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Transmission efficiency of *Fusarium oxysporum* f.sp. cepae in bulbs of shallot (*Allium cepa* L. group *aggregatum*)

Andini Hanif[1,2*], Suryo Wiyono[1] , Abdul Munif[1] and Sri Hendrastuti Hidayat[1]

*[1]Departement of Plant Protection, Faculty of Agriculture, IPB University, Jl. Kamper Babakan Dramaga, Bogor 16680, West Java, Indonesia. [2]Departement of Agrotechnology, Faculty of Agriculture, Universitas Muhammadiyah Sumatera Utara, Jl. Kapten Muchtar Basri 3, Medan 20238, North Sumatera, Indonesia. *E-mail: andinihanif@umsu.ac.id*

Abstract

Fusarium oxysporum f. sp. *cepae* (Foc) in bulb is a source of inoculum for *Fusarium* basal rot disease of shallot. This research aimed to determine the transmission efficiency of Foc carried by bulb of shallot to plants, and the relationship between shallot variety and soil origin with the efficiency of pathogen transmission. This research used two varieties of shallots 'Batu Ijo' and 'Bima', with six different seed lots. The bulbs of shallot were tested for seed health using the blotter test and growing on test methods and tested for transmission efficiency on several sources of soil. The results showed a correlation between the infection of Foc in the bulbs of shallot and the incidence of basal rot on shallot plants. The results also showed a relationship between plant varieties and soil properties on the efficiency of Foc transmission Foc to shallot bulbs. Shallot varieties that are resistant to Fusarium basal rot disease have lower transmission efficiency of Foc to bulbs compared to susceptible varieties.The study also revealed that soil from Samosir, which had higher nitrogen and clay content, along with a larger population of soil microorganisms, showed increased suppression of *Fusarium* basal rot disease. This, in turn, helped reduce the transmission efficiency of Foc in shallot bulbs.

Key words: *Fusarium* basal rot disease, soil, varieties

Introduction

Shallots (*Allium cepa* L. group *aggregatum*) are one of the important horticultural crops. Shallots are used as a cooking spice and have chemical compounds that are useful for health. Shallot is cultivated by farmers in almost all provinces in Indonesia. Disruption of pests and diseases is one of the main problems in shallot cultivation. One of the main diseases in shallots is Fusarium basal rot disease which is caused by infection with *Fusarium oxysporum* f.sp. *cepae* (Foc). Incidence of Fusarium basal rot disease on shallots has been reported in several countries such as India (Dinakaran *et al.*, 2013), Vietnam (Le *et al.*, 2020), Iran (Motlagh *et al.*, 2010). The incidence of Fusarium basal rot disease in India reached 74.33 % (Shamyuktha *et al.*, 2020), and reported of yield losses due to this disease reached 45 % in Africa (Sintayehu *et al.*, 2011). Fusarium basal rot disease can lead to yield losses of up to 50 to 100% in Indonesia (Herlina *et al.*, 2021). Reports of fusarium basal rot disease incidence in Indonesia include Brebes and Probolinggo regencies in Jawa Timur Province, with rates of 32.1% and 80%, respectively (Supyani *et al.*, 2021; Sholeh *et al.*, 2023), as well as 33.9% in Bantul and in Yogyakarta province (Aisyah *et al.*, 2021).

Pathogenic infections in the generative growth phase can cause seed-borne diseases. Several types of shallot pathogenic fungi that are seed-borne are *Fusarium* species, *Rhizoctonia solani*, *Alternaria alternata, A. porri, Botrytis allii, Aspergillus* sp., *Colletotrichum* sp., and *Sclerotium* sp. (Rajapakse and Edirimanna 2002; Adongo *et al.*, 2015). Foc is one of the dominant fungi found on shallots and causes Fusarium basal rot disease (Fadhilah *et al.*, 2014; Saputri *et al.*, 2018). Foc in shallot bulbs as a seed-borne pathogen is one of the main problems in agriculture and disease management. The movement of shallot bulbs as seeds for trade and germplasm exchange increases long-distance dispersal and permanent establishment of Foc in new areas. Foc carried by shallot bulbs has the potential to cause Fusarium basal rot disease in shallot plants. Fungal infection on bulbs is higher than on shallot seeds. Foc infection in shallot bulbs was 22.5 %, whereas Foc was not found in shallot seeds (Saputri *et al.*, 2018).

