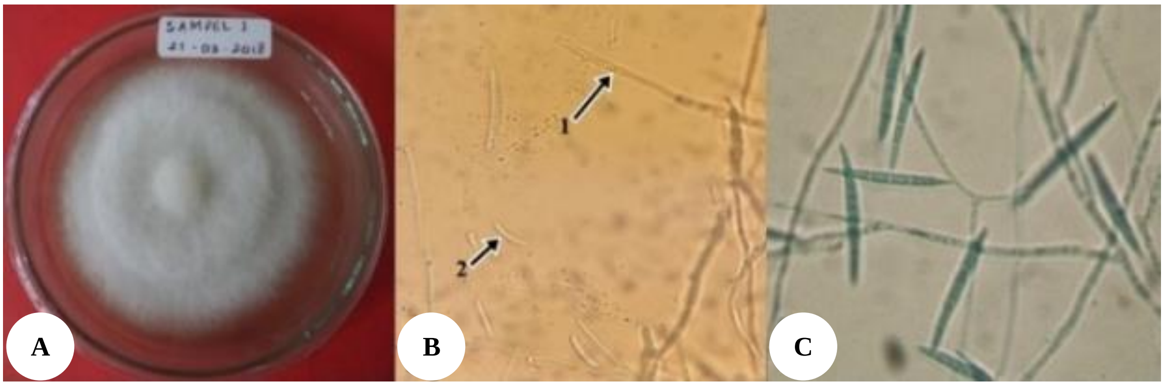

Figure 1. *Fusarium oxysporum* on shallots. A. The Morphology of colony 8th day on PDA medium, B. Hyphae, and Microconidia (1 and 2); C. Macroconidia under the contrast phase microscope ML 2000 (400x magnification)

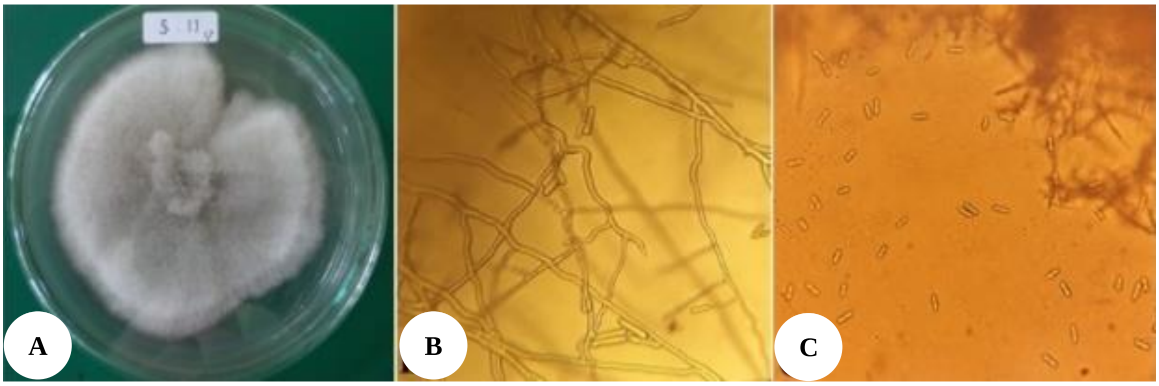

Figure 2. *Colletotrichum* sp. on a shallot plant. A. The morphology of colony on media PDA medium at 8th day of observation, B. Hyphae, C. Conidia under the Contrast phase microscope ML 2000 (400x magnification)

Table 2. The characteristics of *Colletotrichum* sp. fungi on shallots plants

Characteristics	***Colletotrichum* sp.**
The part which the symptoms are visible (the part of shallots plant)	Part of leaf (spotted)
The color of colony	Gray blackish
The diameter of colony (4th day) in PDA medium	± 4,74 cm
The color of spore	Pale white
The shape of hyphae	No insulate
The shape of mycelium	Cotton-like
Conidia shape	Tube-like

Another symptom in shallot plants was *Anthracnose* disease, caused by *Colletotrichum* sp. fungi. In the medium of Potato Dextrose Agar (PDA), *Colletotrichum* sp. fungi could grow well at room temperature. The fungi attached to shallots had the isolate characteristics as presented in Table 2. The morphology of the colony and the shape of conidium were shown in Figure 2.

The observation of diseases in the shallot field

Based on the field observations, the symptoms of Moler disease were discovered in all locations. Latifah et al. (2011) showed that pathogens can be carried out by the seeds or seedlings of shallots. This will lead to variations in the first symptoms and the incubation period of Moller disease on shallot plants. Nugroho et al. (2011) showed that the use of different shallot varieties caused the different intensity of the diseases. Meanwhile, the symptoms of Anthracnose were discovered in the six locations only (Table 3). The percentage of infected plants is presented in Table 4.

The area distribution of shallots in South Kalimantan was presented in Figure 3. The Moller disease distribution on shallots plant in South Kalimantan was shown in Figure 4.

Based on the observation of the disease distribution area in South Kalimantan, Anthracnose was observed in several districts. The data were collected according to the symptom estimation on the shallots after the isolation and identification stages. Anthracnose disease was discovered at several districts in South Kalimantan namely Balangan, Kotabaru, Tabalong, Tanah Laut, and Tapin, each with a different percentage of infected area. However, Anthracnose was not observed in Banjarbaru (Table 5). Anthracnose attacking shallots in Tabalong District was as many as 26% of the total infected area. This was the largest attack among other districts. Meanwhile, no Anthracnose disease attack was observed in Banjarbaru city. The data of Anthracnose disease in South Kalimantan can be seen in Figure 5.

The environmental effect towards the pathogen reproduction

The soil pH range of six shallot cultivation districts in South Kalimantan was 5.79-7.55, with the characteristics of more acidic until more basic (Table 6). The rainfall, humidity, and temperature from November 2017 until March 2018 in South Kalimantan were also measured. The rainfall ranged from 86 to 545 millimeters, the humidity was between 84.2%-88.2%, and the temperature was between 26.1°-28.4°C.

Fusarium sp. and Anthracnose in shallots are very dependent on environmental conditions, i.e., soil pH, soil temperature, humidity, and nutrients. Conditions in the field can be very supportive of the growth of these pathogens. This is consistent with the statements of Agrios (1996) and Sastrahidayat (2011), which stated the development of pathogens increases with high temperatures and low soil pH. At a temperature of 18°C, there is a little infection in plants, in temperatures, 25-28°C pathogens will become malignant while at a temperature of 38°C the pathogen will die. At a soil temperature of 25-30°C the spores will germinate, so that the attack rate will increase due to increased softening of the root plants, which causes the wound to become easily injured to facilitate pathogens in the healing process in the host plant. While at lower temperatures the germination process will be hampered.

Table 3. The disease symptom observation of shallots cultivated in South Kalimantan, Indonesia

District/City	Village	Varieties	Cultivation Area (m^2)	Total cultivation area (m^2)	Symptoms		Infected area (m^2)	
					Moller	Anthracnose	Moller	Anthracnose
Tabalong	Jaro Bawah	Super Philips	10000	20000	+	+	3468	5202
	Jaro Atas	Super philips	10000		+	-	2890	-
Balangan	Batu Mandi	Bima brebes	289	289	+	+	86.7	28.9
Tanah Laut	Ambungan	Bima brebes	2312	3757	+	+	867	144.5
	Ujung Batu	Bima brebes	1445		+	+	578	289
Kotabaru	Sehapi	Bima brebes	10000	10000	+	+	2312	1445
Tapin	Asam Rendah	Batu Ijo	10000	20000	+	+	5202	2890
	Harapan masa	Bima brebes	10000		+	-	1156	-
Banjarbaru	Loktabat	Bima brebes	289	289	+	-	86.7	-

Note: +: present, -: absent

Table 4. The distribution of Moller disease in South Kalimantan, Indonesia

District/City	Village	Plant area (m^2)	Infected area (m^2)	Total infected area (m^2)	Percentage of attack (%)
Balangan	Batu Mandi	289	86.7	86.7	30
Banjarbaru	Loktabat	289	86.7	86.7	30
Kotabaru	Sehapi	10000	2312	2312	23.1
Tabalong	Jaro Atas	20000	2890	6358	31.8
	Jaro Bawah		3468		
Tanah Laut	Ambungan	3757	867	1445	38.5
	Ujung Batu		578		
Tapin	Asam Randah	20000	5202	6358	31.8
	Harapan Masa		1156		

Table 5. The distribution of Anthracnose disease attack in South Kalimantan, Indonesia

District/city	Village	Cultivation area (m^2)	Infected area (m^2)	Total infected area (m^2)	Percentage of attack (%)
Balangan	Batu Mandi	289	28.9	28.9	10
Banjarbaru	Loktabat	289	0	0	0
Kotabaru	Sehapi	10000	1445	1445	14.4
Tabalong	Jaro Atas	20000	0	5202	26
	Jaro Bawah		5202		
Tanah Laut	Ambungan	3757	144.5	433.5	11.5
	Ujung Batu		289		
Tapin	Asam Randah	20000	2890	2890	14.4
	Harapan Masa		0		

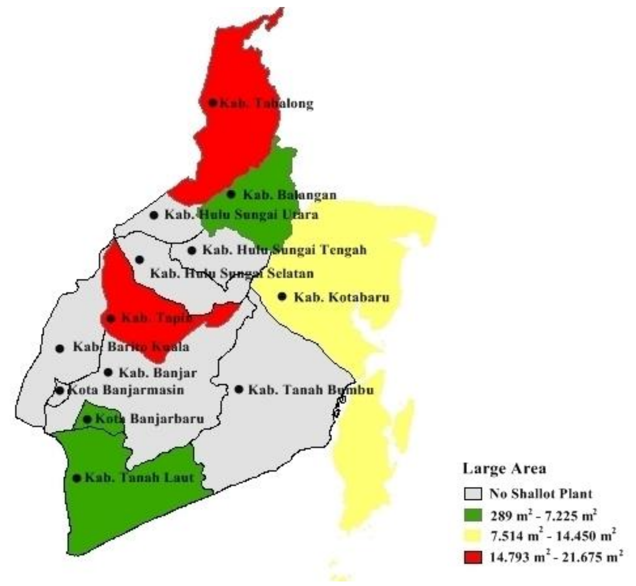

Figure 3. Map of area distribution of shallots in South Kalimantan, Indonesia

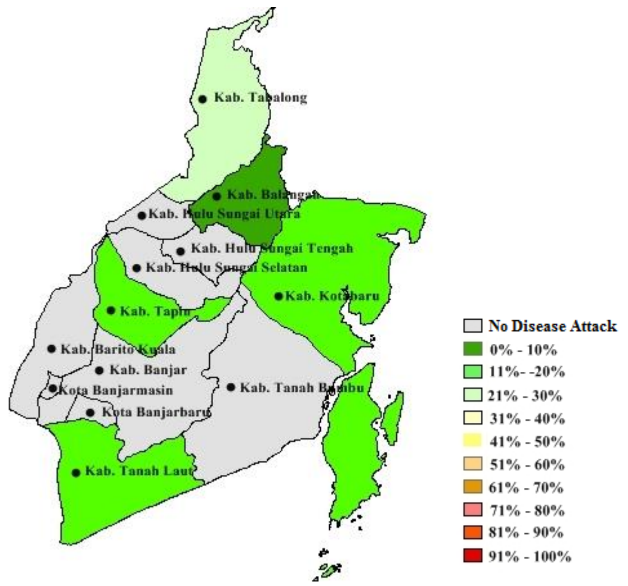

Figure 5. The map of Anthracnose disease distribution on shallots plant in South Kalimantan, Indonesia

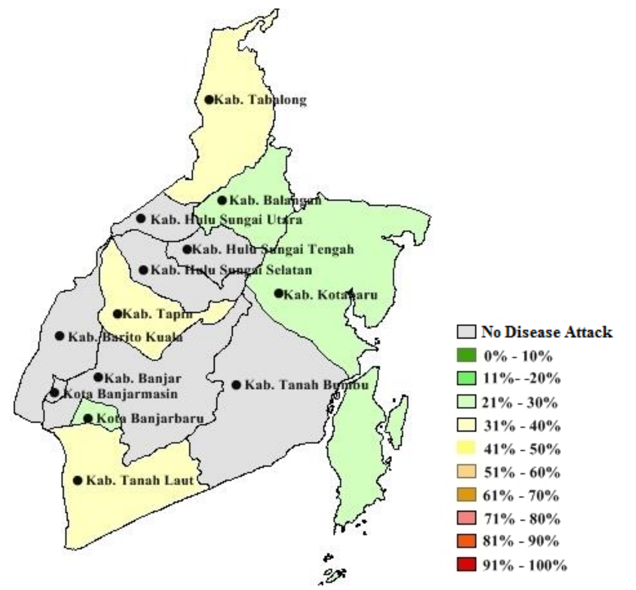

Figure 4. The map of Moller disease distribution of shallots in South Kalimantan, Indonesia

Table 6. The soil pH on shallots in South Kalimantan, Indonesia

District/City	Village	pH	Indication
Tabalong	Jaro Bawah	5.79	More acidic
	Jaro Atas	7.20	More basic
Balangan	Batu Mandi	6.48	Neutral
Tanah Laut	Ambungan	7.55	More basic
	Ujung Batu	6.14	Neutral
Kotabaru	Sehapi	5.96	More acidic
Tapin	Asam Rendah	6.67	Neutral
	Harapan masa	6.26	Neutral
Banjarbaru	Loktabat	6.17	Neutral

The Koch Postulate test result

The Koch Postulate test was conducted by re-inoculating the isolate, from an isolated infected plant in the field and from the pure culture. The result of inoculation on healthy plants is seen in Figure 6. The result of inoculation with the second isolate suspected due to Anthracnose is shown in Figure 7.

Figure 6. Symptoms resulted from inoculation with the isolate of *Fusarium oxysporum* on shallots. A. Initial symptoms of attack; B. Advanced symptoms of Moler disease

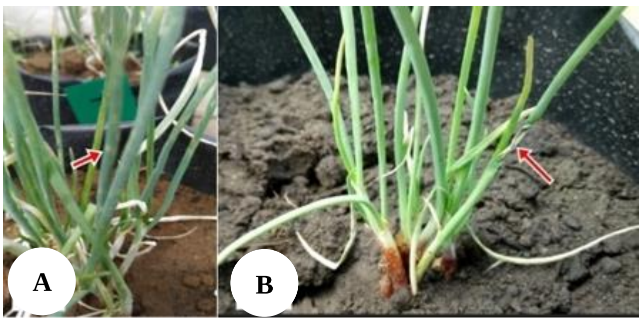

Figure 7. Symptoms of sick plant inoculated with the isolate of *Colletotrichum* sp. A. Initial symptoms spots appeared; B. Advanced symptoms, spots become larger, and the leaf twisted

Figures 6 and 7 showed that the symptom of *F. oxysporum* and anthracnose on shallots. Figure 6.A showed the early symptoms of *Fusarium* and Figure 6.B showed the late symptoms. Whereas Figure 7a showed the early symptoms of Anthracnose caused by *Colletotrichum* sp and Figure 7b showed the late symptoms of anthracnose diseases.

Discussion

According to the results of the field observations at six districts in South Kalimantan, there were two types of diseases attacking the shallots: Moler disease and Anthracnose disease. Moler disease was caused by *Fusarium oxysporum* fungi. The characteristics of this disease are started by shriveling at the tip of the leaf and pervading to its base (Samadi and Cahyono 2005). This type of fungi spreads through seed bulbs, air, soil, and water. If the contagion is throughout the seed bulbs, the symptoms appear about 7 until 14 days after panting. However, the symptoms appear 14 days after planting if the contagion is throughout the soil, air, and water (Suryanto 2010). According to Semangun (2000), the first symptom of Moler disease on shallots is the leaf turned yellow starting from its base, then pervading to the middle part until the whole plant withered and dried. The leaves of shallots infected with Moler disease would most likely be twisted. On the other hand, Anthracnose disease, according to Suhardi (1991) and Suhendro et al. (2001) would cause the leaves to have brown spots which can make the leaf break and fall. However, the bulb part would turn dark green and black. Subsequently, the leaf would become twisted (rolled). If the root is infected, the leaf cannot grow up like usual. The infected bulbs cause the plants to decay. According to Hadiwiyono (2004); Suhardi and Hadisutrisno (1994); and Suhardi et al. (1994; 2000) and Semangun (2007), Moler disease caused by *Fusarium* can attack all types of onions. This fungus can infect shallots in all growth stages of the plant, and this was supported by Abawi and Lorbeer (1972). On the other hand, Anthracnose disease can be unbearable for some shallots' clones, even though Sumenep cultivation was considered tolerant to this disease.

Based on Agrios (1996) and Semangun (1996), the fungi had a colony that was colorless until cream or light yellow and could be discovered in light red or purple with insulating mycelium. The microconidia are round-shaped, ovoid kidney, and lancet, meanwhile its macroconidia is crescent-shaped with many septa. In the PDA medium, fungi *Colletotrichum* sp. can grow well at room temperature. Liu et al. (2016) and Semangun (2007) reported that the fungi colony had pale grey blackish color, oval-shaped conidia, uninsulated, hyaline with the blunt end.

The result of soil pH analysis showed that the soil taken from six districts in South Kalimantan had pH ranging from 5.79-7.55 with characteristics of more acidic and more basic. According to Tjahjadi (1989) and Sastrahidayat (1989), these fungi could live with temperatures ranging from 10°C-24°C and more acidic soil with pH ranging from 4.5-6.0, and the wet soil conditions caused *Fusarium* sp. fungi to grow fast. However, *Colletotrichum* sp. fungi according to Yulianty (2006) need a pH of 5 to grow well and quickly.

The distribution area of shallot cultivation in South Kalimantan

The survey of shallot cultivation in South Kalimantan resulted in the area distribution map of shallots in several districts in South Kalimantan. This showed both the different extensive planting methods and that half of the districts did not cultivate shallots. The distribution of shallots in South Kalimantan is presented in Figure 1. When the survey was conducted, some farmers were not planting the shallots. The plant areas changed from time to time and varied for each district or city.

The diverse areas of shallots were in Balangan, Banjarbaru, Kotabaru, Tabalong, Tanah Laut, and Tapin districts. The rest of the districts did not plant shallots at that time. The rainfall conditions when the survey was conducted probably affected the shallot planting activity. The survey was conducted from November 2017 to March 2018 when the rainfall level was high, and some farmers were not planting the shallots. The largest areas of shallot cultivation were in Tabalong and Tapin with a total plant area of 20.000 m^2; and the smallest areas were in Balangan and Banjarbaru about 289 m^2.

The high level of rainfall would inhibit shallot growth and increase the chance of disease infection. Rahayu (2009) reported that shallots are well cultivated in the dry season. It can be seen from the length of plants, the width of leaves, the rate of plant growth, the diameter of seed bulbs, the weight of dry seeds (under the sun) of each clump, and the productivity of plants per hectare that these were slower in the peak of rainy season. This case was one of the reasons for the farmers refusing to plant shallots in this period or even speed up the harvest. This was in accordance with what Purba (2009) stated that the decrease of shallot production in the rainy season was because of the disease attack and the growth disruption was due to the leaves that were hard to photosynthesize.

The areas of shallots surveyed also had different altitudes. The areas of shallots in Asam Randah village in the district of Tapin had an uphill shape and uneven land. The same condition was in the planting area in Sehapi village located on the hillside of highland and uneven land in the district of Kotabaru. The differences in altitude made the growth rate of shallots vary from place to place. The high altitudes had a lower temperature, thus affecting the shallots growth rate and production. The altitude was the main factor that change the diversity of heat, the average temperatures decreased along with the increase of the altitude approximately 0.6°C every 100 m (Anshari et al. 2011).

The varieties of shallots planted in the surveyed locations were mostly Bima Brebes variety. Only three locations that used other varieties, including Super Philips variety used in Jaro Atas and Jaro Bawah village and Batu Ijo variety, was used in Asam Randah village, Tapin district. The uSuper Philips variety was the most used variety since this one is more tolerant to several diseases.

This was a similar reason for Asam Randah village farmers in Tapin in using Batu Ijo variety in shallots cultivation.

The distribution of Moler disease on shallots in South Kalimantan

The field survey showed that Moler disease symptoms were discovered in some districts in the shallot cultivated areas in South Kalimantan. Moler disease is one of the major diseases attacking shallots in South Kalimantan. The data was supported by Safitri et al. (2018) that the Moler diseases were identified as one of two major diseases that attacked shallots in South Kalimantan.

The percentage of Moler disease attacks ranges from 23.1-38.5%. The largest area attacked by Moler disease according to its symptoms was in Tanah Laut District, where the total of the infected area reached 38.5%. On the other hand, the lowest attack was recorded in Kotabaru with the attack percentage being only 23.1%.

The distribution of Moler disease in South Kalimantan spread to whole districts with the largest attack being in the district of Tabalong with the total planting areas of 6358 m^2. The lowest attack was in Balangan District and Banjarbaru city with the total plant areas 86.7 m^2. However, according to the percentage of attacks, Tanah Laut District was the largest area attacked by this disease with a total percentage of 38.5% from the total cultivated area. Tapin district was the lowest area attacked by the disease with a percentage of only 23.1% of the total cultivated area in the whole district of Kotabaru. The environmental condition is one of the factors determining the Moler disease development. The significant weather change caused this disease to increase. Soil nutrient leaching caused by the runoff made the nutrient deficiency and increased disease growth. Wiyono (2007) reported that climate change, such as the increase of temperature, increased the disease condition. Besides, the low organic content in the soil also contributed to the increasing disease severity.