Seed transmission refers to the transfer of seed-borne pathogens from seeds to seedlings and plants. Seed transmission efficiency is an indicator of the ability of pathogen inoculum in seed to cause disease symptoms in plants. Transmission efficiency of seed-borne pathogens is the fraction of seed-borne infections causing disease symptoms in plants (Agarwal and Sinclair, 1997; Akem and Melouk, 1990). Pathogen transmission efficiency directly determines whether pathogen seed inoculum can cause disease in plants (Nallathambi *et al.*, 2020). The efficiency of seed-borne pathogen transmission can be measured based on the transmission ratio, the ratio between pathogen infection in seeds and the incidence of plant disease in the field. Information of efficiency of pathogen transmission is needed for the management of seed-borne diseases, for example to recommend plant genotypes for planting (Ojiambo *et al.*, 2003). The efficiency of transmission has been studied in several plant disease pathogens including Cucumber green mottle mosaic virus on cucurbit crops, *F. proliferatum* and *F.verticilloides* on maize, *Alternaria sesami* on sesame seed (Li *et al.*, 2015; Al-Juboory and Juber 2013; Ojiambo *et al.*, 2003). There are no reports regarding Foc transmission efficiency on shallot bulbs, as well as its relation to soil properties and plant varieties.

Therefore this research aimed to determine the efficiency of Foc transmission carried by shallot bulbs and its relationship to a variety of shallots and soil source. This is important to determine the potential of shallot varieties and soil source in the transmission of Foc from shallot bulb to plants, as a basis for developing methods for controlling the disease.

Materials and methods

Shallot bulbs sample: The shallot bulbs of two varieties, 'Batu Ijo' and 'Bima'used were bulbs as planting material (seed bulbs). The bulbs were collected from six different seed lots from different source of harvesting location, hereinafter referred to as 'Batu Ijo' 1 to 'Batu Ijo' 6, and 'Bima' 1 to 'Bima' 6 (Table 1). The bulbs were sorted based on the criteria for shallot bulbs used for seeds, including having a uniform shape and size of the bulbs, and bulbs not rotten.

Table 1. Source of harvesting shallot bulb

Varieties	Sample	Source
Batu Ijo	Batu Ijo 1	Ngantang, Malang Regency, East Java, Indonesia
	Batu Ijo 2	Bersole, Tegal Regency, East Java, Indonesia
	Batu Ijo 3	Cikole, Bandung Barat Regency, West Java, Indonesia
	Batu Ijo 4	Nagalingga, Karo Regency, North Sumatera, Indonesia
	Batu Ijo 5	Pandai Sikek, Tanah Datar Regency, West Sumatera, Indonesia
	Batu Ijo 6	Badan Standarisasi Instrumen Pertanian (BSIP), North Sumatera, Indonesia
Bima	Bima 1	Sisalam, Brebes Regency, Central Java, Indonesia
	Bima 2	Siwungkuk, Brebes Regency, Central Java, Indonesia
	Bima 3	Bersole, Tegal, Central Java, Indonesia
	Bima 4	Marelan, Deli Serdang Regency, North Sumatera, Indonesia
	Bima 5	Kedondong, Demak Regency, Central Java
	Bima 6	Pesantunan, Brebes Regency, Central Java, Indonesia