Sastrahidayat (1992) stated that the growth of Moler disease was affected by the condition of the environment such as soil pH level, soil temperature, humidity, and soil nutrient. *F. oxysporum* fungi could grow in low soil pH levels and temperature between 18°C and 38°C. High humidity would affect the growth of host plant which turned into succulent. Therefore, the immune system of the plant to the pathogens would decrease (Supriyadi et al. 2013). The low soil pH level was good for Moler disease but was not good to plant because it would make the plant sensitive to disease infection.

The distribution of Anthracnose disease on shallots in South Kalimantan

The field survey showed that Anthracnose disease had attacked cultivated shallots in several districts in South Kalimantan. The same indication was observed by Safitri et al. (2018) that had successfully isolated and identified the Anthracnose diseases in the shallot cultivation areas. Anthracnose was found in several districts in South Kalimantan such as Balangan, Kotabaru, Tabalong, Tanah Laut, and Tapin with different intensities of attack. However, there was no attack in Banjarbaru city. Anthracnose disease attacked shallots in Tabalong with a percentage of 26%. This was the largest attack among other districts.

The largest infected areas of Anthracnose disease were in Tabalong and Tapin. It reached 26% of the whole planting area in both districts. The smallest infected area of Anthracnose disease was Balangan, which was only 10% of total planting area.

The high intensity of rainfall when the research was conducted was one of the factors that can influence disease growth. Humidity will increase if the temperature is low due to high rainfall. High levels of moisture make pathogens grow faster. Semangun (2011) reported that the growth rate of Anthracnose disease will be faster when the humidity is high, or higher than 80%. The thickness of the onion tuber epidermis affects the intensity of the attack of *C. gloeosporioides*. The thicker the epidermis layer, the more resistant to the attack of *C. gloeosporioides* (Marlitasari et al. 2016).

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ASIAN JOURNAL OF AGRICULTURE
Volume 3, Number 2, December 2019 E-ISSN: 2580-4537
Pages: 41-46 DOI: 10.13057/asianjagric/g030202

Plant resistance to leaves and their effects on paddy rice production in Kutai Barat District, East Kalimantan Province, Indonesia

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Manuscript received: 22 April 2019. Revision accepted: 16 August 2019.

Abstract. *Akhsan N, Sopialena, Fahrizal. 2019. Plant resistance to leaves and their effects on paddy rice production in Kutai Barat District, East Kalimantan Province, Indonesia. Asian J Agric 3: 41-46.* This study was aimed to evaluate the effect of fertilizer application on the resistance of lowland commercial rice cultivars (*Oryza sativa L.*) against leaf spot diseases in Kutai Barat District, East Kalimantan Timur, Indonesia and to determine the factors influencing the resistance. A field experiment was conducted in rice fields within Long Iram and Linggang Bigung Sub-districts, West Kutai District and the disease identification was performed at the Laboratory of Pests and Diseases, Faculty of Agriculture, Mulawarman University. The field experiment was designed in a split-plot design arranged in a Randomized Block Design (RBD) using four replications. The main plot was fertilizer application (P) consisting of two fertilizer application treatments, i.e., 200 kg.ha^{-1} Urea (p1), and 200 kg.ha^{-1} Urea + 200 kg.ha^{-1} NPK (p2). The sub-plots were varieties (V) consisting of three varieties, i.e., Ciherang (v1), Mekongga (v2) and Inpari 6 (v3). The disease identification was performed by identification of leaf spot disease, isolated from the sample plants using morphological observation under a microscope. The number and density of stomata, intensity of leaf disease infection, and yield of the rice were observed. The humidity was also measured at the time of observation of leaf spot disease intensity. The results showed that different fertilizer treatments did not affect the leaf spot disease intensity, but the varieties affected the disease intensity at 7, 14, 21.35 and 49 days after planting. The number of stomata of *Ciherang*, *Mekongga* and *Inpari 6* varieties was 230,182 and 236 stomata/mm^2, respectively. *Ciherang* variety was more resistant against the leaf spot disease compared to other varieties. Stomatal density does not always affect the intensity of leaf spot disease in lowland rice. There was a correlation between air humidity and the intensity of leaf disease infection. The interaction between fertilization and varieties was significant for the rice yield and the highest yield was obtained by *Ciherang* variety fertilized with 200 kg.ha^{-1} Urea + 200 kg ha^{-1} NPK about 3.58 Mg.ha-1 (grain wet weight).In conclusion, Fertilizer application does not affect the leaf spot disease infection and Ciherang variety is the most resistant plant against leaf spot disease compared to *Mekongga* and *Impari 6* varieties.

Keywords: Fertilizer application, Kutai Barat District, leaf spot disease, plant resistance

INTRODUCTION

Rice (*Oryza sativa* L.) is a cultivated plant that has a very important role in Indonesia for food and other needs. Indonesia is the third-largest rice producer in the world after China and India (FAOSTAT 2014). Rice is a staple food for most people in Indonesia, as well as one of the largest importing countries in the world after Myanmar, Vietnam, and Bangladesh (Oishimaya 2017). With an increasing population in Indonesia, there is an increase in the demand for rice. On the other hand, Indonesian rice production is not still sufficient to fulfill the Indonesian people rice consumption. Indonesian government endorses many efforts to achieve rice self-sufficiency by farming intensification and extensification.

Intensification and extensification of rice cultivation in Kutai Barat District, East Kalimantan Province was carried out in seven sub-districts, i.e., Penyinggahan, Muara Pahu, Mook Manaar Bulan, Barong Tongkok, Linggang Bigung, Long Iram and Bongan. In Long Iram and Linggang Bigung sub-districts, paddy fields have been extended with each planting area of 361.4 ha and 493.9 ha carried out in 2016 (Central Statistics Agency 2016). Furthermore, Long Iram Seberang, and Linggang Amer sub-districts, are two regions that have different altitudes compared to other regions. In these two areas, the planting season is carried out twice a year, since the water supply is abundant to support lowland rice cultivation. Therefore, the rice production in these areas is higher than in other areas.

At present, the pest and disease attacks are increased significantly by the time of the increase of rice area cultivation. Efforts of pest and disease control have been conducted, but those were limited and lacked efficiency. Spotting and exploding diseases are some of the most destructive and are increasing from year to year. In addition, the attention of the Agricultural Agency responsible for pest and disease control in those areas is also still limited (UPTD. Food Crop Agriculture and Horticulture 2017). Rice plants attacked by leaf spot disease will reduce the function of leaves to conduct photosynthesis, consequently, It will reduce the plant yield (Debona et al. 2014).

Disease attacks will occur if there is interaction between susceptible plants, virulent pathogens, and a supportive environment (Semangun 2001). Climate is an environmental factor that supports the interaction of virulent pathogens and susceptible plants. One such climate factor is humidity. The development of pathogens which are generally microorganisms, is strongly influenced by humidity. As a result, the disease will develop well if the humidity level is appropriate (Wiyono 2007). Plant resistance to pathogens is also influenced by nutrients that can strengthen plant tissue. Abdulrachman and Yulianto (2001), stated that application of nitrogen, phosphor, and potassium fertilizer to rice plants could reduce the intensity of brown leaf spot disease from 57.81% to 32.05% and striped spot disease from 8.55% to 2.48%. Suryadi (1995) also reported that the application of potassium fertilizer in rice plants can also reduce the intensity of leaf blight disease by 20-30%, compared to without using the potassium fertilizer application. The plant resistance against leaf spot diseases is also influenced by the genetic properties of the plant itself. Plants that are compatible with pathogens will cause a higher intensity of disease, however, plants that are tolerant to disease can still survive and produce grains (Horsfall and Cowling 1980). Therefore, it is necessary to conduct a study to evaluate the resistance of the plants of three rice varieties to leaf spot disease and rice production and the factors that influence them with different fertilization applications.

MATERIALS DAN METHODS

The study site

The research was conducted at rice farms in Long Iram Seberang, Long Iram, Linggang Amer, and Linggang Bigung Sub-districts of Kutai Barat District, East Kalimantan Province, Indonesia. Identification of leaf spot infected plants was carried out at the Plant Pest and Diseases Laboratory, Faculty of Agriculture, Mulawarman University, Samarinda, Indonesia.

Leaf spot pathogen isolation and identification

The media used for the pathogen isolation and identification was Potato Dextrose Agar (PDA) as an ingredient for the isolation media of pathogenic fungi and methylene blue (Tuite 1969). Isolation was carried out by cutting the leaves between symptomatic and healthy parts, approximately 0.5 cm^2 in leaf area. The leaves were then sterilized with 70% alcohol, washed with distilled water, dried, and placed in PDA media on an aseptic petri dish. Furthermore, the fungus that was growing from the leaves was purified, observed in the microscope, identified, and documented. Finally, the procedures of the Koch postulate were carried out. The identification of the diseases was based on identification key literature for pathogens (Barnett 1960; Booth 1971; Alexopoulos dan Mims 1978; Agrios 1996).

Experimental design

The experiment employed a split-plot design arranged in a Randomized Completely Block Design (RCBD) with 4 replications. The main plot was fertilizer application (P) consisting of (*p1*) 200 $kg.ha^{-1}$ Urea (46% Nitrogen) fertilizer, and (*p2*) 200 $kg.ha^{-1}$ Urea (46% Nitrogen) fertilizer + 200 $kg.ha^{-1}$ NPK (15% Nitrogen, 15% Phosphor, 15% Potassium). The subplot had different cultivated lowland varieties (V), i.e., (*v1*) Ciherang, (*v2*) Mekongga and (*v3*) Inpari 6 varieties. The soil tillage was carried out by hand tractor. The size of an experiment unit plot was 2 x 2 m^2. Transplanting was carried out for 15-20 days of rice seedling in the nursery after sowing. The plant spacing was 25 cm x 25 cm. Twelve plants were determined as samples for each variety, with 6 sample points in each plot. Insecticide and rodenticide were used to control the pest mainly insects and rats. The intensity of leaf spot disease, air humidity, number and density of stomata and rice production were observed.

The intensity of leaf spot infection measurement

The leaf spot infection intensity was observed weekly and 12 observations were carried out during the experiment. The observation was started one week after planting. The infection intensity was calculated using the following formula:

$$I = \frac{\sum (n_i . v_i)}{Z.N} X 100\%$$

Note*:*
I = Infection Intensity (%)
ni= Number of infected leaves
vi= Infection score of infected leaf (Table 1)
N= Total of number of leaves observed
Z = The highest infection score observed (Table 1)

Table 1. Score, and the category of spots infection.

Leaf score	Infection level
1	1 – 5% infection of the total leaf area
3	5 – 11 % infection of the total leaf area
5	11 - 25% infection of the total leaf area
7	25 - 75% infection of the total leaf area
9	75 - 100 % infection of the total leaf area

Source: Directorate of Crop Protection (2007)

After obtaining data on the intensity of rice leaf spot infection, it was followed by calculating the rate of infection of the disease.

Air humidity measurement

The measurement of air humidity was carried out in two research locations starting from the first time of rice planting to the last observation in harvest time using the *HTC-1* digital hygrometer. Furthermore, the relationship between humidity and the rate of disease infection per week was analyzed.

Number and density of stomata observation

The stomata number and density were observed in the Plant Anatomy and Systematics Laboratory, Faculty of Mathematics and Natural Sciences, Mulawarman University, using binocular microscope.

Rice yield

The rice yield was calculated using formula:

$$Y = \frac{10.000 m^2}{L\ (m^2)} \times \frac{X\ \ kg}{1000\ kg}$$

Where:
Y = Rice production ($Mg.ha^{-1}$)
X = Rice yield for each plot (kg)
L = Plot area (m^2)

Data analysis

Data on disease intensity and production were analyzed using Analysis of Variance (ANOVA), and if there were significant differences the post-test of Least Significant Different (LSD) the level of α = 5% were used to compare the mean values among treatments.

RESULTS AND DISCUSSION

Leaf spot infection intensity

Results of variance analysis on the intensity of leaf spot disease infection in three rice varieties at the age of 7 - 49 days after trans-planting (dat) showed a significant effect, but there was no effect of application of fertilizer treatment. The highest disease infection intensity was observed in the Inpari 6 and the lowest was in Ciherang variety (Figures 1 and 2). Furthermore, at the age of 56-84 dat, both treatments did not give significant effect. There was a tendency for higher intensity of leaf spot disease attack in the treatment of *p2* (200 $kg.ha^{-1}$ Urea (46 % Nitrogen) fertilizer + 200 $kg.ha^{-1}$ NPK compared to *p1* (200 kg ha^{-1} Urea). In addition, the infection intensity of leaf spot disease increased from time to time of observation (Figures 1 and 2).

Results of isolation and identification of leaf spot diseases collected from the study sites found most of diseases were disease-related-fungi. In all varieties, i.e., the leaf spot disease was caused by *Culvularia* sp., *Bipolaris* sp., *Fusarium* sp. and *Pyricularia* sp. (Figure 3). The first *Culvularia oryzae* fungus was reported to cause black grains of rice; this disease is the main disease of upland rice (Busi et al. 2009; Butt et al. 2011; Hsuan et al. 2011), but at the experimental site which is planted in lowland, the diseases were also found and observed. In Pakistan, such kind of disease was also found to cause rice leaf spot disease (Majeed et al. 2016). *Bipolaris* sp., *Fusarium* sp. and *Pyricularia* sp. are very common infecting rice plants (Shabana et al. 2008; Pinciroli et al. 2013; Pagliaccia et al. 2018). According to Ikrarwati and Yukti (2014), the fungi attacking rice especially Ciherang variety in seedling stage are *Alternaria* sp., *Fusarium* sp., *Drechslera* sp., *Synonym Bipolaris* sp., and *Curvularia* sp. The symptom of Fusarium at the leaves shows small dark brown spots and the edges of the spots are light brown and slightly wilted. This fungus is often found in rice plants as well as infecting parts of roots, stems, midribs, leaves, and fruit (Semangun 2001).

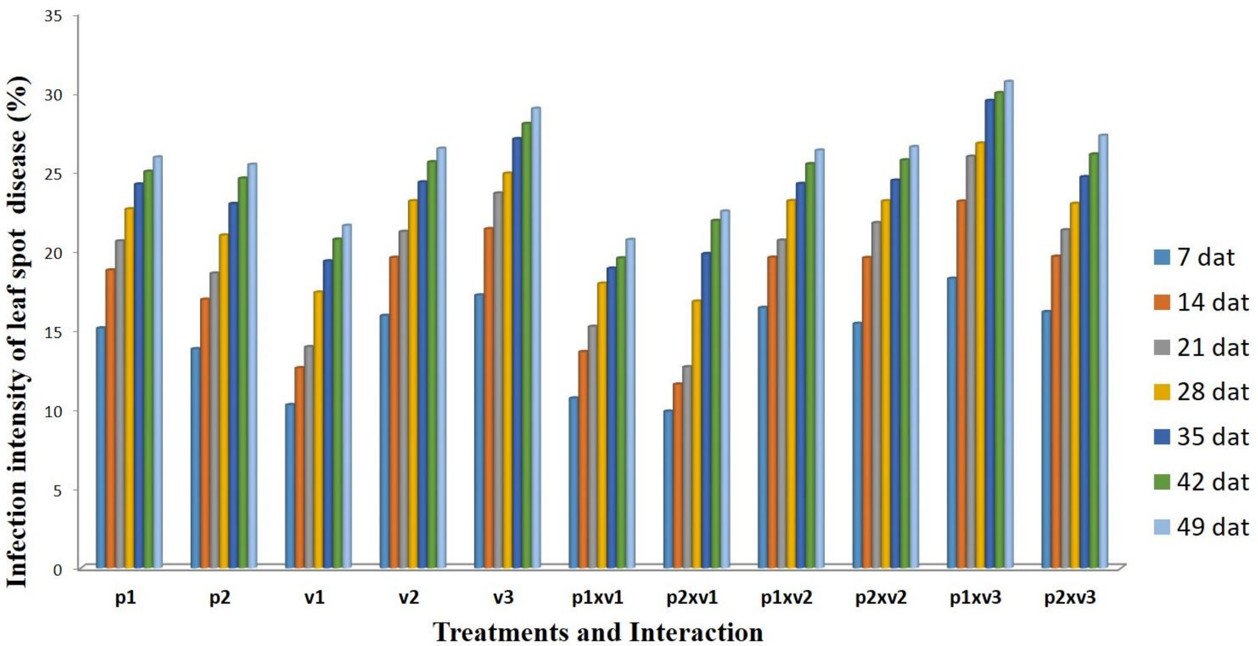

Figure 1. Intensity of leaf spot disease infection on rice plant from 7- 49 dat of observation. *p1*: 200 $kg.ha^{-1}$ Urea, *p2*: 200 $kg.ha^{-1}$ Urea + 200 $kg.ha^{-1}$ NPK, *v1*: Ciherang, *v2*: Mekongga, *v3*: dan Inpari 6, and *p1*x*v1*, *p2*x*v1*, *p1*x*v2*, *p2*x*v2*, *p1*x*v3*, *p2*x*v2* are interaction between variety and fertilizer application treatment.

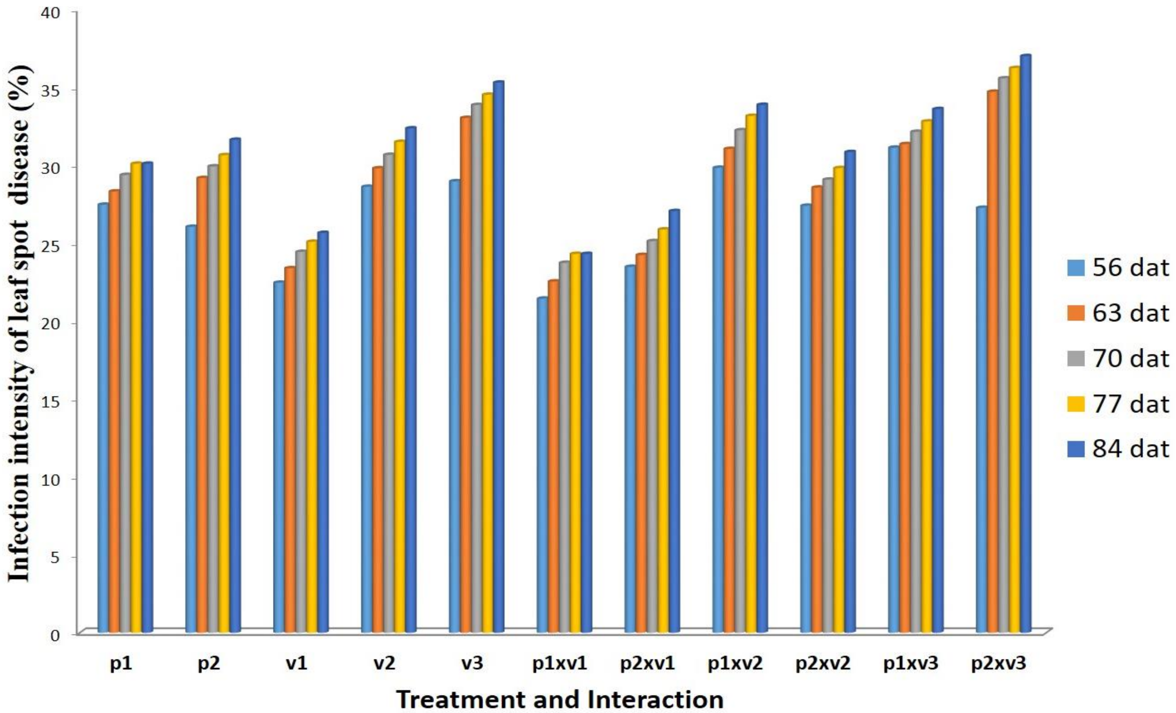

Figure 2. Intensity of leaf spot disease infection on rice plant from 56-84 dat of observation. *p1*: 200 kg.ha^{-1} Urea, *p2*: 200 kg.ha^{-1} Urea + 200 kg.ha^{-1} NPK, *v1*: Ciherang, *v2*: Mekongga, *v3*: dan Inpari 6, and *p1xv1*, *p2xv1*, *p1xv2*, *p2xv2*, *p1xv3*, *p2xv2* are interaction between variety and fertilizer application treatment.

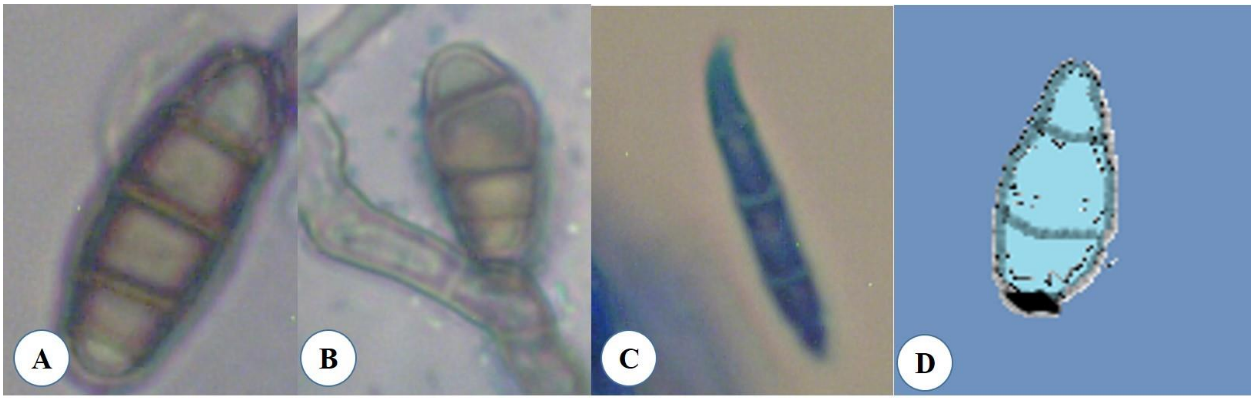

Figure 3. Fungus conidia of *Bipolaris* sp. (A), *Culvularia* sp. (B), *Fusarium* sp. (C), and *Pyricularia* sp. (D).