Seed health testing using the Blotter test: A total of 200 shallot bulbs were prepared for each seed lot. After surface sterilization with NaClO_2 % for 1 minutes and rinsed with sterile distilled water 3 times, the shallot bulbs were placed in a plastic box filled with three sheets of moistened sterile filter paper. Each box containing 25 bulbs was repeated for eight repetitions. The bulbs were incubated for eight days in a room temperature (25-27 ^{O}C) and the percentage of Foc infection and the percentage of necrosis in the basal plate of shallot bulbs were calculated (Fadhilah *et al.*, 2014). The percentage of Foc infection in the tubers and the number of necrotic tubers in the basal plate were calculated using the following formula:

$$\text{Foc infection (\%)} = \frac{\text{Total of infected bulbs}}{\text{Total of bulbs observed}} \times 100$$

$$\text{Necrosis basal plate (\%)} = \frac{\text{Total bulbs necrosis basal plate}}{\text{Total of bulbs observed}} \times 100$$

Seed health testing using the growing on test: A total of 200 shallot bulbs were prepared for each seed lot. Growing on test was carried out in a screen house by planting prepared shallot bulbs in sterilized sand media. Shallot bulbs that have been surface sterilized and cut off 1/3 of the tip of the bulb were planted in sterile sand media. Each box contained 25 bulbs, repeated for eight repetitions. The shallots were incubated in screen house at temperature 27-30 ^{o}C and humidity at 55-75 %. The observation were made up to 21 days after planting. The observation parameter was the incidence of Fusarium basal rot disease in shallot plants. The percentage of Fusarium basal rot disease incidence was calculated using the formula:

$$\text{Disease incidence (\%)} = \frac{\text{Total plants with fusarium basal rot symptoms}}{\text{Total of plants observed}} \times 100$$

Testing of transmission Foc by bulbs shallot in several soil sources: This research uses soil from several areas in North Sumatera, Samosir Regency, Karo Regency and Binjai Regency. The soil that has been prepared was mixed with compost in a ratio of 3:1, and filled into polybags. The treatment was repeated three times, with 15 plants in each replication. Shallot bulbs were planted in prepared polybags, and disease incidence was observed weekly for 21 days after planting. To assess the efficiency of Foc transmission carried by the shallot bulbs, the transmission ratio was used, which compares the ratio of Foc infection on the bulbs to the incidence of Fusarium basal rot disease in the field.

The soils used were analyzed to determine its chemical, physical and biological properties. The soil chemical properties analyzed include soil pH, CEC, soil chemical compound content (C-Organic, total N, Phosphate, Ca, Mg, K, and Na). The physical properties of the soil analyzed were soil texture (sand, clay and dust). The biological properties of the soil analyzed were the population and diversity of soil microbes, the population of phosphate solubilizing, potassium solubilizing and nitrogen fixing microbes. Analysis of the chemical and physical properties of the soil was carried out by sending samples to the laboratory of PT Scofin Indonesia North Sumatera.

Data analysis: Data on Foc infection in shallot bulbs and the incidence of Fusarium basal rot disease in shallot plants were collected and processed using ANOVA test with SPSS 25.0. The relationship between Foc infection in shallot bulb seeds in the blotter test and growing on test for each seed lot sample was analyzed by linear regression using SPSS 16.0.

Results and discussion

Infection of *Foc* in shallot bulbs: Infection of Foc in shallot bulbs is indicated by necrosis in the basal plate of the bulb. The level of Foc infection in shallot bulbs can be demonstrated by measuring the percentage of necrosis in the basal plate of the bulb. Blotter test showed that the highest infection of Foc in bulbs shallot was in 'Batu Ijo' 3, with a percentage of necrosis in basal plate of 64.6 %. This result showed that 3 % of the other bulbs were infected with non-pathogenic Fusarium, so there were no symptoms of necrosis on basal plate. The highest infection of Foc in bulbs shallot of the 'Bima' variety was found in 'Bima' 6, with a percentage of basal plate necrosis of 79 % and Foc infection of 74.5 %. The results showed that all Fusarium infections in shallot bulbs were pathogenic, while other bulbs showing symptoms of necrosis were infected by pathogens other than Fusarium (Fig. 1). Foc infection in shallot bulbs was characterized by the presence of white hyphae at the true stem and root. When the bulbs are cut open, necrosis is observed in the basal plate tissue (Fadillah *et al.*, 2014).