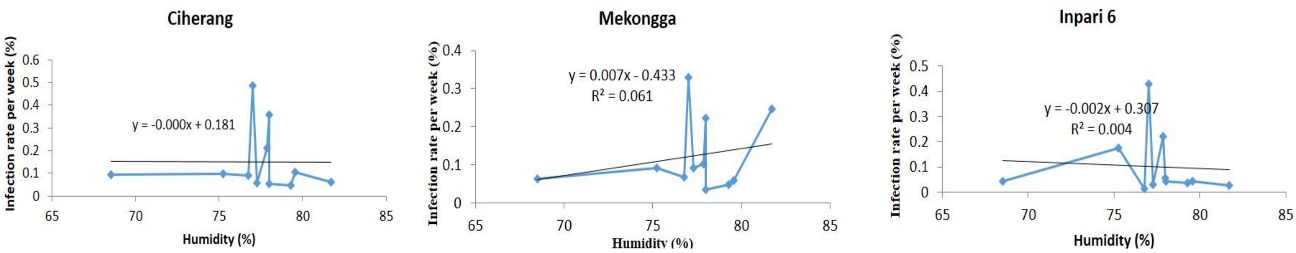

Figure 4. Correlation between air humidity and infection rate of leaf spot disease in Ciherang, Mekongga, Inpari 6 varieties cultivated in Long Iram Sub-district, Kutai Barat District, East Kalimantan Province, Indonesia.

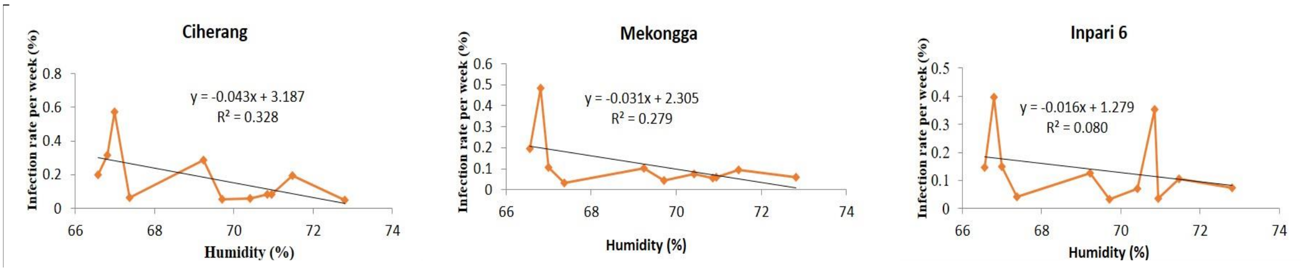

Figure 5. Correlation between air humidity and infection rate of leaf spot disease in Ciherang, Mekongga, Inpari 6 varieties cultivated in Linggang Bigung Sub-district, Kutai Barat District, East Kalimantan Province, Indonesia.

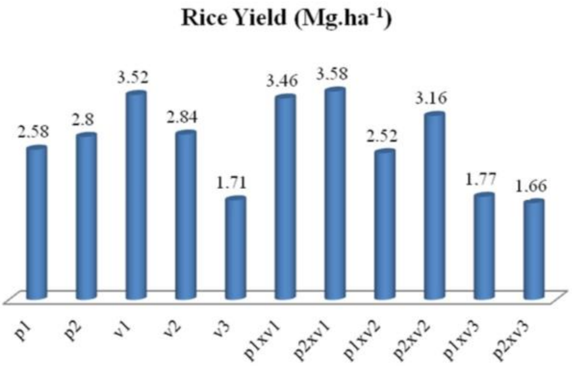

Figure 6. Rice yield due to the variety and fertilizer application associated with leaf spot pathogen attack from 56- 84 dat of observation. *p1*: 200 kg.ha^{-1} Urea, *p2*: 200 kg.ha^{-1} Urea + 200 kg.ha^{-1} NPK, *v1*: Ciherang, *v2*: Mekongga, *v3*: dan Inpari 6, and *p1xv1*, *p2xv1*, *p1xv2*, *p2xv2*, *p1xv3*, *p2xv2* are interaction between variety and fertilizer application treatment.

Air humidity and the correlation with disease infection intensity

In the concept of triangle disease, climate factors as physical environmental factors that greatly influence the occurrence of disease. Its influence on pathogens can pathogenic life cycle, virulence, transmission, and reproduction (Zeng et al. 2011). Air humidity greatly influences the development, growth, breeding, and reactivity of disease both directly and indirectly. The development of the disease is strongly influenced by the dynamics of climate factors. Relatively high humidity is a potential condition for the onset of disease. The occurrence of pathogenic infections is often determined by the humidity around the plantations, especially for fungal pathogens (Wiyono 2007). The air humidity in these two experimental sites was varied during the study. In Long Iram (altitude 14-16 m asl) air humidity was between 68-83% while in Linggang Bigung (altitude 19-21 m asl) was between 66-73%. The infection rates increased in air humidity from 76 to 78% in Long Iram, and in air humidity from 65 to 67% in Linggang Bigung. The results of the correlation analysis (r) showed that the correlation between air humidity and the rate of infection of rice leaf spot disease in Long Iram was in Ciherang variety r = -0.0092, Mekongga r = 0.2485, and Inpari 6 r = 0.0696 (Figure 4). Furthermore, the correlation between air humidity and the rate of infection of rice leaf spot disease in Linggang Bigung was in Ciherang variety r = -0.5731, Mekongga r = -0.5288, and Inpari 6 r = -0.2843 (Figure 5). These results indicate that the increasing humidity was not always accompanied by an increase in the infection rate of leaf spot disease.

Stomatal number and density

The observations of the stomata density showed that there were 230, 182 and 236 stomata per mm in Ciherang, Mekongga and Inpari6 varieties, respectively. The previous observation concerning the leaf spot disease showed that the highest infection intensity of leaf spot disease was observed in Inpari 6, whereas the lowest intensity was observed in the Ciherang followed by Mekongga varieties. The greater the number of stomata, the greater the possibility of pathogen penetration as showed in Seraiwangi plant tissue (Idris and Nurmansyah 2016). Plant resistance against leaf spot disease is associated with low stomata density. Our results were in line with the study of Pradana et al. (2017), which reported that the higher the density of stomata in the leaves, the more susceptible to penetration or infection of pathogens. Pathogens can penetrate through natural holes in plant, one of which is stomata. The number of stomata can be used as an indicator of plant resistance to a disease, the higher the number of stomata on the leaves, the higher the penetration and pathogen infection to leaf tissue. The fact that the Ciherang variety had high stomatal density, but was more resistant to leaf spot disease is contradictive with the previous study. Therefore, in this case, the stomatal density cannot always be used as an indicator of plant resistance to leaf spot disease. The narrow opening of the stomata acts as a structural defense mechanism (Yudiwanti 2007). Stomata openings are the main route of entry of pathogens into plants and plants have evolved a mechanism to regulate stomata openings as an immune response to bacterial invasion. Closure of the stomata causes fewer pathogens to enter the plant, thus having a negative impact on pathogenesis (Zeng et al. 2011).

Rice yields (Mg.ha^{-1})

The results of the analysis of variance on rice yield due to the interaction effects of varieties and fertilizer application showed a significant effect. The rice production yield is presented in Figure 6. If there is an association between the rice yield with the intensity of leaf spot disease, the low intensity of the disease will produce high yield as observed in Ciherang variety. Conversely, if the high intensity of the disease will produce low rice yield as shown in Inpari 6 variety.

Fertilizer treatment has a significant effect on rice production, but not significantly on the level of disease intensity both in the vegetative and generative phases. Taufik (2011) stated that in generative phase, the resistance response of rice plants varies but there is a tendency that varieties resistant or vulnerable in the vegetative phase also tend to be more resistant or vulnerable at the generative level. The intensity of leaf spot disease was higher the treatment of (*p1*) 200 $kg.ha^{-1}$ Urea compared to 200 $kg.ha^{-1}$ Urea + 200 $kg.ha^{-1}$ NPK fertilizer. This is comparable for rice yield, where the rice yield at the treatment of 200 $kg.ha^{-1}$ Urea fertilizer is lower (2.58 Mg. ha^{-1}) compared to at the treatment 200 $kg.ha^{-1}$ Urea +200 $kg.ha^{-1}$ NPK fertilizer (2.8 $Mg.ha^{-1}$). Walkey (1985) and Dewi et al. (2013) stated that the resistance of plants tolerant to diseases, in general, has good growth and can compensate the level of pathogenic infections to produce high yield. According to Agrios (2005), tolerance or resistance is a trait that can be inherited, these traits allow pathogens to develop and multiply within the host while the host does not have receptor parts to activate toxic substances released by pathogens, so that plants are still able to produce.

In conclusion, from the study, we can see that the resistance of rice against leaf spot diseases is strongly influenced by the varieties. Ciherang variety is the most resistant to the leaf spot disease in the study site at 7- 49 days after planting of observation. More than one pathogenic fungus was identified to cause leaf spot diseases of rice plants cultivated in the study sites. There is a positive correlation between the humidity and the rate of infection of leaf spot disease. Stomata density is not always to be an indicator of plant resistance to diseases. Varied interaction was observed between varieties and fertilization on rice yield. The highest yield was produced by Ciherang variety with fertilizing of 200 $kg.ha^{-1}$ Urea + 200 $kg.ha^{-1}$ NPK (3.58 Mg.ha-1).

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ASIAN JOURNAL OF AGRICULTURE
Volume 3, Number 2, December 2019 E-ISSN: 2580-4537
Pages: 47-54 DOI: 10.13057/asianjagric/g030203

Potential of *Pseudomonas stutzeri* strains isolated from rhizospheric soil endowed with antifungal activities against phytopathogenic fungus *Stemphylium botryosum*

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Manuscript received: 20 September 2019. Revision accepted: 21 October 2019.

Abstract. *Mokrani S, Bejaoui B, Belabid L, Nabti E. 2019. Potential of* Pseudomonas stutzeri strains *isolated from rhizospheric soil endowed with antifungal activities against phytopathogenic fungus* Stemphylium botryosum. *Asian J Agric 3: 47-54.* In this study, two *Pseudomonas* strains P4 and P5 were isolated from rhizospheric soil and characterized for PGP (Plant Growth Promoting) traits production like HCN (hydrogen cyanide), siderophores and IAA (Indole Acetic Acid). A phylogenic tree based on 16S DNAr identification-related the two strains P4 and P5 to *Pseudomonas stutzeri* NR 116489 and NR 113652.1. One phytopathogenic fungus St-bt (*Stemphylium botryosum*) was isolated from *Phaseolus vulgaris* L. Macroscopic and microscopic identification attributed it to the genus *Stemphylium*. Antifungal activities of the two *Pseudomonas* strains P4 and P5 against fungus isolate St-bt had revealed very highly significant inhibition percentages of 38.46± 3.85% and 56.56± 2.22% for each strain, respectively.

Keywords: Antifungal activity, PGP traits, *Pseudomonas stutzeri*, *Stemphylium botryosum*

INTRODUCTION

Agriculture is the groundwork for food industries on a global scale. Sustainable agriculture strives to continue improving without compromising the environment (Gimenez et al. 2018). It is described as a system protecting manufacturing in the long conduct, except degrading of the natural properties, including the useful resources of the low-input applied sciences utilization (Altieri 1989; Saleh et al. 2017). On different sides, contemporary agriculture faces the extensive assignment of supplying a world population within continuous increase with food, at the same time as the natural sources augment to remain equal (González de Molina et al. 2017). Especially with abiotic stress prerequisites such as drought, heat, and salinity causing big losses in conformity with agricultural production worldwide. Also, the environment heterogenic conditions and world climatic changes are only a few of the challenges facing modern agriculture (Mittler and Blumwald 2010).

Furthermore, biotic stress is described as caused by plant living microorganisms that comprise fungi, bacteria, viruses, parasites, weeds, insects, and distinctive native and cultivated plants (Newton et al. 2011). Although fungal phytopathogens are considered causal agents on primary plant diseases, they can infect a range of plant components. This can include roots, stems, leaves, vegetation, and fruits; inducing characteristic visible symptoms as spots, blights, anthracnose, wilts, rots, etc. (Thilagam et al. 2018). In addition, microbial diseases caused about 16% of the loss of annual manufactured tonnage worldwide at the start of the 21st century. 70-80% of these losses have been caused by fungi (Moore et al. 2011). To face all its constraints, chemical fungicides are extensively used for many plant disease treatments. Whereas these products can cause environmental pollution and disastrous effects on human health (Agrios 1988). The intensive uses of certain chemical compounds have influenced the improved resistance of the targeted organisms against certain chemicals (Goldman et al. 1994). However, biological control may remain a promising alternative in imitation of chemical elements to manage phytopathogenic fungi (Wallace et al. 2017; Tagele et al. 2018) and afford attractive dietary supplements among methods due to the limited environmental pollution (Compant et al. 2005; Nogórska et al. 2007).

The biological control entails the utilization of salutary organisms, their genes and/or gene products, such as metabolites that lessen the negative effect of plant pathogens and promote high-quality plant products (Vinale et al. 2008). Several microorganisms such as bacteria, fungi, viruses, algae, and protozoa have been recommended as individual or combined effective biopesticides (Chauhan et al. 2018).

The microbe can control a variety of pests (Nobutaka 2008), particularly a wide range of bacterial genera that can be used as biocontrol agents PGPR (Plant Growth Promoting Rhizobacteria) such as *Azospirillum*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Burkholderia*, *Enterobacteria*, *Pseudomonas*, *Rhizobium* and *Serratia* (Goswami et al. 2016). Among these bacteria, *Pseudomonas* spp. are aerobic, gram-negative bacteria, ubiquitous in agricultural soils, and adapted in broad range growing area in the rhizosphere (Weller 2007). *Pseudomonas* possesses many traits to make them suitable for biocontrol and growth-promoting agents (Wei et al. 1996; Morrissey et al. 2004a; Stockwell and Stack 2007; Mishra and Arora 2017). The current study aimed to characterize the antifungal activities of two *Pseudomonas* strains endowed with PGPR characteristics against some phytopathogenic fungus isolate St-bt.

MATERIALS AND METHODS

Origin of *Pseudomonas* strains

In a previous study (Mokrani et al. 2019) two *Pseudomonas* strains P4 and P5 were isolated in 2010 from rhizospheric soil of *Phaseolus vulgaris* L from Tighanif Mascara (35°24' N 0°19'E). The strains were stored in TSB (Tryptic Soy Broth) broth supplemented with 25% glycerol by sub-culturing every 12-18 months.

Phylogenic identification of *Pseudomonas* strains

DNA extraction and PCR amplification

Genomic DNA of the two strains, P4 and P5, were extracted using the technique of phenol/chloroform/ isoamyl alcohol according to (William et al. 2012). PCR amplifications were performed using the following primers: Amorce Formed 16S Forward (5'-AGA GTT TGA TCC TGG CTC AG-3') and Amorce reverse 16S Reverse (5'-CTA CGG CTA CCT TGT TAC GA-3') for 16S rRNA gene. 35 cycles were realized with the following conditions: preheating at 94°C for 3 min; denaturation at 94°C for 45 sec; hybridization at 55°C for 1 min; elongation at 72°C for 2 min; final elongation at 72°C for 7 min and final cooling at 4°C for 7 min (Mokrani et al. 2018a).

16S DNAr sequencing

Partial 16S DNAr was compared with the nucleotide sequences from international databases using BLAST (Basic Local Alignment Search Tool Program). The alignments of the nucleotide sequences were conducted out by using the Clustal W algorithm. The phylogenetic tree was obtained by MEGA 7 software program and the neighbor-joining algorithm. Bootstrap values have been determined from 100 replicates.

PGPR analysis

Production of HCN

Hydrogen cyanide (HCN) production was carried out as described by Lorck (1948). Bacterial strains were streaked on nutrient agar supplemented with 0.44% L-glycine. The change of color from yellow to light brown or red-brown indicated a moderate or high production of HCN, respectively (Trivedi et al. 2008).

Siderophores synthesis

Siderophores determination was carried out by qualitative detection on a Chrome Azurol-S (CAS) agar medium. *Pseudomonas* strains were streaked on Petri dish plates containing CAS medium and incubated at 30◦C/48 h. Formation of orange halos surrounding colonies indicated siderophore production (Yeole et al. 2001).

IAA production

Qualitative IAA production. Bacterial strains were examined to produce Indole Acetic Acid (IAA) by the technique described by Bric et al. (1991). Bacterial strains were inoculated in Petri dish plates containing Luria Bertani (LB) medium supplemented with 5 mM L-tryptophan. Production of IAA and/or its analogs in the medium manifested by leading to a red-pink color. For other indoles, color produced was yellow or brown-yellow.

IAA chromatography. To confirm the IA chromatography of IAA extracts was done according to the technique reported by (Kuang-Ren et al. 2003, modified). *Pseudomonas* strains were cultured on TSB (Tryptic Soy Broth) and incubated at 30◦C/24 h. After incubation, centrifugation was carried out and the supernatants were recuperated and mixed with ethyl acetate (1:2). After vigorous stirring, the partial extracts of the solvent layer were allowed to rest for 10 min. The procedure was repeated 3 to-4 times. Then, IAA chromatography was executed using dissolvent system propanol: water (8:2). Chromatogram of two IAA extracted samples and control (10 mg/100 mL about IAA) were developed with Salkowski's reagent.

Fungus isolation and identification

The phytopathogenic fungal isolate St-bt was isolated from *P. vulgaris*, and planted under a greenhouse in Tighanif Mascara (35◦24' N 0◦19'E). Small slices of leaves of an infected plant showing characteristic symptoms were cut sterilely. The slices were then placed on Petri dishes containing a PDA (Potato Dextrose Agar) medium followed by incubation at 25◦C/5-7 days (Grewal and Jhooty 1984). The purification of the fungal isolate was performed by successive culturing 2-3 times of mycelium on a new PDA medium. The St-bt isolate was identified by observation of the macroscopic and microscopic aspects (Terbeche 2011). Colonies of the isolate St-bt obtained on PDA medium after incubation at 25 C°/7 days were examined for their macroscopic aspects like perception, colonies border, and color (Lecellier 2013).

The microscopic observation was carried out for the appearance of the mycelium and the prospective presence of specific forms of reproduction using the technique reported by Su et al (2012). The method consisted of observation of a part of the mycelium taken from a PDA agar culture at 25°C/7days under an optical microscope (10x and 40x). The activation of sporulation of the St-bt isolate was stimulated by the application of UV light for 16 h at room temperature followed by incubation in the darkness at 25 °C from 3 to 5 days. The identification of the isolate St-bt was then performed using the keys proposed by Pitt and Hocking (2009); Hernandez-Perez and du Toit (2000) for the identification of species belonging to the genus *Stemphylium*.

Pathogenicity test

The pathogenicity test was reproduced on *Phaseolus vulgris* L plants by the modified method reported by Ben Hassena (2009). 50 mL of distilled water was added to an old fungal culture of 10 to 15 days on PDA medium. Then, the surface was thoroughly scraped and mixed to release the sporulated forms. The suspension containing (spores + mycelium) was used for contamination by pouring into a pot containing a *P. vulgaris* plant for 2-3 weeks. The onset of symptoms was taken within one week after contamination.

Antifungal activity

Antagonist activity of the *Pseudomonas* strains against the isolate St-bt was carried out by the method of dual culture described by Landa et al (1997). Evaluation of the antifungal activity was estimated by calculation of the percent inhibition of the mycelium growth according to the following formula:

$$PI = \frac{(\text{r control} - \text{r test})}{\text{r control}} \times 100$$

Where: PI: percent inhibition;

r test: maximum radial distance of fungus on a line towards the antagonist bacteria in dual culture;

r control: maximum radial distance of fungus inoculated in the center of petri plate).

Statistical analysis

Results of antifungal activity were represented as the percent inhibition means±SD (standard deviation). Those percentages were then analyzed using one-way variance analysis with a significance level of $p<0.05$. Similarly, when significant differences were found, multiple comparisons were performed using Dennett's test.

RESULTS AND DISCUSSION

Microorganisms growing in the rhizosphere, particularly *Pseudomonas* spp., are ideal for use as biocontrol agents of many bacterial and fungal plant diseases. They are known for their high diversity, colonization capacity, plant growth traits involved in stimulation or inhibition plant growth and suppression of different plant pathogens.

Phylogenic identification of *Pseudomonas* strains

Phylogenic identification of the two *Pseudomonas* strains, P4 and P5, was completed by 16S DNAr identification. Agarose gel electrophoresis revealed two clear bands of 1500 bp (Figure 1). This showed that the conserved regions of the 16S rRNA gene were amplified by the SF and SR primer pair**.** The phylogenetic analysis related to the two *Pseudomonas* strains (100%) to *Pseudomonas stutzeri* NR 116489 and NR 113652.1 was presented (Figure 2).

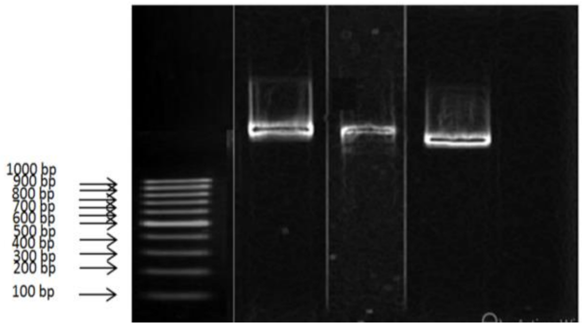


Figure 1. 16SDNAr gene PCR amplicons on 1.5% agarose gel. DNA bands of about 1500 bp were obtained from the two *Pseudomonas* strains after 16S RNAr gene PCR. A. SM: size marker; B. Strains P4; C. Strain P5; D. Positive control established by 16S DNAr of strain P25 *Pseudomonas grimontii* isolated from *Vicia sativa* (Mokrani et al. 2019); E. Negative control

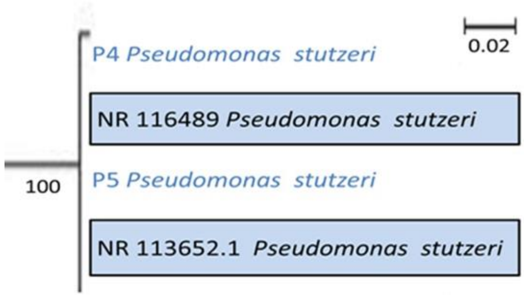


Figure 2 Neighbor-Joining tree obtained using MEGA7, revealing the phylogenetic relationship of the *Pseudomonas* strains. Bar indicates 2% sequence divergence

The molecular identification via 16S DNAr of the two *Pseudomonas* strains attributed them to *Pseudomonas stutzeri* species. The genus *Pseudomonas*, is one of the most varied and ecologically widespread groups of microorganisms on the planet (Spiers et al. 2000). Phylogenetic affiliation of different *Pseudomonas* sp based on 16S rRNA gene suggests that molecular divergence contributes to the genetic range of *Pseudomonas* sp. The strain determination performed on 16S rRNA gene was once related to the nucleotide diversity of *Pseudomonas* sp in soil rhizosphere community amongst distinct agricultural vegetation (Adhikari et al. 2015). *P. stutzeri* is a non-fluorescent bacterium widely distributed in the environment. Over the last few years, many improvements have been made in elucidating the taxonomy of this variety of taxonomical groups, demonstrating the clonality of its populations. The species has obtained a lot of interest due to the fact of its unique metabolic properties (Lalucat et al. 2006). Furthermore, strains of *P. stutzeri* have structured in at least seven DNA-DNA similarity groups known as genomovars (Ursing et al. 1995). Confirmation of this system of internal subdivisions was once said following different methods to bacterial phylogeny, which included 16S rRNA gene sequencing (Bennasar et al. 1996). Two individuals of the same genomovar share increased than 70% DNA-DNA similarity, the common species threshold, and these values are reduced and typically much less than 50% when individuals of distinct genomovars are compared (Rosselló et al. 1991).

Production of HCN

Pseudomonas strains are known to synthesize a large variety of antibiotics that act effectively in controlling plant pathogens. From those, cyanide hydrogen is the most volatile antibiotic largely described as *Pseudomonas* biocontrol agents. Evaluation of the capacity of the two *P. stutzeri* stains to produce hydrogen cyanide, revealed a qualitative change of Whatman paper N°1. Color changes from yellow to reddish or reddish-brown confirmed HCN production (Figure 3).

Soil microorganisms make contributions to soil fertility, soil structure, biodiversity and have a few PGP traits (Barea et al. 1996). One secondary metabolite produced commonly through rhizosphere *Pseudomonads* is Hydrogen Cyanide (HCN), a gas identified to negatively have an impact on root metabolism and root extension (Alström and Burns 1989; Schippers et al. 1990). This suggests that cyanogenesis, by way of rhizobacteria in the rhizosphere, can adversely influence plant growth (Kremer and Souissi 2001). The degree of HCN produced by means of the use of the rhizobacteria *in vitro* does not correlate with the determined biocontrol effects, as a result disproving the biocontrol hypothesis. But alternatively, is implicated in geochemical procedures in the substrate, indirectly developing the availability of phosphate (Rijavec and Lapanje 2016). Also, the function of HCN manufacturing by using *Pseudomonas fluorescens* strain CHA0 as an antagonistic aspect that contributes to biocontrol of *Meloidogyne javanica*, the root-knot nematode, in situ (Siddiqui et al. 2006).

Siderophores synthesis

It has been suggested that the siderophore-mediated competition for iron with soil-borne pathogens is an important mechanism for biological control. Qualitative production of siderophores on CAS agar media by the two *P. stutzeri* strains, showed appearance of characteristic orange halos surrounding growth. This indicated Fe chelation and revealed siderophores production (Figure 4).

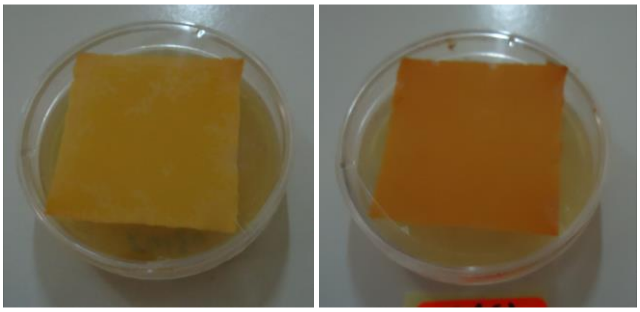
To facilitate iron (III) acquisition, plants and microorganisms, such as fungi and bacteria, produce and excrete strong iron (III) chelators called siderophores (Neilands 1984). Important groups of siderophores consist of hydroxamate siderophores, catecholate (phenolates) siderophores and carboxylate siderophores (Pattan et al. 2017). Various species amongst non-fluorescent Pseudomonas group are capable to produce siderophores (Meyer et al. 2002; Bultreys et al. 2001). The *P. stutzeri* strain CCUG 36651, was once shown to produce ferrioxamine E (nocardamine) as the major siderophore. This collectively with ferrioxamine G and two cyclic ferrioxamines having molecular masses 14 and 28 atomic mass units decrease than that of ferrioxamine E, suggested to be ferrioxamine D2 and ferrioxamine X1, respectively (Essén et al. 2007). Furthermore, siderophores act as a possible biocontrol agent in opposition to negative phytopathogens and hold the potential to alternative hazardous pesticides (Saha et al. 2016).

IAA production

Indole acetic acid is one of the most physiologically active and important auxins regulating a range of plant cellular and physiological processes. Qualitative determination of the indole acetic acid revealed that the two strains produced IAA on Luria-Bertani agar supplemented with 5 mm L-tryptophan. The color obtained was pink-red for the two strains, signifying a moderate IAA production (Figure 5).

Production of IAA was confirmed by paper chromatography compared with the authentic control (10 mg/mL). After revelation with Salkowski's reagent, chromatogram of IAA extracts showed two pinkish spots (R_f =0.53) corresponding to the authentic IAA (Figure 6).

Indole acetic acid production is another essential PGPR trait implicated directly in stimulation of plant increase. IAA is a major property of rhizosphere bacteria that plays a main function in plant growth and development; specifically in modifying cell division, cell differentiation,, cell expansion, and lateral root formation (Pant and Agrawal 2014). Equally, IAA incites in the production of longer roots with multiplied wide variety of root hairs and root laterals which are involved in nutrient uptake (Datta et al. 2000).



A B

Figure 3. Qualitative production of hydrogen cyanide by *P. stutzeri* strains on agar medium (a: weak reddish color for strain P4 indicating low production; b: reddish-brown color for strain P5 meaning moderate production)

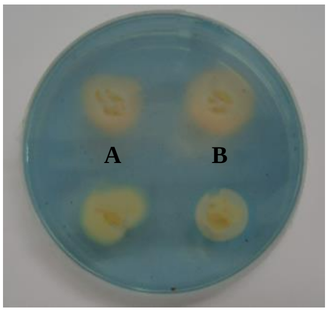
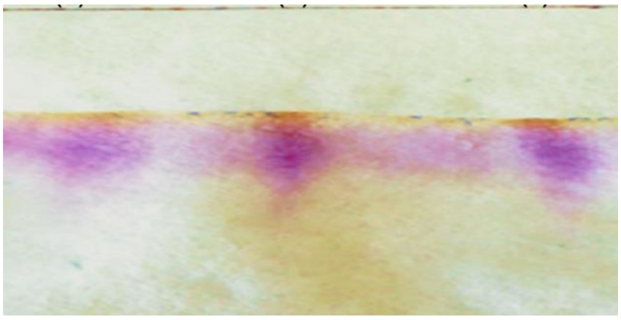


Figure 4. Qualitative productions of siderophores by *Pseudomonas stutzeri* strains on CAS-medium (a: small orange halo surround growth for strain P4 meaning low production; b: large orange halo surround colonies for strain P5 revealing moderate production)

Figure 5. Production of indole acetic acid by strain P5 *Pseudomonas stutzeri* (red color reflecting moderate IAA production)



A **B** **C**

Figure 6. IAA chromatogram. A. Control; B. IAA extract of strain P4; C. IAA extract of strain P5

Also, it stimulates cell elongation via enhancing positive conditions like: extend in osmotic contents of the cell, rising in permeability of water into cell, limiting in wall pressure, amplifying in cell wall synthesis, inducing RXA, and protein synthesis. Furthermore, IAA enhances embial activity, inhibits or prolongs abscission of leaves, induces flowering and fruiting (Zhao 2010).

Isolation and identification of fungus

Isolation of fungus

The fungus isolates St-bt was isolated from *P. vulgaris* showing leaf blight disease symptoms. Characteristic small yellow-brown tasks were observed on the leaves area (Figure 7).

In this study, isolation of fungal diseases occurring in *P. vulgaris* cultures revealed fungus St-bt showing characteristic symptoms of leaf blight disease. Riley et al (2002) had reported that control measures of plan pathogens depend on proper identification of diseases and the causal agents. Therefore, diagnosis is one of the most important aspects of a plant pathologist's training. It includes identification of affected plants, recognizing healthy plant appearance, identifying attribute symptoms, and checking distribution of symptoms.

Figure 7. Leaf blight caused by fungus isolate St-bt on *Phaseolus vulgaris* leaves

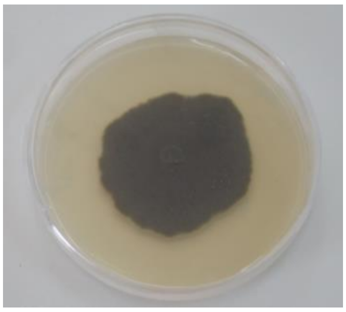


Figure 8. Macroscopic aspect of isolate St-bt on PDA medium

Macroscopic and microscopic identification

After isolation on PDA medium, fungus isolate St-bt revealed characteristic black colonies surrounded with white color in the edge (Figure 8). Furthermore, colonies were velvety with irregular shape, black in the revers, marked by less speed growth forming colony of 26 mm at the end of 7 days of incubation.

Microscopic examination of isolate St-bt showed mycelium characterized by ramified septa, typical mature and immature conidia. Evenly, conidia were always isolated and never in chains, an essential distinguishing feature between genera *Alternaria* and *Stemhyllium* (Figure 9*)*. Immature conidia were ovate to cylindrical, brown with one to two transverse septa. Mature conidia were dark brown cylindrical, with a pointed apex; they assume cottony longitudinal and transverse septations with an irregular outline.

In this present study, macroscopic and microscopic identification of the isolate St-bt had attributed it to *Stemphylium botryosum*. Hosen et al (2009) mentioned that the colonies of *S. botryosum* on PDA medium are velvety to cottony in texture with a grey, brown, or brownish-black color colony. Also, conidia are colored and septated were determined amongst *S. botryosum* strains (Caudillo-Ruiz et al. 2017). Furthermore, Boutkhil (2012) had reported that *S. botryosum* is a frequent fungus isolated from bean cultures. The fungus *Stemphylium botryosum* has been suggested to be very widespread in temperate and subtropical regions, where it is established in a wide range of economically essential vegetation (Mwakutuya 2006).

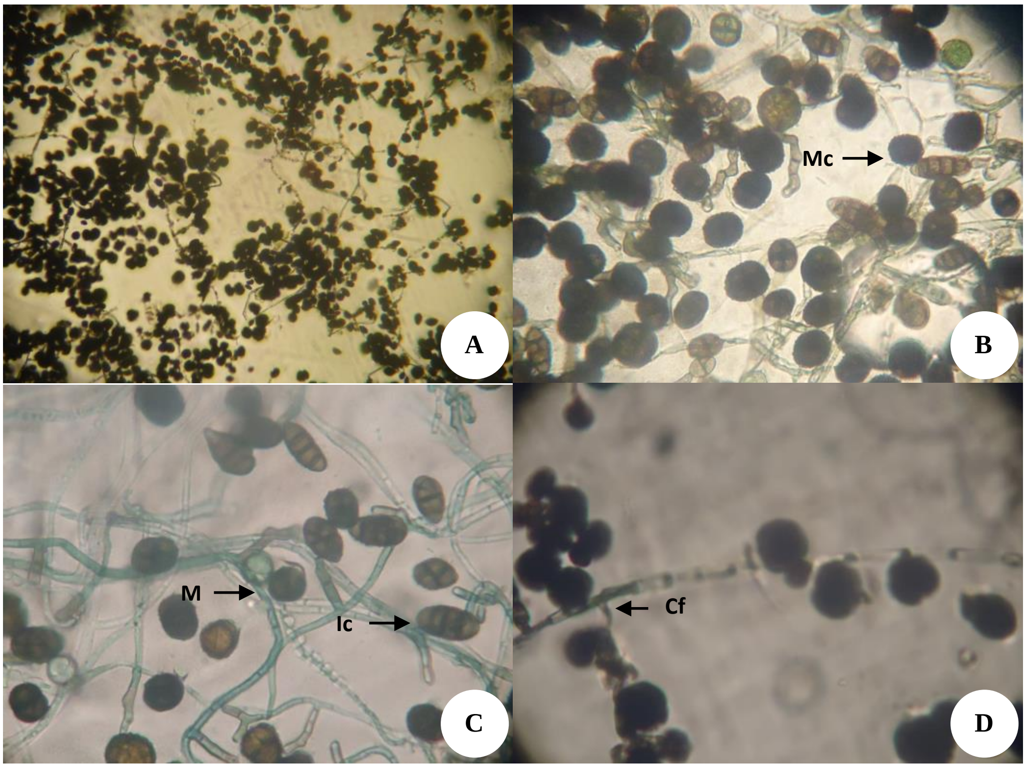


Figure 9. Microscopic observation of isolate St-bt (A and B: showed abundant spores after activation of fungus culture by UV light for 16 h at room temperature (10x) and (40x), respectively; C: plentiful mycelium without culture activation (40x); D: indicated conidiophore (Cf) (40x); Ic: immature conidia; Mc: mature conidia; M: Mycelium

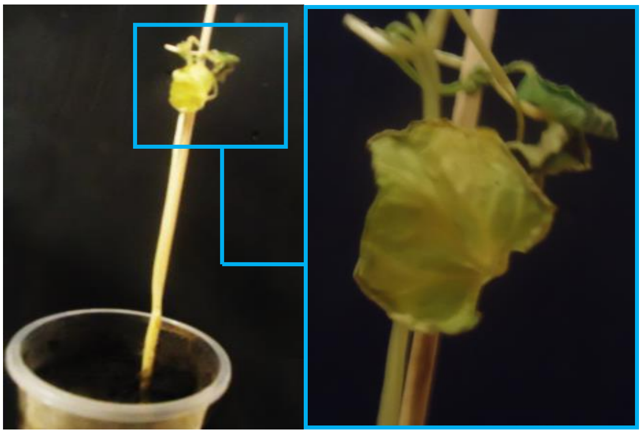


Figure 10. Pathogenicity test of isolate St-bt *Stemphylim botryosum* on *Phaseolus vulgaris* (A and B: characteristic symptom indicating yellow-brown tasks of the infected leaves)

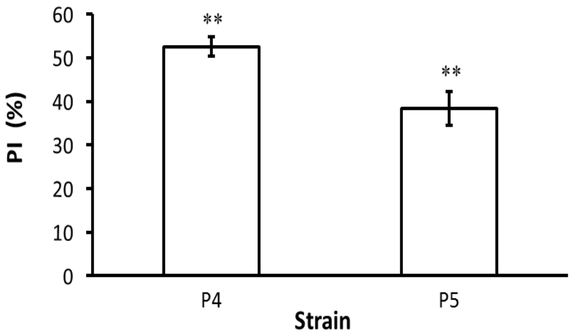


Figure 11. Antifungal activities of *Pseudomonas stutzeri* strains against isolate St-bt (PI: percent inhibition; **: very significant difference at p<0.05).

Pathogenicity test

The purpose of the pathogenicity test was to reproduce disease symptoms observed on *P. vulgaris* leaves a moment of isolation to confirm the pathogenicity of the isolate St-bt. This test revealed symptomatic stemphyliose leaf blight disease. It was characterized by yellow-brown tasks observed throughout the leaves area (Figure 10).

In conclusion, current findings led to conclude that *P. stutzeri* strains are potent antifungal agents against isolate St-bt *in vitro* trials. Thus, by producing effective PGP traits like HCN and siderophores involved in the pathogen suppression. However, further evaluation of the effectiveness of strains P4 and P5 for biological control of bean leaf blight caused by isolated St-bt on-field culture and the design formulations and applications should be done in the field.

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ASIAN JOURNAL OF AGRICULTURE
Volume 3, Number 2, December 2019
Pages: 55-62
E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g030204

Diversity of diseases of rice (*Oryza sativa*) in Kutai Kartanegara, Indonesia

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Manuscript received: 19 August 2019. Revision accepted: 29 October 2019.

Abstract. *Sopialena, Sofian, Nurdiana J. 2019. Diversity of diseases of rice* (Oryza sativa) *in Kutai Kartanegara, Indonesia. Asian J Agric 3: 55-62.* This research aimed to identify the diversity of diseases that are becoming the main threat to four paddy varieties in Kutai Kartanegara, Indonesia. Though similar studies have been conducted, to our knowledge, this research brings significant findings related to the diverse categories of plant diseases in the specific geographical area. The study was performed in Sidomulyo village, Anggana district using a purposive sampling method. From the selected paddy fields in the size of 2x2 square meters, the samples then were identified. Further analysis was performed on every suspected infected part of the plants. The results showed that there are five dominant plant diseases found, i.e., Blast disease, brown spot, narrow brown spot, false smut, and Sheath blight. Whereas the main cause of the diseases recorded are fungus and bacteria, i.e., *Pyricularia grisae* (Cke) Sacc., *Cercospora oryzae* Miyake., *Rhizoctonia solani* Kuhn., *Helminthosporium oryzae* L., *Ustilaginoidea virens* (Cke) Tak.), and *Xanthomonas campestris* pv. *oryzae* Dye.

Keywords: Bacteria, diseases, fungus, rice

INTRODUCTION

Rice is one of the worlds' primary staple foods and provides food security for many countries (Remans et al. 2014; Hafiz et al. 2015; Zhang et al. 2017; Chang et al. 2018), including for Indonesians (Widyanti et al. 2014). However, there is still a shortage of rice production (Bantacut 2014). One of the main reasons for this issue is the reduced harvested area and thus the decrease in productivity, which mainly happened in Kutai Kartanegara and Berau regencies (Badan Pusat Statistik 2016). In 2015, rice production reached 408.78 thousand tons of dry grain (GKG), but reduced by 17.78 thousand tons compared to 2014 (Badan Pusat Statistik 2016). Therefore, it is necessary to identify the possibility of other potential lands that can be expanded into crop-based agriculture land, e.g., tidal water rice fields (Mareza et al. 2016; Setyo and Elly 2018). Besides tidal fields, agroecosystems can be identified as irrigated rice fields, rain-fed rice fields, deepwater rice fields and upland rice fields (Jayanthi et al. 2006). In East Kalimantan alone, tidal areas can be found in many places, including at Sidomulyo Village, Anggana Sub-district, Kutai Kartanegara District at about 700 ha. (Dinas Pangan, Tanaman Pangan dan Hortikultura 2017). From the existing land area, most of them have been used the paddy varieties such as Ciherang, Cimelati, and Mekongga (Susilastuti 2018). Though the use of plant-based pesticides is common among farmers (Sopialena et al. 2018b), the pathogenic microorganisms seem to be potential problems still. In this regard, this study found that Pandan Ungu variety is rarely used due to its susceptible characteristics to pathogens.

The types of plant-disturbing organisms in host plants may vary (Saunders et al. 2010; Suyadi et al. 2017; Sopialena et al. 2018). In East Kalimantan, the most common pathogens experienced are *blast* (*Pyricularia grisae* (Cke) Sacc.), *hawar daun bakteri* (*Xanthomonas campestris* pv. *oryzae* Dye.), *bercak daun coklat* (*Helminthosporium oryzae* L.), and *tungro* (Sopialena and Pratiwi 2017). Disease can lead to crop failure and may attack both the vegetative and generative phases of the root, leaves, stems, or panicles of rice plants (Rodriguez et al. 2009; Gao et al. 2010; Ariyanto et al. 2013). The blast disease (*Pyricularia grisae* (Cke) Sacc.) may lead to death for rice, nursing plants, and the decrease of the quality of grain; which is mainly caused by the *H. oryzae*, whereas *hawar pelepah* or *upih* disease may cause the seeds failed to germinate (Kihoro et al. 2013; Sopialena and Pratiwi 2017). Also, there are other diseases caused by pathogens that are harmful to the plants, such as false smut disease and sheath blight (Sopialena and Pratiwi 2017). The need to fulfill the rice production and the potential of tidal land, especially in the village of Sidomulyo, Kutai Kartanegara, became the central issue for this research. Further, this identification provides a broader picture of how to combine the planting management (e.g., the use of paddy varieties) within the geographical characteristics (Stringer et al. 2016).

This study was intended to enrich the discussion on the type of diseases at different local paddy varieties for tidal fields, in particular in the area of East Kalimantan, Indonesia.

MATERIALS AND METHODS

This research was conducted in tidal rice fields at Sidomulyo Village, Anggana Sub-district, Kutai Kartanegara District, East Kalimantan Province, Indonesia. It has climatic conditions of an average rainfall of 2051.50 m asl., a temperature of 26.80ºC and relative humidity of about 60–80%. The tidal rice fields are known as the lands that experience the overflow of tidal water in a specified period. The research was conducted in the cropping areas of 1 ha crop, and the area was further divided into 50 m x 12 m. The information was collected using purposive sampling based on specific criteria, i.e., the identification of infected plants, both in vegetative and generative phases. Direct observations were performed to identify the symptoms of the diseases in four different local paddy varieties (i.e., Pandan ungu, Cimelati, Mekongga, Cigeulis). In the area of paddy tile (*ubinan*) with a size of 2x2 square meter, for each suspected part of the plants (leaf and stem) which were infected, they were cut to about 5-10 cm and analyzed in the laboratory.

To identify diseases caused by fungus, the samples of the plants were cut into small pieces first and sterilized using aquadest before soaking in a 70% alcohol solution for about 15-20 seconds. The next step was to dry the sample over the tissue, before transferring it into a cup that has contained Potato Dextrose Agar (PDA) media using nippers and left to isolate for 2-3 days. An ose of the needle and a methylene blue liquid was used to examine the growth of the fungal colonies. On the other hand, the identification of suspected diseases is because the bacteria is made through bacterial isolation to see the colony's morphological features, colonic features, bacterial cell shape, and colony color (Saunders et al. 2010). The isolation of the bacteria taken from the infected plants was conducted by preparing the media on Petri cultivation for some time waiting for it to be cool and stable.

For leaf spot or stem spot, the isolation is carried out by bringing the leaf or stem part into a plastic bag and wet cotton to keep the material from wilting. Furthermore, the diseased material was cleaned with alcohol and cut in the section between the leaves or the stems of the sick and healthy, then grown on the PDA agar media. They were then incubated until the pathogen grew and both colonies and microbes that developed were identified under a microscope. For rust disease, identification was carried out by directly scraping the rust on the rice leaf and looking directly under a microscope.

RESULTS AND DISCUSSION

The findings

The observations in the vegetative phase were performed when the plants were about 1-8 weeks, while the generative phase was conducted when the plants reached 9-14 weeks after planting. The information on the type of diseases at different paddy varieties in vegetative and generative phases was presented in Table 1.

Diseases in the vegetative phase

The results of the identification process to the paddy varieties of Cimelati, Cigeulis, Pandan ungu, and Mekongga, indicated that the types of main potential diseases in the vegetative phase could be classified into three types, i.e., *hawar daun bakteri, blast,* and *bercak coklat.* As presented in Figure 1.A, *hawar daun* disease is caused by the infection of *Xanthomonas campestris* pv. *oryzae* Dye. It begins with the appearance of gray (yellowish) spots on the leaf edge. In its development, the symptoms will expand, forming blight, and eventually cause the leaves to dry out. This disease also attacks Cimelati, Cigeulis, and Mekongga varieties.

Table 1. The identification of rice diseases in different paddy varieties.

Variety	Growth phases	
	Vegetative	Generative
	1-8 weeks after plantation	9-14 weeks after plantation
Cimelati	*Hawar daun bakteri* (*Xanthomonas campestris* pv.*oryzae* Dye.) Blast (*Pyricularia grisae* (Cke) Sacc.) *Bercak coklat* (*Helminthosporium oryzae* L.)	*Hawar daun bakteri* (*Xanthomonas campestris* pv.*oryzae* Dye.) *Bercak coklat* (*Helminthosporium oryzae* L.) Blast (*Pyricularia grisae* (Cke) Sacc.) *Gosong palsu* (*Ustilaginoidea virens* (Cke) Tak.)
Cigeulis	*Hawar daun bakteri* (*Xanthomonas campestris* pv.*oryzae* Dye.) Blast (*Pyricularia grisae (Cke) Sacc.)*	*Hawar daun bakteri* (*Xanthomonas campestris* pv.*oryzae* Dye.) *Bercak coklat* (*Helminthosporium oryzae* L.) Blast (*Pyricularia grisae* (Cke) Sacc.) *Hawar pelepah or upih* (*Rhizoctonia solani* Khun.) *Bercak daun sempit* (*Cercospora oryzae* Miyake.)
Pandan ungu	*Bercak coklat* (*Helminthosporium oryzae* L.) Blast (*Pyricularia grisae* (Cke) Sacc.)	*Hawar daun bakteri* (*Xanthomonas campestris* pv.*oryzae* Dye.) *Bercak coklat* (*Helminthosporium oryzae* L.) Blast (*Pyricularia grisae* (Cke) Sacc.) *Hawar pelepah/ upih* (*Rhizoctonia solani* Khun.)
Mekongga	*Hawar daun bakteri* (*Xanthomonas campestris* pv.*oryzae* Dye.) *Bercak coklat* (*Helminthosporium oryzae* L.) Blast (*Pyricularia grisae* (Cke) Sacc.)	*Hawar daun bakteri* (*Xanthomonas campestris* pv.*oryzae* Dye.) *Bercak coklat* (*Helminthosporium oryzae* L.) Blast (*Pyricularia grisae* (Cke) Sacc.) *Hawar pelepah or upih* (*Rhizoctonia solani* Khun.)

On the other hand, *Pyricularia grisae* (Cke) Sacc.*blast* caused blast infection (Figure 1.B). It started with the onset of symptoms on the leaves in the form of spots such as rhombus with a pointed tip. The center of the spots is gray or whitish, with a reddish-brown or brown edge. This disease attacks varieties Cimelati, Cigeulis, Pandan ungu, and Mekongga. The disease *H. oryzae* attacks the leaves of rice plants, with brown, oval-shaped brown spots spread evenly on the leaf surface with gray or white dots, gray or white dots in the middle of the spots. Young spots are dark brown or purplish-shaped size of sesame seeds. Different from *Xanthomonas campestris* pv. *oryzae* Dye, this disease attacks the Cimelati, Pandan ungu, and Mekongga varieties, as shown in Figure 1.C.

Disease in the generative phase

On the other hand, in the generative phase, there are at least six main different diseases that are identified (i.e., *Hawar daun bakteri, Blast, Bercak coklat, Gosong palsu, Hawar pelepah/upih,* and *Bercak daun sempit*). *Hawar daun bakteri* was indicated from the leaves that are colored gray to yellow, folded, and rolled. In a severe state, the entire leaf rolls, withers, and dies. Cimelati, Cigeulis, Pandan ungu, and Mekongga varieties are susceptible to this strike (Figure 2.A).

While the plants were affected by the blast (Figure 2.B-C), the symptoms were indicated by the appearance of shaped leaves like a rhombus and a pointed tip (blast leaves). The center of the spots are gray or white. It usually has brown or reddish-brown edges and heavily influences all the paddy varieties, i.e., Cimelati, Cigeulis, Pandan ungu, and Mekongga. Also, Figure 2.D shows the symptoms of *H. oryzae* which caused the leaves to have oval-shaped brown spots which spread evenly on the surface of leaves with gray or white dots. Similar to *Pyricularia grisae* (Cke) Sacc., *H. oryzae* may attack the four paddy varieties, i.e., Cimelati, Cigeulis, Pandan ungu, and Mekongga.

On the other hand, the symptoms of *Cercospora oryzae* Miyake began with the stricken leaves having narrow patches lengthwise, reddish-brown, parallel to the leaf bone, and commonly attacking the *Cigeulis* variety (Figure 3.A). Whereas, as shown in Figure 3.B, the infection of *Rhizoctonia solani* Kuhn on the plant as indicated by the spotted formation on a gray-shaped oval-gray midrib and then gray-white with a brown fringe. This disease was typically found on the sheath or the leaf midrib, and sometimes on leaf strands if the conditions were supportable for the development of pathogens. This disease was commonly found in the varieties of Cigeulis, Pandan ungu, and Mekongga. Further, as seen in Figure 3.C, the symptoms of *Ustilaginoidea virens* (Cke) Tak were indicated by the strained rice seeds turning into golden spores (sometimes green). In most cases, this disease attacks Cimelati variety, and only a few grains of rice were discovered on the panicle.

Based on the results of the macroscopical observations, the colony of the bacteria *Xanthomonas campestris* pv. *oryzae* Dye was identified by the appearance of rounded convex with a yellow surface area, as presented in Figure 4.A and 4.B. While Figure 4.C presented the isolation of microcystic observation of *bacilli* colony from fungus *Pyricularia grisae* (Cke) Sacc. Based on the microscopic observation, the fungus seemed to have a long, sparse, single, and gray-colored conidiophore, which formed a conidium at the end. As shown in Figure 4.D, conidium looked like an oval with a pointed tip and was insulated into three-room with gram-negative color (red). While Figure 4.E indicated the colonies of *H. oryzae*., which appeared to be white on the fourth day. With microscopic observation, this fungus was seen to have a slightly curved *Conidia* (curve), enlarged in the center, smeared, brownish, and germinated from both end cells (Figure 4.F).

A B C

Figure 1. A. The sympton of *hawar daun bakteri* in vegetative phase**.** B. The symptom of *blast* in vegetative phase**.** C. The symptom of *bercak coklat* in vegetative phase.

Figure 2.A. The symptom of *hawar daun bakteri* in generative phase, B. The symptom of *blas*t leaves, C. The symptom of neck blast, D. The symptom of *bercak coklat* in generative phase.

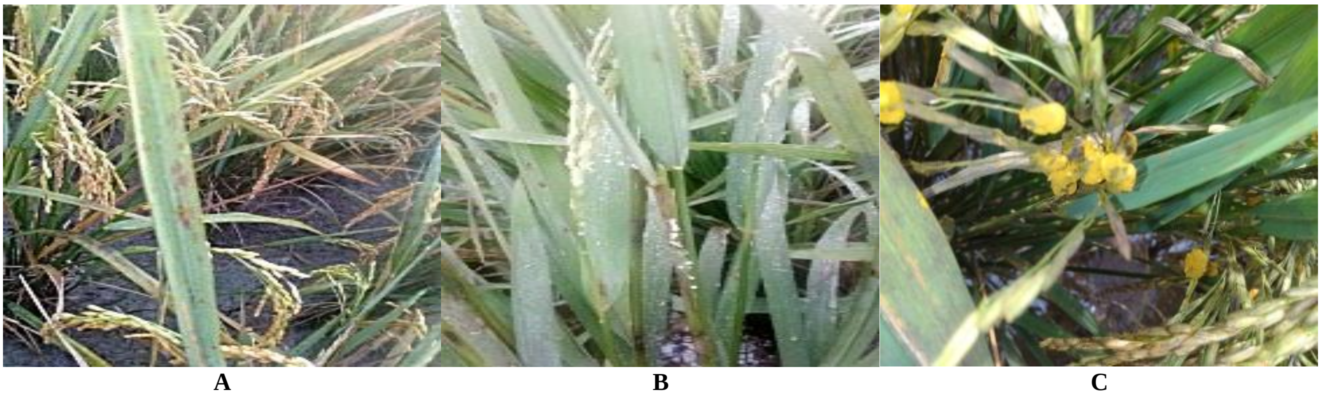

Figure 3.A. The symptom of *bercak daun sempit* in generative phase. B. The symptom of *hawar pelepah/upih* in generative phase. C. The symptom of *gosong palsu* in generative phase.

As seen in Figure 5.A and 5.B, the colony of the fungus *C. oryzae* appeared to be grayish and was white-colored on the fourth day. By using microscopic observation, the fungus seemed to have cylindrical conidia which consisted of 3-10 septa, brown conidiophore, and growing on individual patches, where the conidium was formed on top of a conidiophore. While Figure 5.C and 5.D implied the colonies of the *R. solani* fungus. It was likely to be white, and this fungus was capped with hyphens in and many branches. The colony of *U. virens* appeared to be grayish-brown on the fourth day. Microscopically, this fungus has a spore, spiked, and germinated by forming a smaller secondary conidium and hyaline where the wind propels the conidium. Further, the highest spore production occurs at night where the hyphae are attached. The form is an oval-shaped lump and has propagules (Figure 5.E-F).

Discussion

Hawar daun bakteri

In this study, it was revealed that the bacteria which caused the *hawar daun bakteri* (HBD) disease is *Xanthomonas campestris* pv. *oryzae* Dye. The bacteria of *Xanthomonas campestris* pv. *oryzae* Dye. is a significant disease in many rice-producing countries. The main reason is that HDB can reduce the rice yields at a different level of degrees, depending on the growth stage of the infected plants, and the surrounding environment (Sopialena and Palupi 2017). Regarding the characteristics, *Xanthomonas* is a slimy colony and produces yellow xanthomonadin pigments (Cao et al. 2018). HDB disease often occurs in the rainy season, especially when there is a strong wind, which plays a role in the transmission and its spreading. Meanwhile, persistently irrigated crops may create environmental conditions that lead the disease to better develop(Bansal et al. 2017). Similarly, the cropping arrangement, which is too rigid, provides a massive influence on the development of the disease. Conversely, planting with a far distance also allows the transmission from one plant to another. The jolt and tipping between the infected leaves and the healthy ones may trigger the rate of pathogen infection.

According to Abadi (2003), the characteristics of bacteria are short stems, rarely seen in pairs, and having a size of (1-2) x (0.8-1) μm. It has one polar flagellum with a size of 6-8 μm which serves as a mobile device. These bacteria are characterized by a narrow host range (Albuquerque et al. 2011) and are considered aerobic and can produce *extracellular polysaccharides* (EPS) which have an essential influence to cause the exudate which may infect the leaves. *Xanthomonas* are slimy colonies and generate a yellow pigment that may differ from another genus, i.e., *Pseudomonas* sp. *Xanthomonas* enter the plant tissue through the hydathodes which may happen at the leaf edge, broken roots, or the cut of the leaves. Seeds, farming tools, infected tillers, and weeds could be the primary cause of the bacterial inoculum, and the moisture on the leaf surface can dissolve the bacterial cells and make them spread freely. Akhavan et al. (2013) and Karavina et al. (2011) suggest that wind, rain, and irrigation channels may help the spread of the inoculum also. The bacteria can survive in the soil for 1-3 months, or 7-8 months in the seed.

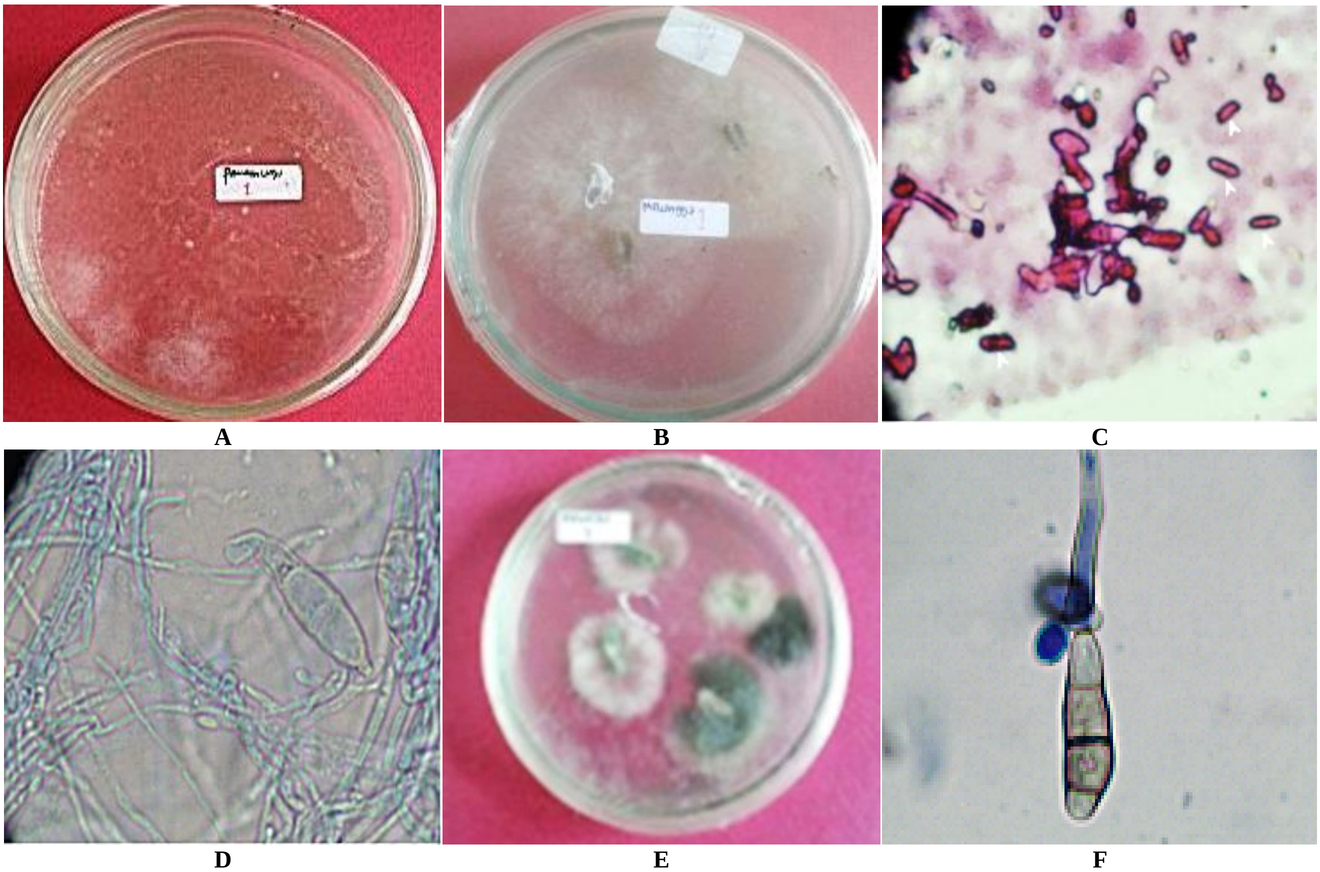

Figure 4.A. The colony of *Xanthomonas campestris* pv. *oryzae* Dye. B. *Pyricularia grisae* (Cke) Sacc. colony. C. Bacilli bacteria in gram-negative color (400x). D. *Pyricularia grisae* (Cke) Sacc. (400x) conidia. E. Fungus colony *Helminthosporium oryzae* L., F. *Helminthosporium oryzae* L. (400x) conidia.

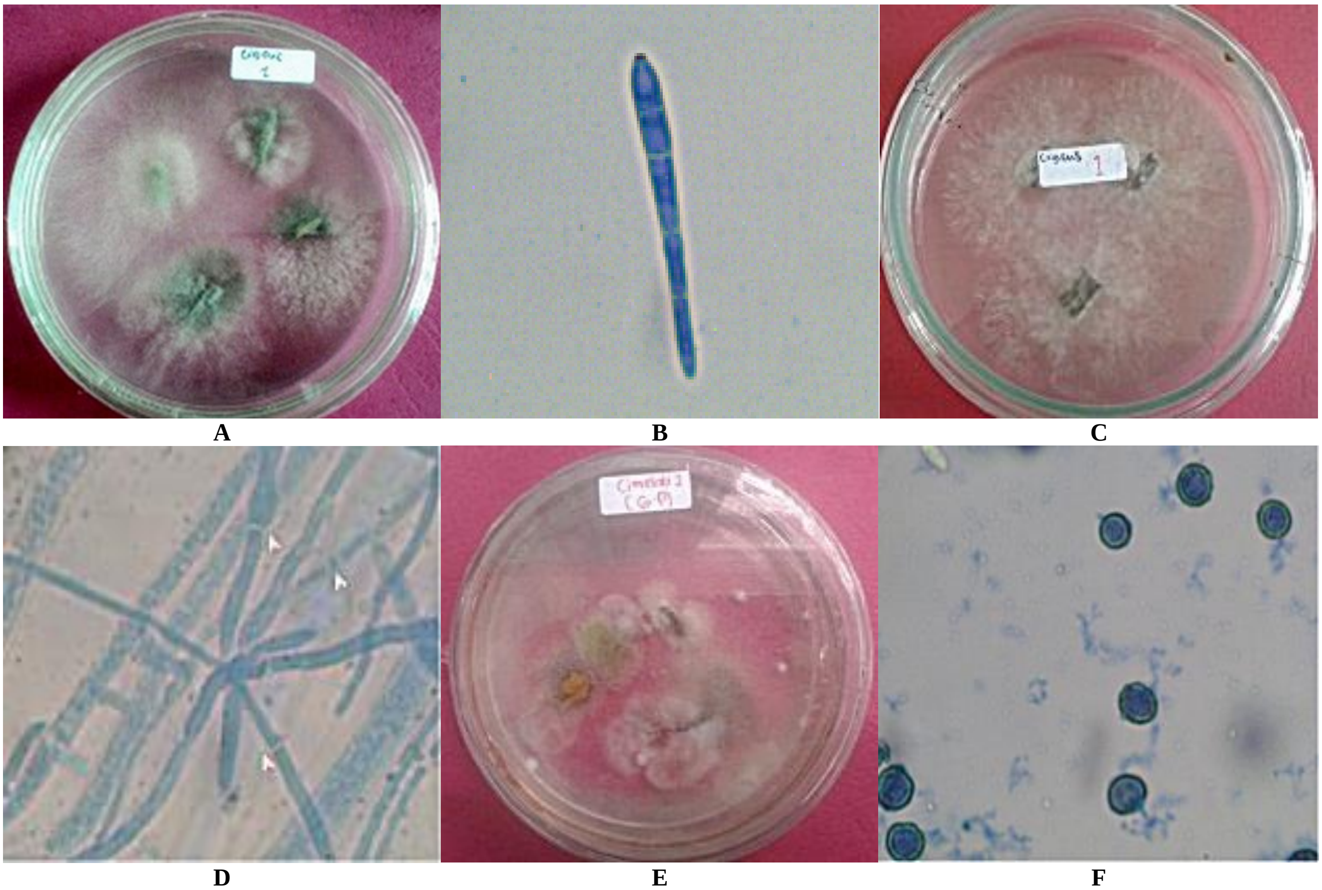

Figure 5.A. *Cercospora oryzae* Miyake colony, B. *Cercospora oryzae* Miyake (400x) conidia, C. *Rhizoctonia solani Kuhn.* colony, D. *Rhizoctonia solani* Kuhn. (400x) hypha, E. *Ustilaginoidea virens* (Cke) Tak colony, F. *Ustilaginoidea virens* (Cke) Tak. (400x) spores.

Blast (*Pyricularia grisae* (Cke) Sacc.)

Pyricularia grisae (Cke) Sacc. is found on all the paddy varieties in tidal rice fields and may infect either in the vegetative and generative phases. *P. oryzae* showed good growth at room temperature and optimum temperature ranging from 25-30°C (Channakeshava and Pankaja 2018). *P. grisea* works by disturbing the growth process. It initially infected the young leaves and forced the leaves to become dry and die. When the disease attacks in the growth phase, it is likely to cause *puso* (crop failure), particularly for vulnerable varieties. However, it does not result in excessive yield loss if the infection occurs in the phase of tiller development. The use of Si application also indicated a positive effect to reduce the intensity of leaf and neck blast attack, in particular for Ciherang variety (Siregar et al. 2016). Also, Amir et al. (2003) reported that the blast neck might potentially damage the rice crop if there is ample moisture at the beginning of flowering; either happening in morning or afternoon. In such climatic conditions, the temperature is not the limiting factor. At a temperature of 30-32°C, the neck blast is still capable of forming itself. There was a report mentioning that in Southeast Sulawesi, neck blast has infected the IR42 variety (at the average paddy age is two months) within the area of 300 ha. The main reason is that it was planted with a relatively high population, and there was a lot of dew/moisture at the beginning of the flowering season.

Bercak coklat (*Helminthosporium oryzae* L.)

Helminthosporium oryzae appeared to attack the four local paddy varieties either in the vegetative or generative stage. According to Iqbal et al. (2015), the main indication of the disease was seen on leaves and later found on young seedling and panicle branches in older plants. The most severe symptoms come into sight approximately one month before the harvest. *Bercak coklat* affects the leaves and grains which could reduce the quality. The infection passes through stomata or directly to the epidermal cell wall after forming appressoria. The fungus may survive longer in plant tissue, depending on the supporting environment (Hafiz et al. 2015). The conidia of *H. oryzae* has specific characteristics, i.e., having a brown color, insulated into 6-17 parts, cylindrical shape, slightly curved, and somewhat widened in the middle. Environmental factors, i.e., wind speed and sunshine hours are the most decisive factor for epidemic development (Biswas et al. 2018). Further, *H. oryzae* lives as a parasite, which may destroy the sprouts and fruit, and cause a blemish on the host's leaf. The life cycle of this disease begins with a fungal conidium propagated by the wind and infection occurs through the mouth of the skin, while a new symptom may emerge 30 days or more after infection.

Bercak daun sempit (*Cercospora oryzae* Miyake)

This study found that *C. oryzae.* appeared in the Cigeulis variety and occurred in the generative phase. According to Mahmud et al (2018), *C. oryzae* is a harmful disease because it could damage the production, by drying the leaves before the time and harming the midrib. The spots mostly increased at seedlings. In severe attacks, *bercak daun sempit* is visible on leaves, stems, and flowers. However, when the plant starts to ripen, heavy symptoms begin to appear on the flag leaves. In most cases, the severe symptoms cause the leaves to dry out. The infections which happen in the midrib and stem, may initiate the stem and rot leaves which make the plant collapse (Wahyuno et al. 2017).

The disease usually transmits through the air and an alternating host. The hot air temperature and interspersed with rain are likely to accelerate the growth of the fungus, of which the fungus generates *bercak daun sempit* (Purnomo 2013). The infection happens through stomata, and new symptoms may appear about 30 days or more after the contamination. The symptoms pass slowly, even for young plants. However, the time of formation and filling of the panicles is the most vulnerable time for the entire stage.

Hawar pelepah/upih (*Rhizoctonia solani* Kuhn.)

Rhizoctonia solani came across in Cigeulis variety, Pandan ungu, and Mekongga and occurred in the generative phase. What is more, many species of *Rhizoctonia* cause important diseases (Bolton et al. 2010; Madbouly et al. 2014). It can subsist in the soil, where the perennial plants existing and can transform into dormant structures when the host plant is unattainable (Tirtana 2013). Though the dispersion of the pathogens can pass through the parts of plants that are buried in the soil, seeds also further the spreading across rainwater, irrigation flows or floods. The use of agricultural tools that contaminated these pathogens from the soil turned out to be a common way of dispersal. The ideal temperature for most of this fungus is 15-18^{o}C. However, a small number of them can grow up to 35^{o}C. The consequence of *hawar pelepah* infection is decelerating plant growth. When it grows in Potato Dextrose agar medium (PDA), the mycelium will initially appear white, and gradually the color of mycelium turns into light brown to old.

Gosong palsu (*Ustilaginoidea virens* (Cke) Tak.)

Ustilaginoidea virens was found in *Cimelati* variety and happen in the generative phase. It is a disease-causing pathogenic fungus, formerly also known as *U. virens*. Conidia are seen in an oval shape, very small, and have several green spore balls from sclerotia in the middle. The sclerotia can endure in the winter and flourish by spring. The fungus seems to be a solid, soft, and yellow-covered membrane, and the color changes from yellow to orange, yellowish-green, and eventually into a greenish-black.

Gosong palsu diseases occur in rice grains. The fungus turns the panicle grains into a greenish-yellow spore where it looks like velvet. The spores are initially small and visible among the glomes and covered with a membrane because of further growth. The color of the spore ball becomes orange, then turns greenish to greenish. At this stage, when the surface of the spore bulb is broken, the outer layer of the ball will consist of mature spores along with the remaining fragments of mycelium. It has three layers of which, for the outermost layer, the green spore is blackish, the middle layer is orange, and the inner layer is yellow. According to Fan et al (2015) pathogen, *U. virens* produces a toxin known as Ustiloxins. Ustiloxins are tetrapeptides and Ustiloxins A-F is isolated from *gosong palsu* spore water extract. Toxins cause mycotoxicosis and may inhibit brain tubulin polymerization at micromolar concentrations. Pathogens can produce sclerotia in the life cycle stage, which germinate and produce Asco spores that will infect at the beginning of flowering. The continued rate of infection is an important part of the cycle of *gosong palsu* disease.

G*osong palsu* diseases are prevalent in humid weather. In relatively high humidity, low temperature, and precipitation accompanied by cloudy days during flowering rice plants are favorable for the fungus *U. virens. Gosong palsu* disease develops in the rice husk and turns the rice endosperm into a large fungal sclerotium, which protrudes to the outside, golden yellow. Usually, in one panicle, there are only a few grains of rice that are exposed to the conidium emitted by wind and spores are present in the air around the night, while in the daytime very little. In general, the infection can occur, before or after the fertilization in panicle seed.

To this end, this study provided a better understanding of the types of paddy diseases and it's natural occurrence in four different paddy varieties in Kutai Kartanegara, Indonesia. The information is useful for the farmers to predict and identify the management of the appropriate diseases to paddy planting. *Gosong palsu* was commonly found in Cimelati variety, but *hawar pelepah/upih* emerged in Cigeulis, Pandan ungu, and Mekongga varieties. Further, Cigeulis was susceptible to *bercak daun sempit,* while the rest of four paddy varieties were susceptive to *bercak daun coklat*, *blast*, and *hawar daun bakteri*.

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ASIAN JOURNAL OF AGRICULTURE
Volume 3, Number 2, December 2019
Pages: 63-76
E-ISSN: 2580-4537
DOI: 10.13057/asianjagric/g030205

Functional food plants in Debre Markos District, East Gojjam, Ethiopia

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Manuscript received: 11 May 2019. Revision accepted: 16 November 2019.

Abstract. *Belay H, Wondimu T. 2019. Functional food plants in Debre Markos District, East Gojjam Ethiopia. Asian J Agric 3: 63-76.* An ethnobotanical analysis of functional food plants was conducted in Debre Markos District, East Gojjam, Ethiopia, to record indigenous knowledge. Ethnobotanical methods were used to collect data from October 2015 to July 2016. Ten villages were chosen at random from the district's 21 villages to perform the analysis. A total of 80 informants (61 men and 19 women), ranging in age from 20 to 79, were chosen to participate in the study and provide information on functional food plants grown and used in the District. Twenty of the 80 informants were chosen (as key informants) based on recommendations from elders and kebele administrators, while the rest were chosen at random. Semi-structured interviews and group discussions were used to gather ethnobotanical data. As functional food plants grown in the District, 29 species from 27 genera and 19 families were identified. The Poaceae and Fabaceae families proved to be the most diverse, with 5 and 4 species, respectively. Herbs (23), shrubs (3), and trees are the species' habits (growth forms) (3). Grains (23.4%) and leaves (23.4%) were the most used plant components (13.8%). Cooking/boiling seeds was the most common form of preparation (23.4%), followed by raw/unprocessed seeds (12.9%). According to informant consensus, *Lupinus albus* L. (Gibto) was the most recorded species (90%) followed by *Eragrostis tef* L. (Dabbo teff) (78.8%). *Linum usitatissimum* L. (Telba) was found to be the most favored species for preventing multiple diseases in a preference rating. Based on the general use-value of functional food plants, a direct matrix analysis showed that *Eragrostis tef* L. (Dabbo teff) was the most important species. *Cinnamomum verum* L. was identified by the fidelity level index. *Lupinus albus* L. (Kerefa) (Gibto) to have a high practical benefit in the prevention of diabetes mellitus and hypertension. The key threat to functional food plants, such as *Lupinus albus* (Gibto), in the study area was the loss of agricultural lands due to new building construction. The woreda is the central city of East Gojjam Zone. As a result, stalk owners must focus their efforts on resolving the problem.

Keywords: Debre Markos, ethnobotany, functional food plants, indigenous knowledge, phytochemicals

INTRODUCTION

Ethiopia is a country with a diverse range of climatic and ecological conditions and a large variety of flora and fauna. Furthermore, the country's high geographic heterogeneity, combined with the diverse cultural diversity of the different ethnic groups, resulted in a wide range of traditional expertise and practice (Asfaw 2001). Maintaining sustainable food production while delivering high-quality food products with added versatility to prevent lifestyle-related diseases, is one of the most difficult food productivity and development research challenges (Wang and Bohn 2012).

Beyond the standard diet, functional foods have health benefits. Functional foods differ from medicinal foods and dietary supplements in that they can be consumed freely as part of daily life. Medicinal foods and drugs are typically used to treat disease in specific cases. On the other hand, dietary supplements overlap with foods developed for specific dietary purposes and fortified foods (Ross 2000).

Most functional foods have been shown to reduce lipid and cholesterol levels, increase bone mineral density and antioxidant status. They also possess anticancer properties, that are derived from plants (legumes, cereals, grains, fruits, and vegetables) with a few exceptions (Eskin and Tamir 2006). However, only a handful of the hundreds of plant-based functional foods that have been documented have been integrated into everyday foods (Wang and Bohn 2012).

Healing in Ethiopian traditional medicine, according to Kassaye et al. (2006), is concerned with curing diseases but also with preserving the human physical, spiritual, social, emotional, and material well-being. This means that Ethiopians have a long history of traditional knowledge about the role of food plants. Functional food plants are still widely used in Ethiopia, and their acceptability, availability, and popularity are undeniable given that most of the population consumes them. People in Debre Markos district, like people in Ethiopia, have cultural activities that are passed down from generation to generation to avoid such human diseases. However, according to FAO (1995), indigenous knowledge is rapidly disappearing in many parts of the world, including Ethiopia. This is because of changes in lifestyle, occupational habits of household members, and the death of knowledgeable people.

According to information obtained from Debre Markos University's Botanical Department and the researcher's opinion, no ethnobotanical research on "*functional food plants*" has been conducted in the Debre Markos district. By researching and recording historically used functional food plants in Debre Markos district, the aim of this study was to fill a gap in the country's limited inventory of Functional Food Plant use. The research will provide useful information on the status of functional food plants and their application in the study field. This will serve as a baseline for future studies.

The goals of the study were (i) to identify and record the people's indigenous knowledge of functional food plants in the study area, (ii) to classify and record the most significant functional food plants used by Debre Markos district residents, (iii) to show the way local people in the study area use plant parts as functional foods and the way they prepare them.

MATERIALS AND METHODS

Study area

Geographical location of the study area

Debre Markos is one of 18 districts of East Gojjam Zone and one of 151 districts in Amhara National Regional State (DMDARDO 2015). The largest town in the East Gojjam Region, located about 295 kilometers from Addis Ababa and 265 kilometers from Bahir Dar, the ANRS's capital. It is made up of 7 kebeles. Its elevation is between 2420 and 2509 meters above sea level, and its geographical coordinates are 10^{0} 20¨ N. 37^{0} 43‘‘ E.\ 10^{0} 333¨ N. 37^{0} 717¨ E. (Figure 1). The district is bordered on the east, north, and west by Gozamin district and the south by Aneded district. The woreda's topography is mostly 98% plane and 2% plateau (DMDFEDO 2015).

Soil and drainage

Clay soil and black soil are the two most common soil types in Debre Markos district, accounting for roughly 73% and 27% of the total area. The district's topography, vegetation, and rainfall pattern promote the existence of a few perennial rivers, such as the Wetrin, Weseta, and Chemoga, which have been used for irrigation in the past (DMDARDO 2015).

Climate and ecology

The district's agro-climatic region is traditionally known as "weina-dega," with average annual temperatures varying between 15.0 and 20.0 ° C (Figure 2). The district is known for its unimodal raindrops, with the total annual rainfall falling between the end of April and the end of October, with an average annual range of 1200mm and 1380mm (Figure 3)(DMDARDO 2015).

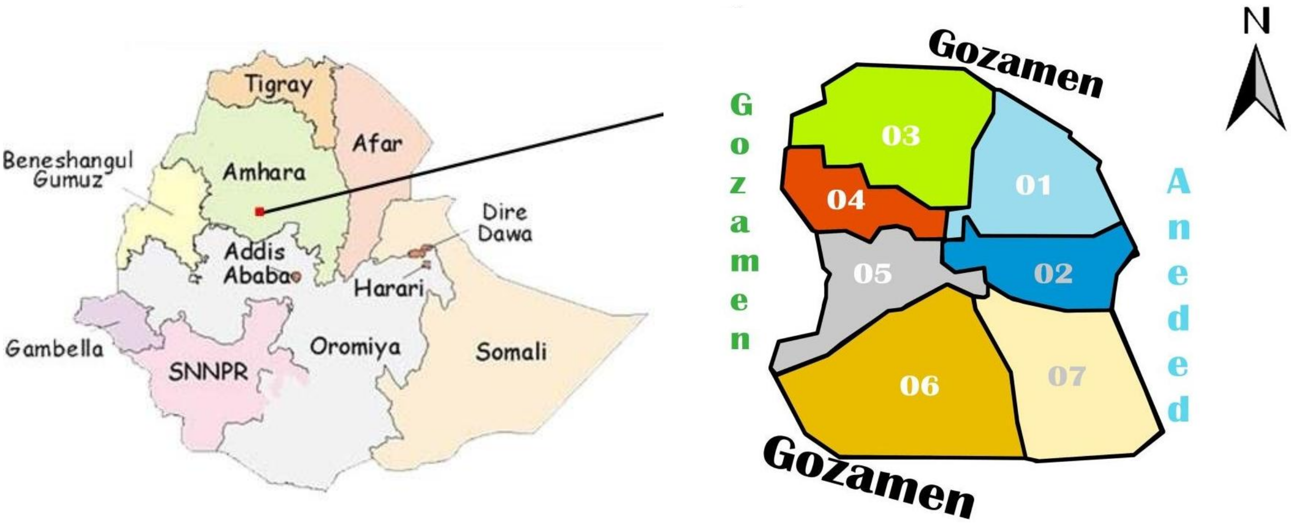

Figure 1. -Map of Debre Markos District (source: DMDFEDO 2015).

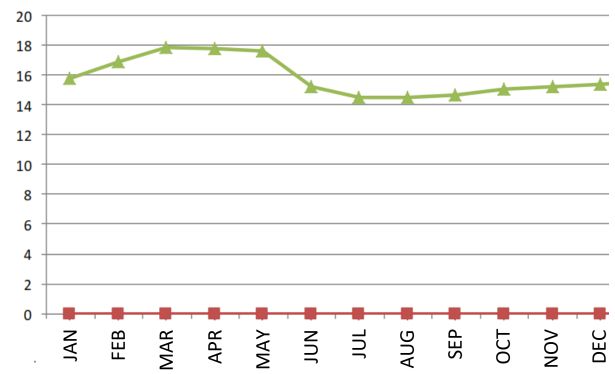

Figure 2. Monthly temperature of Debre Markos District 2015 (Source of data: -Versat International LLC).

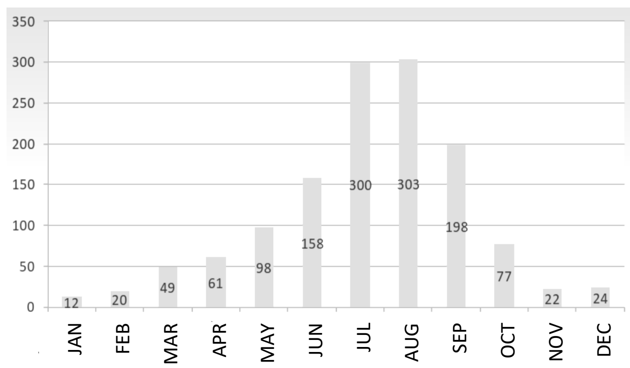

Figure 3. Monthly precipitation of Debre Markos District 2015 (Source of data:-Versat International LLC).

Crop production is largely dependent on rain and small-scale irrigation. There is only one rainy season (known locally as "kiremt"), which is critical for the cultivation of both long and short-cycle crops.

The area's agricultural system is a mixed farming system, in which crop production is multiple cropping with a small amount of land used intensively. Crop rotation, which alternates cereal production with legume crop production to preserve soil fertility, has traditionally been used to practice continuous cropping. The food crops grown in the district include: cereal (wheat, teef, maize, barley, engedo, oat), fruits (papaya, pom, cocks, lomes, oranges, mangoes, bananas, gibberries), vegetables (garlic, chocolate, tomatoes, pumpkins, salad, kosta), root crops (sweet potatoes, potatoes, reddishes, carrots) and cash crops, including the cereal crop, (cereals, pumpkins, gibberries, etc.) (DMDARDO 2015).

The district covers a total area of 6160 hectares, the majority of which is land for farming purposes (2363 ha, and 38,36%), followed by residential, road and water areas of the area of 1995 ha according to information obtained from DMDARDO (2015). (32.4%). Other land characteristics share significant areas, as listed in Table 1.

Population structure and medical service

The district has a total population of 107,433 people, with 50,036 males and 57,397 females. Rural dwellers account for 1,268 (1,053 males and 215 females) of the total population, while urban dwellers account for 106,165 (48,983 males and 57,182 females). The Amhara (98%), Oromo (0.8%), and other ethnic groups are the most numerous in the district (1.2%). Amharic is the first language of 99.3% of the population, and Orthodox Christianity is practiced by the majority (96%) of the population, whereas Protestantism and Islam are practiced by the remaining population (DMDFEDO 2015).

According to DMDHHIV/AIDSPCO (2015), the top ten human diseases in the area are acute upper respiratory infection with 3,431 (20.53%) of infected people, followed by Dyspepsia (gastritis) with 1,984 (11.87%) of infected people and the others are mentioned in Table 2.

However, according to DMDHHIV/AIDSPCO (2015), few people of Debre Markos are affected by chronic diseases such as diabetes (104), hypertension (432), heart disease (5), and cancer (12).

Table 1. Land use patterns of Debre Markos District, Ethiopia.

Land use	Area (ha)	Percentage
Agricultural land	2363	38.36
Residence, road, and water bodies	1995	32.40
Grazing land	815	13.23
Forest areas	624	10.12
Irrigation areas	363	5.89
Total	6160	100

Source: DMDARDO (2015)

Table 2. Top ten human diseases in Debre Markos District, Ethiopia.

Type of disease	No. of people affected	Percentage
Acute upper respiratory infection	3431	20.53
Dyspepsia (gastritis)	1984	11.87
Acute febrile illness	1891	11.31
Parasitic disease	1600	9.57
Disease of musculoskeletal system and connective tissue	1506	9.01
Urinary tract infection	1407	8.42
Diarrhea	1338	8.01
Un specified digestive system disease	1305	7.81
Eye disease	1159	6.93
Pneumonia	1093	6.54
Total	16714	100

Source: DMDHHIV/AIDSPCO (2015)

MATERIALS AND METHODS

Design of the research

The purpose of this cross-sectional study was to investigate and document the functional food plants in the Debre Markos district. From September 30, 2015, to November 12, 2015, the collection of journals and other literature was employed. From October 5 to November 5, 2015, a reconnaissance survey was carried out. The deadline for developing and submitting proposals was November 27, 2015. Data collection took place from December 22, 2015 to the first two weeks of March 2016, and thesis development took place from the middle of April to the end of June 2016.

Reconnaissance survey and selection of the study sites

Before starting the actual study, a reconnaissance survey was conducted from October 5 to November 5, 2015, to gather information about functional food plants and identify sampling sites. During the survey, ten representative villages were chosen from seven kebeles (Table 3). To determine the number of representative villages, the population size and number of villages in each kebele were considered, and random selection was used to determine sample sites from each kebele. As a result, the study sites were chosen using a systematic simple random sampling method.

Selection of informants

A total of 80 informants (61 males and 19 females), aged 20 to 79 years were chosen, with the number of informants from each kebele determined systematically based on the proportion of the population size. In addition to this, twenty elders and knowledgeable people were identified as key informants through purposive sampling (Martin, 1995), and other informants were chosen at random from the sampled villages. The key informants were chosen based on the recommendations and comments of community elders, farmers, students, religious leaders, and the researcher's observations. Age group, educational and marital status of informants were recorded.

Table 3. Study kebeles with their corresponding sampling site and no. of informants participated.

Study kebeles	Sample villages	Population size	Number of informants	Total
1	Endegem	12762	9	9
2	Engecha	13505	10	10
3	Shola amba	27163	13	21
	Yebragie		8	
4	Yemeka	14000	10	10
5	Kebi achira	18016	7	13
	Muakuat		6	
6	Yenora	10921	7	7
7	Abedeg	11066	6	10
	Chemoga		4	
Total	10	107433	80	80

Note: Source of population data: DMDFEDO, (2015)

Table 4. Demographics.

	Age group (in yrs.)	Sex and no of informants		Total
		Male	Female	
Age group	20-30	13	5	18
	35-49	21	10	31
	50-64	20	3	23
	65-79	7	1	8
Education level	Illiterate	12	3	15
	Only read and write	22	9	31
	Modern education	20	7	27
	Church education	7	-	7
Marital status	Single	6	2	8
	Married	53	15	68
	Divorce	2	2	4

In the study area, eighty informants were divided into four age groups. They were: young (20-34), middle-aged (35-49), elder (50-64), and elderly (65-79). The middle-aged groups had the highest number of informants. Males outnumbered females (61 to 19). Most of the informants (31) were able to read and write. Approximately 68 of the total informants were married (Table 4).

Ethnobotanical data collection

From December 22, 2015, to March 21, 2016, ethnobotanical data was collected. Ethnobotanical data covered the different types of functional food plants, cured diseases, parts used, and preparation methods; following the data collection tools recommended by Martin (1995).

Semi-structured interview-Ethnobotanical data was collected through semi-structured interviews using checklist item questions written in English (Appendix 2) and later translated into Amharic. The items include information on the informant's personal identity, a local health problem, the names of functional food plants, the part(s) used, preparation methods, and disease management. The entire informant interview was conducted through direct communication between the researcher and the informants (Figure 4). Before starting data collection, informants' willingness was confirmed. This was accomplished by raising informant awareness by describing the future significance of the research for the study area and the country.

Group discussions, including both short and precise group discussions, were held with informants about the functional food plants in the study sites. Local names of functional food plants, their uses, methods of preparation, indigenous knowledge about them, and other data were collected.

Functional food plant specimen collection and identification

During a guided field walk, the voucher specimens were collected, numbered, pressed, and dried for identification. Flora of Ethiopia and Eritrea was used for identification, with assistance from Biology Department of Debre Markos University, Ethiopia.

Figure 4. Photographs showing group discussion to key informants during ethnobotanical data collection.

Data analysis

Descriptive statistics

The ethnobotanical data was analyzed using survey and analytical techniques for ethnobotanical methods that Martin (1995) and Alexiades (1996) recommended. The data on functional food plants and associated information obtained from informants in the study area were analyzed and summarized using descriptive statistical methods. Using appropriate software and descriptive research methods, the most useful information gathered on functional food plants identified by local people, including functional importance and method of preparation, was analyzed.

Informant consensus

Informants were consulted to assess the reliability of information provided during the interview; however, if the informant's idea differed from the original information, it was dismissed as irrelevant information. Only the most important ones were considered and statistically analyzed; Alexiades' approach was used (1996). Similarly, for six classes of plant uses identified by informants, the informant consensus factor was quantitatively analyzed.

ICF=nur-nt /nur-1

Where,
ICF: Informants Consensus Factor
Nur: Number of use citation in each category
Nt: Number of species used

Preference ranking

For most of the functional food plants used to prevent the most prevalent disease in the study field, preference (priority) ranking was performed using seven primary informants. As a result, five functional food plants were selected to be rated preferentially by key informants on a numerical scale for preventing Gastritis (0 for no value, 1 for lowest value and 5 the highest value, and the rest with intermediate values). The values provided by the main informants were combined to determine the community's preference for functional food plants in the study area. The highest score was placed first, while the lowest was placed last, and the rest were assigned different ranks based on the results of the study. A similar study was carried out on six functional food plants to see how effective they were at preventing various diseases.

Direct matrix ranking

To compare multiple purposes of plants commonly identified by informants, direct matrix ranking is used (Cotton, 1996). Thus, a direct matrix ranking exercise was conducted on eight functional food plants based on the relative benefits obtained from each plant during the general group discussion and semi-structured interviews with key informants. Use values to each attribute assigned as (5-best, 4-very good, 3-good, 2-less used, 1-least used, and 0-no used) based on their perceived degree of usefulness; later, the values assigned for each plant were added together to decide its rank.

Fidelity level index

The fidelity level index (FL) is utilized to prove that certain plant species are used for the same main reason (Alexiaden, 1996). As a result, the fidelity level for functional food plants used to prevent diabetes and hypertension was determined. The degree of fidelity is measured as follows:

FL (%) = (NP/N) X 100

Where;
NP: Number of informants that claim use of a plant species to prevent a particular disease
N: Number of informants that use the plant as a functional food, as described by Alexiades (1996).

RESULTS AND DISCUSSION

Status of indigenous knowledge related to functional food plants in the study area

The study area residents use a variety of functional food plants to prevent and treat various diseases. Several health problems, except for a few (such as tuberculosis, pneumonia, and acute diarrhea), are thought to have plant or spiritual remedies in the region. These people use a variety of functional food plants to prevent and treat various diseases and improve their immunity. According to informants, information regarding functional food plants as a health feature was collected informally from friends or elders of neighbors, often through conversation and mostly during the outbreak of a health-related issue. The second most common source of information acquisition was from family members who learned from their parents, accounting for 20% of all responses.

Most of the indigenous knowledge is acquired orally from elders, parents, or relatives, as it is done elsewhere (Abbasi et al. 2013). As a result, the information-transfer mechanism can pose a challenge to indigenous knowledge. Indigenous information is rapidly disappearing in many parts of the world, including Ethiopia, due to changes in lifestyle, changing occupational habits of household members, and the death of village elders (FAO, 1995). Many of the people interviewed for this study believe that modernization is endangering the information system.

Functional food plants in the study area

Distribution of functional food plants among taxa

During the research, 29 species from 27 genera and 19 families were described and reported. The Poaceae family proved to be the most numerous, with six species divided into five genera, followed by the Fabaceae family, which had four species divided into four genera. This could be because these are the most abundant, widely used, and cultivated food plants in the area. Solanaceae had two species and two genera, Rutaceae had two species under one genus, and the remaining (15) each had one species (Table 5). This shows a high diversity of functional food plants in the study field and their use.

Diversity of habits (growth forms) of functional food plants

Herbs are the most common group of functional food plants in the study field, accounting for 23 species (79.3%), followed by shrubs and trees, each with 3 species (10.34%) (Figure 5).

Human diseases and the corresponding number of Functional Food Plant species used in the study area

A total of 29 food plant species have been shown to have anti-disease and immunity-boosting properties. Many of them are local species that are widely used in the study area's culture. Apart from that, most of them are traditional foods, and their preparation methods are close to those used in daily diets (Table 6).

As a result, about 19 human diseases have been reported as being avoided or healed by 29 plant species. In the study region, 13 ailments are prevented by using two or more Functional Food Plant species, and six are prevented by using only one plant species (Table 7). Chronic diseases which were very rare among the top ten diseases identified by DMDHIV/AIDSPCO in 2015, indicated that the population's lifestyle is more closely linked to the use of Functional foods; Functional foods have a beneficial impact on improving health, preventing and reducing chronic disease risk factor (Roberfroid 2003).

The documentation demonstrates the use of significant numbers of functional food plants by Gastrontia with 8 species followed by hypertension using 7 species; cancer 6; microbial and diabetes with 5 species each and then constipation with 4 species each (Table 8). This shows that various functional food plants in the field of study can prevent a particular disease.

Plant parts used as functional food

Different sections of the plant were stated to be used for functional purposes in this research. According to the results of the interviews, grains were the most widely used plant components, accounting for (14, 48.3%), followed by leaves (4, 13.8 percent), tuber (3, 10.3 percent), fruits and grains (3, 10.3 percent), fruits (2, 6.9 percent), roots, barks, and bulb (1, 3.4 percent) (Figure 6). At an appropriate stage, functional food plants are part of a diverse diet regularly. As a result, grain is the most common regular edible component of plant species expected to have functional value in the study field.

Methods of preparation of functional food plants

Diverse processing of functional food plants is practiced in the local communities of Debre Markos district. Most of them are cooked/boiled (37.9%) followed by raw (20.7%), powder/baking (17.2%) and fluid/juice account (17.2%). Other forms of preparations are also shown (Table 9).

Table 5. Number of functional food plant species in each family in the studying area.

Family	Scientific name	Vernacular name	Habit	No. of genera	No. of species	Percentage of plant species	Abundance
Alliaceae	*Allium sativum*	Nech shinkurt	Herb	1	1	3.44	Common
Apiaceae	*Daucus carota*	Carrot	Herb	1	1	3.44	Common
Asteraceae	*Guizotia abyssinica*	Nug	Herb	1	1	3.44	Common
Brassicaceae	*Brassica carinata*	Yabeshagomen	Herb	1	1	3.44	Common
Caricaceae	*Carica papaya*	Papaya	Tree	1	1	3.44	Common
Chenopodiaceae	*Beta vulgaris*	Key sir	Herb	1	1	3.44	Common
Cucurbitaceae	*Cucurbita pepo*	Duba	Herb	1	1	3.44	Common
Fabaceae	*Cicer arietinum*	Shimbra	Herb	4	4	13.79	Common
	Lupinus albus	Gibto	Herb				Common
	Trigonella foenum graecum	Abish	Herb				Common
	Vicia faba	Bakela	Herb				Common
Lamiaceae	*Thymus schimperi*	To sign	Herb	1	1	3.44	Rare
Lauraceae	*Cinnamomum verum*	Kerefa	Tree	1	1	3.44	Rare
Linaceae	*Linum usitatissimum*	Telba	Herb	1	1	3.44	Common
Pedaliaceae	*Sesamum orientale*	Selit	Herb	1	1	3.44	Rare
Poaceae	*Avena sativa*	Engedo	Herb	5	6	20.68	Common
	Eragrostis tef	Dabbo teff	Herb				Common
	Hordeum vulgare	Gebis	Herb				Common
	Sorghum bicolor	Zengada	Herb				Common
	Triticum dicoccon	Ajja	Herb				Common
	Triticum aestivum	Sendie	Herb				Common
Ranunculaceae	*Nigella sativa*	Tikurazemud	Shrub	1	1	3.44	Rare
Rutaceae	*Citrus aurantifolia*	Lomi	Shrub	1	2	6.89	Common
	Citrus sinensis	Birtukan	Shrub				
Solanaceae	*Lycopersicon esculentum*	Timatim	Herb	2	2	6.89	Common
	Solanum tuberosum	Dinich					Common
Theaceae	*Camellia sinensis*	Shay kitel	Tree	1	1	3.44	Rare
Urticaceae	*Urtica simensis*	Sama	Herb	1	1	3.44	Common
Zingiberaceae	*Zingiber officinale*	Zingible	Herb	1	1	3.44	Common

Table 6. List of functional food plants.

Scientific name of functional food plants	Family	Local name in Amharic	HbH	Pp	Disease type	Method of preparation
Allium sativum L.	Alliaceae	Nech shinkurt	H Hg	Bulb	Cancer, hypertension. Microbial disease	Crush the bulb of Allium sativum and daily prepare it as in "watt." Crush the bulb and add with tea or honey.
Avena sativa L.	Poaceae	Engedo	H CU	Grain	Diabetes, general weakness, osteoporosis.	Grind and bake the grain of *Avena sativa* to form "injera" or mix the grain powder with *Hordeum vulgare* and *Triticum dicoccon* and boil it to make "atmit" (gruel).
Beta vulgaris L.	Chenopodiaceae	Keysir	H Hg	tap root	Hypertension	Roast or cook the tap root of *Beta* vulgaris.
Brassica carinata A.Braun	Brassicaceae	Yabeshago men	H Hg	Leaf	Constipation and cancer	Chop the leaf of *Allium cepa* and cook it with oil for few minutes.
Camellia sinensis L.	Theaceae	Shay kitel	T Cu	Leaf	Heart disease	In water, boil the leaf of *Camellia sinensis.*
Cicer arietinum L.	Fabaceae	Shimbra	H Cu	Grain	Diabetes and general weakness	Roast the grain of *Cicer arietinum* as "Kolo" or cook it as Nifro or utilize the roasted powder as "Shiro".
Carica papaya L	Caricaceae	Papaya	T Hg	Fruit and Grain	Gastritis Helminthiasis	Without processing or in raw condition, eat the fruit of *Carica papaya* Dry and crush the grain of *Carica papaya* and drink it with tea.
Cinnamomum verum L.	Lauraceae	Kerefa	T Cu	bark	Diabetes	Boil the bark of *Cinnamomum verum* in water and mix it with tea or honey.
Citrus aurantifolia Christm.	Rutaceae	Lomi	Sh Hg	Fruit	Microbial disease	Pour the juice of the fruit of *Citrus aurantifolia* on uncooked vegetables as salad or mix it with tea.
					Cancer	Eat the fruit as it is.
Citrus sinensis L.	Rutaceae	Birtukan	Sh Hg	Fruit	Microbial disease	The fruit or juice of the plant.
Cucurbita pepo L.	Cucurbitaceae	Duba	H Hg	Fruit	Gastritis Helminthiasis	Eat the fruit of *Cucurbita pepo* after being boiled. Roast the dried seed of the plant.
Daucus carota L.	Apiaceae	Carrot	H Hg	root	Cancer	Eat the fresh unprocessed, or roasted and cooked, of juice of *Daucus carota* root.
Eragrostis tef L.	Poaceae	Dabbo teff	H Cu	Grain	Anemia	Consume the grain powder of *Eragrostis tef* as "injera" or "atmit" gruel.
Guizotia abyssinica L.f.	Asteraceae	Nug	H Cu	Grain	Bronchitis	Eat the grain of *Guizotia abyssinica* after being roasted, well ground. Prepare the "Kolo" from *Hordeum vulgare* and *Cicer arietinum* by boiling them with water and drunk after being filtered.
Hordeum vulgare L.	Poaceae	Gebis	H Cu	Grain	Gastritis	Dehull, roast and then ground the grain of *Hordeum vulgare* and mix the powder with water and honey or sugar as "beso" or the roasted hulled grain of the plant as" Kolo".
					Osteoporosis or general weakness	Hull the seed of *Hordeum vulgare*, roast and grind it and cook it like porridge.
Linum usitatissimum L.	Linaceae	Telba	H Cu	Grain	Gastritis constipation, Breast cancer, attention deficit	Soak the seed of *Linum usitatissimum* with water and boil it then cool it and drink it. Place the seed of *Linum usitatissimum* in any form, but usually the roasted and ground one, in the glass and mix it with warm water.

Lupinus albus L.	Fabaceae	Gibto	H	Cu	Grain	Hypertension	Consume the seed of *Lupinus albus* after being soaked for 2-3 days to remove its bitter taste, and then being roasted and boiled and being spiced with onion and pepper or being processed in the way which local areki synthesize to prepare Gibto areki.
Lycopersicon esculentum Mill.	Solanaceae	Timatim	H	Hg	Fruit	Anemia, heart disease and cancer	Consume the juice, fresh or canned, of *Lycopersicon esculentum*; whole fresh or canned or crushed diced fruit or as tomato paste or soup.
Nigella sativa L.	Ranunculaceae	Tikur azmud	Sh	Hg	Grain	Constipation, liver disease and general weakness	Eat the powdered seed of *Nigella sativa* which was mixed with honey or in the bread or chew the seed directly.
Sesamum orientale L.	Pedaliaceae	Selit	H	Cu	Grain	Diarrhea	Consume the seed of *Sesamum orientale* or the roasted seed mixed on bread as spice.
Solanum tuberosum L.	Solanaceae	Dinich	H	Hg, and Cu	Tuber	Gastritis	The tuber of *Solanum tuberosum* in the form of juice.
Sorghum bicolor L.	Poaceae	Zengada	H	Cu	Grain	Hypertension and diabetes	The grain powder of *Sorghum bicolor* as a form of "atmit" or "injera".
Triticum dicoccon Schrank	Poaceae	Ajja	H	Cu	Grain	Diabetes, blood pressure, Rheumatism and osteoporosis	The flour from the grain of *Triticum dicoccon* as bread or "atmit" or the crushed seed as "Kinchie" or soup.
Triticum aestivum L.	Poaceae	Sendie	H	Cu	Grain	Constipation	The whole grain flour of *Triticum aestivum as* "injera" or bread. Also, the seed roasted as "Kolo".
Thymus schimperi R.	Lamiaceae	Tosign	H	Wd	leaf	Hypertension and microbial disease	The leaf of *Thymus schimperi boiled with* water.
Trigonella foenum-graecum L.	Fabaceae	Abish	H	Cu	Grain	Gastritis, hypertension	Grind the germinated and dried seed of *Trigonella foenum-graecum* and Soak it with water for one night, withdraw the filtrate to minimize its bitterness, mix it with water, drink it, or boil and drink it with honey or sugar.
						Poliomyelitis	Grind the grain, mix it with water, put it at the child's back under the sun, and strike it slowly.
Urtica simensis Steudel.	Urticaceae	Sama	H	Wd	leaf	Gastritis	Pick carefully the leaves of *Urtica simensis* with protected hand and cut the leaves and spread them out b/n two hides on the ground and rub them to avoid burning sensation of the leaves, after that, boil and grind them, then put salt on and prepare them.
Vicia faba L.	Fabaceae	Bakela	H	Cu	Grain	Bronchitis	Boil the Vicia faba grain and get water to drink.
						Gastritis	Drink roasted and powdered Vicia faba grains soaked in water overnight.
Zingiber officinale Mill.	Zingiberaceae	Zingible	H	Hg	rhizome	Microbial disease	Place a few pieces of Zingiber officinale in a cup of boiling water, strain, and drink.
						Badmouth odor	Chewing the rhizome as it is and rinsing the mouth with water

Note: Hb: habit, H: herb, T: tree, Sh: shrub; H: habitat, Hg: home garden, Cu: cultivated; Pp: plant parts used

Table 7. List of human diseases in the study area with number of functional food plants (NFFP) used to prevent them.

Disease prevented	Local name of the disease	NFFP	Percentage
Gastritis	Yechegoara beshita	8	27.5
Hypertension	Yedem gfit	7	24.1
Cancer	Cancer	5	17.2
Diabetes mellitus	Yeskuar beshita	5	17.2
Microbial disease	Worershign	5	17.2
Constipation	Yehod dirket	4	13.8
General weakness	Yeakim medakem	4	13.8
Osteoporosis	Yeatint melashek/Sibrat	3	10.3
Rheumatism	Kurtimat	2	6.9
Bronchitis	Yesal beshita	2	6.9
Heart disease	yelib himem	2	6.9
Anemia	Deme manes	2	6.9
Helminthiasis	Yehod telatil	2	6.9
Attention deficit	Yeamro zigemet	1	3.4
Bad mouth odor	Motifo yeaf teren	1	3.4
Breast cancer	Yetuit cancer	1	3.4
Diarrhea	Tekimat	1	3.4
Liver disease	Yegubet beshita	1	3.4
Poliomyelitis	Yelejinet lemisha	1	3.4

Table 9. Methods of preparation of functional food plants (FFP).

Methods of preparation	No. of FFP species	Percentage
Cooking/boiling	11	37.9
Raw/unprocessed	6	20.7
Powdering and baking	5	17.2
Fluid juice	5	17.2
Powdering and boiling	4	13.8
Crushing	3	10.3
Roasting	3	10.3
Roasting and crushing	3	10.3
Crushing and cooking	2	6.8
Boiling and grinding	1	3.4
Roasting, powdering, and soaking	1	3.4
Soaking and roasting	1	3.4
Dehulling and roasting	1	3.4
Powdering soaking	1	3.4

Table 8. List of human diseases that are prevented by more than three functional food plants in the study area.

Family name	Scientific name of the plant	Vernacular name	Disease prevented	Local name of the disease	No. of plant	Percentage
Caricaceae	*Carica papaya*	Papaya	Gastritis	Yecheguara beshita	8	27.6
Cucurbitaceae	*Cucurbita pepo*	Duba				
Poaceae	*Hordeum vulgare*	Gebs				
Linaceae	*Linum usitatissimum*	Telba				
Solanaceae	*Solanum tuberosum*	Dinich				
Fabaceae	*Trigonella foenum graecum*	Abish				
Urticaceae	*Urtica simensis*	Sama				
Fabaceae	*Vicia faba*	Bakela				
Alliaceae	*Allium sativum*	Nech shinkurt	Hypertension	Yedem gfit	7	24.1
Chenopodiaceae	*Beta vulgaris*	Key sir				
Fabaceae	*Lupinus albus*	Gibto				
Poaceae	*Sorghum bicolor*	Zengada				
Poaceae	*Triticum dicoccon*	Ajja				
Lamiaceae	*Thymus schimperi*	Tosign				
Fabaceae	*Trigonella foenum graecum*	Abish				
Alliaceae	*Allium sativum*	Nech shinkurt	Cancer	Nekersa	6	20.7
Brassicaceae	*Brassica carinata*	Yabeshagomen				
Rutaceae	*Citrus aurantifolia*	Lomi				
Rutaceae	*Citrus sinensis*	Birtukan				
Linaceae	*Linum usitatissimum*	Telba				
Pedaliaceae	*Sesamum orientale*	Selit				
Alliaceae	*Allium sativum*	Nech shinkurt	Microbial disease	Worershign	5	17.2
Rutaceae	*Citrus aurantifolia*	Lomi				
Rutaceae	*Citrus sinensis*	Birtukan				
Lamiaceae	*Thymus schimperi*	Tosign				
Zingiberaceae	*Zingiber officinale*	Zinjibil				
Poaceae	*Avena sativa*	Engedo	Diabetes	Yeskuar beshita	5	17.2
Fabaceae	*Cicer arietinum*	Shimbra				
Lauraceae	*Cinnamomum verum*	Kerefa				
Poaceae	*Sorghum bicolor*	Zengada				
Poaceae	*Triticum dicoccon*	Ajja				
Brassicaceae	*Brassica carinata*	Yabeshagomen	Constipation	Yehod dirket	4	13.8
Ranunculaceae	*Nigella sativa*	Tikur azmud				
Linaceae	*Linum usitatissimum*	Telba				
Poaceae	*Triticum aestivum*	Sendie				
Poaceae	*Avena sativa*	Engedo	General weakness	Yeakim medakem	4	13,8
Fabaceae	*Cicer arietinum*	Shimbra				
Poaceae	*Hordeum vulgare*	Gebs				
	Nigella sativa	Tikur azmud				

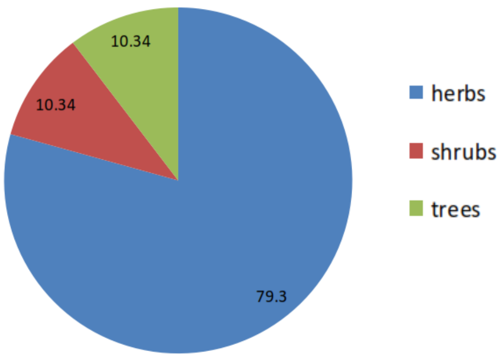

Figure 5. Habits (growth forms) of functional food plants in the study area.

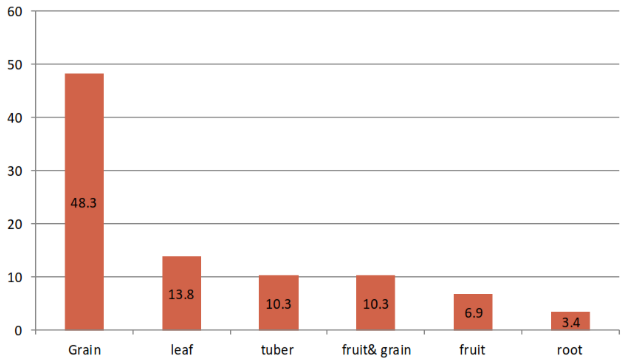

Figure 6. Plant parts used as functional food.

Major plant use categories of functional food plants used by the people in the study area

The research area yielded a total of 29 plants with high Functional Food values. The community uses plants for a variety of purposes in addition to their functionalities. Informants in the study area identified six different types of use (Table 10). As a result, a total of 2,257 use reports (ur) are recorded from 94 frequency of occurrences for the total use category, with each informant reporting on 29 Functional foods Plants. Plants used in construction have the highest ICF value (0.98), followed by functional food plants and forage use-category, both of which have ICF values of (0.97). This research revealed that ethnobotanical knowledge is homogeneous, and plant use reports from informants on various plant use categories are highly consistent.

Informant consensus (FFP use report) of Popular functional food plants

The use of informant consensus is one method of verifying the efficacy of a given plant species. Common functional food plants cited by many local people in an area were recorded and analyzed. As a result, 72 (90 percent) informants cited *Lupinus albus* (Gibto), while 63 (79.8%) informants cited *Eragrostis tef* (Dabbo teff) for their functionality as health care, and others are shown in Table 11. The popularity of these functional food plants may be due to their effectiveness or the ease with which they can be obtained.

Preference ranking of functional food plants used to prevent gastritis

Several plant species can protect against a single ailment. In such situations, the locals give priority to ward plant species based on their ability to avoid a specific disease. Cucurbita pepo (Duba) is the most favored species, followed by *Linum usitatissimum* (Telba), in this study's preference rating for five plants based on preventing gastritis (Table 12). The score was determined by seven primary informants and was focused on the ability of each species to prevent gastritis. The highest value (5) was assigned to species with the highest preventing potential, while the lowest value (1) was assigned to species with the lowest preventing potential as compared to the species identified.

On the other hand, a specific plant species is used to treat a variety of diseases. Similarly, people prefer plant species that can resist different diseases. Based on disease prevention, a preference ranking conducted by seven primary informants (respondents) for six selected plant species (Table 13) revealed that the most common is *Linum usitatissimum* L. (Telba), followed by *Triticum dicoccon* Schrank (Ajja).

Table 10. ICF by use categories of plants for the local people in the study area.

Use-category	Species(#) (nt)	Percentage of species	Use reports (nur)	Percentage of use reports	ICF (nur-nt/nur-1)
Functional food	29	100	809	35.8	0.97
Food/drink	21	72.4	557	24.7	0.96
Spice	10	34.5	171	7.6	0.95
Medicinal plants	22	75.9	317	14	0.93
Forage	9	31.0	306	13.6	0.97
Construction	3	10.3	97	4.3	0.98
Mean ICF					0.96

Direct matrix ranking for multiple uses of functional food plants

To compare multipurpose plants commonly identified by informants, direct matrix ranking was used. As a result, most people in the study area depend on plants for a variety of reasons, including functionality, food/drink, spice, medicinal, forage, construction, etc. According to the results, *Eragrostis tef* (Dabbo teff) was ranked first, followed by Hordeum vulgar (Gebis) and Avena sativa (Engedo) (Table 14). According to the informants' reports and the researcher's perspective, these plants are the most preferred plants by local people for different uses and are the most readily available plant species. Thymus schimperi is the species with the lowest rank in the direct data matrix (Tosign). This is most likely due to its scarcity in the study field. As a result, the utility of resources is determined by their availability.

Table 11. List of functional food plant species reported by thirty and a greater number of informants.

Scientific name	Vernacular name in Amharic	Number of informants	Percentage of informants
Lupinus albus	Gibto	72	90.0s
Eragrostis tef	Dabbo teff	63	78.8
Avena sativa	Engedo	54	67.5
Cucurbita pepo	Duba	51	63.8
Linum usitatissimum	Telba	49	61.3
Citrus aurantifolia	Lomi	49	61.3
Hordeum vulgare	Gebis	48	60.0
Allium sativum	Nech shinkurt	43	53.8
Triticum dicoccon	Ajja	39	48.8
Lycopersicon esculentum	Timatim	37	46.3
Trigonella foenum graecum	Abish	36	45.0

Table12. Preference ranking of functional food plants used to prevent gastritis by key informants (respondents).

Functional food plant	R.4	R.5	R.16	R.20	R.39	R.42	R.53	Total	Rank
Cucurbita pepo	4	5	5	4	4	5	5	31	1
Hordeum vulgare	5	3	4	4	5	1	4	26	3
Linum usitatissimum	3	5	5	4	4	5	4	30	2
Trigonella foenum graecum	4	5	1	3	4	3	2	22	5
Urtica simensis	3	4	3	1	5	3	4	23	4

Table 13. Preference ranking of six selected functional food plants on the degree of preventing several diseases by key informants (respondent).

Functional Food plant	R.11	R.28	R.43	R.62	R.67	R.73	R.80	Total	Rank
Cicer arietinum	3	2	3	4	4	3	3	22	3
Linum usitatissimum	5	5	4	4	5	5	4	32	1
Lycopersicon esculentum	1	1	5	2	1	2	1	13	6
Nigella sativa	2	2	1	3	4	2	1	15	5
Trigonella foenum graecum	2	3	2	2	3	2	5	19	4
Triticum dicoccon	4	5	4	5	5	3	4	30	2

Table14. Direct matrix analyses of selected functional food plants.

Species	Use category						Total	Rank
	Functionality	Food/drink	Spice	Medicinal plants	Forage	Construction		
Avena sativa	5	4	0	5	5	1	20	3
Cicer arietinum	3	5	0	2	4	0	14	7
Eragrostis tef	5	5	0	2	5	5	22	1
Guizotia abyssinica	4	4	2	4	5	0	19	4
Hordeum vulgare	4	5	0	4	4	4	21	2
Lycopersicon esculentum	3	4	4	4	0	0	15	6
Thymus schimperi	3	0	5	3	2	0	13	8
Vicia faba	3	5	0	4	4	0	16	5

Note: Use values are given from 0 to 5; 5: Best, 4: very good, 3: good, 2: less, 1: least and 0: no value.

Table 15. Fidelity level indexes for plant species used to treat Diabetes mellitus and hypertension in the study area.

Disease	Percentage of informants	Species	NP	N	Fidelity level index (NP/N)
Diabetes mellitus	67.5	*Avena sativa*	35	54	0.65
	21.3	*Cicer arietinum*	10	17	0.58
	11.3	*Cinnamomum verum*	7	9	0.78
	78.8	*Eragrostis tef*	26	63	0.41
	10	*Sorghum bicolor*	6	8	0.75
	48.8	*Triticum dicoccon*	28	39	0.72
Hypertension	13.8	*Thymus schimperi*	5	11	0.45
	53.8	*Allium sativum*	38	43	0.88
	22.5	*Beta vulgaris*	10	18	0.56
	10	*Sorghum bicolor*	6	8	0.75
	90	*Lupinus albus*	64	72	0.89
	48.8	*Triticum dicoccon*	26	39	0.67

Fidelity level index of functional food plants used to prevent diabetes and hypertension

The percentage of informants who claim to use certain plant species for the same main reason, or to confirm the effectiveness of plant species in assessing its value, is known as the fidelity level (FL) (Amenu 2007). Fidelity level index was measured for functional food plants used to prevent diabetes and hypertension, and the results showed that *Cinnamomum verum* (Kerefa) and *Lupinus albus* (Gibto) have the highest functional value to prevent diabetes and hypertension, with (FL=0.78) and (FL=0.89), respectively (Table 15).

Description of most cited (ten top) functional food plants in the study area with their functionality

All functional food plants (10 species) that had cited by informants above 45% (Table 11) are described below.

Lupinus albus L. (Fabaceae) GIBTO (Amh.)

Lupinus albus L. is an herbaceous plant with a shortly hairy, bushy steam; lower leaves to have obovate leaflets, and upper leaves to be obovate-cuneate. It has a white to blue corolla. The pod is villous and glabrescent, and the seeds are smooth. The plant is especially popular to be planted in Gojjam. In the study region, the seed is primarily used to prevent hypertension. According to Nigussie (2012), people in northwestern Ethiopia used "Gibto Areki" as a locally-made antihypertensive medicine. In Ethiopia, the fruit and seed of this plant are both used for the same functional reason, according to Ragunathan and Solomon (2009).

Eragrostis tef L. (Poaceae) TEFF (Amh.)

Eragrostis tef L. is a cultivated herb that grows in tufts and can reach a height of 120cm in cultivated plants. The leaf blades are narrow, and the panicle is folded and depressed at the base; the spikes are grey or golden, with up to ten florets, and the seedlings are tiny and delicate. Ethiopia is home to this species. Two types of teff are grown in Ethiopia, one with white grain (Nech teff) and the other with brown grain (Dabbo teff). The seeds of this plant, especially Dabbo teff, are used to prevent anemia and diabetes mellitus in some parts of the world (lower blood glucose). Teff contains omega-3 fatty acids, which have been linked to a reduced risk of heart attacks and prostate tumor development, as well as omega-6 fatty acids, which have been linked to a reduced risk of cardiovascular disease, and docosahexaenoic acids, which have been linked to a reduced risk of breast cancer, according to different authors cited on SUDASR23 (2010).

Avena sativa L. (Poaceae) ENGEDO (Amh.)

The herb Avena sativa L. is cultivated. It can show up as a wheat contaminant or as an escape, near cultivations on rare occasions. Culms are simple, erect, and 40-180cm tall. The uppermost florets are reduced to 1.7-3cm long and non-shuttering, and the florets lack a basal bearded callus. The glumes are narrowly elliptic, oblong, and acute. Lemmas are robust and glabrous hairy around the awn insertion, measuring 1.2-2.5cm in length. In Ethiopia, it is typically grown at altitudes of 2700-3000m, particularly in areas where soil fertility is declining, and it is preferred over barley. Forage is made from crop residue. The plant seed was traditionally used to lower blood sugar, prevent osteoporosis, and improve overall health. According to Lance and Garren (2002), beta-glucan in the seed can lower blood cholesterol and help prevent heart disease, and the seed of this plant can also control gastrointestinal function.

Cucurbita pepo L. (Cucurbitaceae) DUBA (Amh)

Cucurbita pepo L. is an annual herb with ascending, creeping, or bushy, 5-angled stems up to 15 meters long in some varieties. The branched shallow root stem grows from a well-developed taproot. Scabrous and setose stems branch and sometimes root at the noods. It has unisexual yellow flowers that are on the same plant as the male. The fruit was cooked simply with water and eaten when cool to prevent gastritis, and the seeds were roasted and eaten to prevent helminthiasis. In various studies, pumpkin (Duba) and other vegetables are regarded as functional plant-based foods. Braun and Cohen (2007) looked at how beta-carotene in pumpkin affects the immune and hormonal systems and its ability to protect against lung cancer.

Linum usitatissimum L. (Linaceae) TELBA (Amh)

Flax is a slender annual herb that grows to a height of 0.3 to 0.9 meters. It has thin, pale green alternating leaves and branches at the end. One or two delicate blue flowers are tipped on each branch. The seed of this plant is used by the locals to prevent gastritis, constipation, and breast cancer, as well as to boost attention deficiency, but some informants advised that a child taking “absho" for this purpose should avoid flaxseed as a side effect. The alpha-linolenic acid, lignans, and fiber in flaxseed have possible health benefits such as reducing cancer, diabetes, osteoporosis, atherosclerosis, autoimmune, arthritis, cardiovascular disease, and neurological disorder, according to Martinchik et al. (2012). Aside from that, it helps with menopausal symptoms and attention deficit disorder. Furthermore, according to Roizen and Oz (2005), eating flax seeds on a regular basis will reduce real age by 3.4 years due to monounsaturated fats.

Citrus aurantifolia L. (Rutaceae) LOMI (Amh)

Citrus aurantifolia is an evergreen shrub that grows up to 5 meters tall and has many short sharp spines on the stems and beside the leaves. The leaves are oval and delicate, with a gleaming green color. Flowers are white, and the fruit is round or oval, up to 6cm in diameter but typically smaller, with a thin green or yellow peel. The pulp is green, acidic, and juicy. The locals used these plant fruits to protect themselves from microbial diseases and nekersa. According to Wondimu et al. (2000), the community of "Dheerra city" used the fruit of this plant as a nutraceutical to treat flu, wounds, stomachache, and skin rash. On the other hand, Hasegawa, and Miyake (1996) discovered that the phytochemical limonoids contained in citrus fruits were responsible for anticancer activity.

Hordeum vulgare L. (Poaceae) GEBIS (Amh)

Hordeum vulgare L. is an upright, stout, and tufted annual grass. Leaf is truncate and 1-2 mm long. The leaf blades measure 10-15 mm in width. There are three flower anthers, and the ovary is pubescent at the apex. It has a caryopsis fruit with an adherent pericarp. The local community used the plant to prevent Gastritis as a type of "Beso" or underlying "Kolo," and it was also thought to provide extra strength during illness and for mothers during childbirth as a form of "Gonfo." According to Robles-Escajeda et al. (2013), bioactive compounds found in barley (Beta-glucans, tocotrienols, and poly-phendlunasin) have anticancer properties.

Allium sativum L (Alliaceae) NECH SHINKURT (Amh)

Allium sativum L a cultivated herb that can be found in both home gardens and farmland. Bulb ovoid with a white coat that encloses the bulb leaf. The bulbs used to prevent hypertension and microbial and nekersa were all found in the research field. To name a few, the supposed health benefits include cancer chemoprevention, antibiotic, anti-hypertensive, and cholesterol-lowering properties (Srivastava et al. 1995). The bulb of this plant had a nutraceutical benefit to treat flue and lung abscess in the population of "Dheerra city," according to Wondimu et al. (2000). Ernst (1997), on the other hand, discovered that alliums can protect against cancer of the gastrointestinal tract.

Triticum dicoccon Schrank (Poaceae) AJJA (Amh)

Triticum dicoccon is strong or thick-wailed annual cultivated grass culms. Rhachis glabrous or shortly ciliated at the nodes, fragile, disarticulating above the spikelet insertion, spikelet falling attached to the internode below; spikelets 3-10 cm long, laterally compressed; rhachis glabrous or shortly ciliated at the nodes, fragile, disarticulating above the spikelet insertion, spikelet falling attached to the internode below. Glumes 7-10 mm long, coriaceous with a single prominent keel; lemma with an awn up to 15 cm long; spikelet 3(4) flowered. The locals in the study area used this food plant to treat diabetes Miletus, hypertension, and cholesterol in the blood and maintain general health and bone strength often as a form of “Atmit”. Phytochemical saponins and functional component B-glucan in oats help lower LDL cholesterol, which may lower blood pressure and lower the risk of heart disease.

Lycopersicon esculentum Mill. (Solanaceae) TIMATIM (Amh)

Lycopersicon esculentum is an annual or perennial herb with petiolate leaves that alternate. Flowers have two sexes. Above the base, pedicels articulate. The calyx is strongly 5-lobed, and the yellow corolla is stellate. It has 5 stamens of similar length and a bilocular or plurilocular ovary with false septae. The fruit is a berry that is red or orange in color and has elliptical seeds. The locals claimed that eating fresh tomatoes or tomato paste could help them avoid anemia and heart disease. On the other hand, Krinsky and Johnson (2005) say that the phytochemical lycopene found in tomatoes helps prevent prostate cancer. This phytochemical in tomatoes has also been shown to inhibit the growth of breast, lung, cervical, ovarian, and pancreatic tumors, according to Rao and Rao (2007).

In conclusion, the study's findings showed that indigenous people in the study region have their own traditional knowledge and practices on functional food plants that they have learned over centuries. Most the area's usable food plants are grown at home and in the field. Only one species, Urtica simensis Steudel, is purchased from the market for consumption, and it is a wild rare species in the area. Herbs were the most used functional food plants, followed by shrubs and trees. Seeds have also been discovered to be the most used plant parts in Functional Food. Although the residents' favorite method of preparing functional food plants is cooking/boiling, most of them are prepared this way. The status of almost all functional food plants in the study area was common because they are common demands of their daily life activities, but one of the most cultivated functional food plants in Gojjam, *Lupinus albus*, is under threat due to the loss of agricultural lands in the case of construction as the woreda is developed into an administrative town, responsible stakeholders should make so strong effects. It was also stated that at the time of suffering from the disease, information transfer among the population about the functionality of foods was presupposed. As a result, it is critical to continue to build up a safe citizen documentation of functional food plants by passing on information to current and future generations to raise our awareness and avoid chronic diseases triggered by a drastic change in our lifestyle and environmental effects.

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