Widono *et al.* (2022) reported that the basal plate of shallot bulbs showed sensitivity to Foc infection compared to other parts. The Foc pathogenicity test from shallot bulbs showed symptoms of necrosis in the basal plate tissue of the bulb and

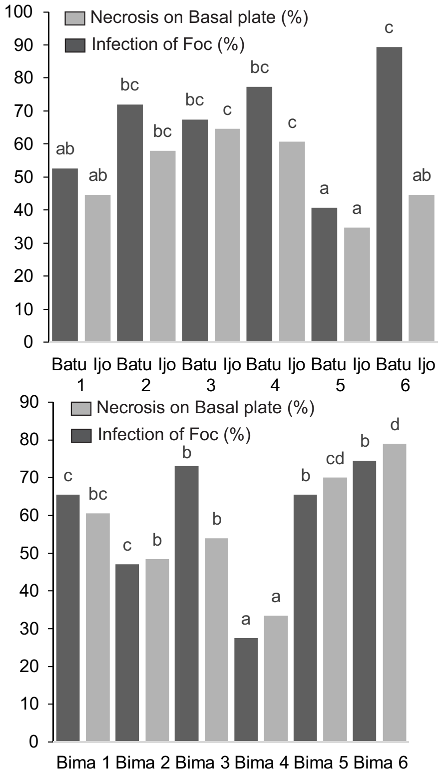

Fig. 1. Infection of Foc in shallot bulbs using the blotter test method and observing necrosis on basal plate of shallot bulbs; Var. 'Batu Ijo' (Above), Var. 'Bima' (Below). The same letter in each column charts are not significantly different in DMRT test at *P*=0.0

has the potential to be a pathogen in plants and cause symptoms of Fusarium basal rot disease in shallot plants (Saputri *et al.*, 2018). According to Fadhilah *et al.* (2014), the presence of Foc infection and pathogenicity in bulbs can be determined from the parameters of necrosis in the basal plate of shallot bulbs.

The results of growing on test proved that the shallot bulbs carried Foc which causes Fusarium basal rot disease. This is indicated by the symptoms in shallot plant as a result of Foc infection carried by shallot bulb, characterized by the color of the leaves turning pale green to yellowish from the top to the base of the leaf, the leaves becoming twisted, the leaves becoming brittle and dry, until finally the plant becomes dry. The symptoms of Fusarium basal rot in the growing on test are similar to the symptoms of Fusarium basal rot disease reported by Lestiyani *et al.* (2016). Foc infection of shallot bulb is important in the spread of Fusarium basal rot diseases. Foc infection in shallot bulbs can reduce seed quality and cause Fusarium basal rot disease in shallot plants (Dabire *et al.*, 2021).

The results of the growing on test showed that the average incidence of disease in variety 'Bima' was 21.2 %, higher than variety 'Batu Ijo' 10.5 % (Fig. 2). These results showed that in testing seed health by growing on test, the incidence of Fusarium basal rot disease in susceptible varieties of shallots is higher than in resistance varieties of shallots. The 'Bima' variety is grouped as a susceptible variety and 'Batu Ijo' is a variety as resistant to Fusarium basal rot disease (Maulidha 2023).

The results also showed that the origin of shallot seed bulbs affected Foc infection in shallot bulbs and plants. Varieties 'Batu Ijo' ('Batu Ijo' 1, 2, and 3) and 'Bima' ('Bima' 1,2,3,5, and 6) originating from Java Island had higher levels of Foc infection in bulbs and plants compared to shallot seed bulbs originating from Sumatera Island. These results indicated that shallot seed bulbs originating from shallot fields infested with Fusarium basal rot disease will carry Foc inoculum on the seed bulb, thus causing Fusarium basal rot disease in planting shallots in new fields. The incidence of Fusarium basal rot disease of shallot has been reported in the center of shallot production on Java island, while in Sumatera island there have been no reports of incidence of Fusarium basal rot disease. The incidence of fusarium basal rot disease in Brebes Central Java reached 60 %, Nganjuk 77.7 % to 100 %, Bantul Yogyakarta reached 33.97 %, (Supriyadi *et al.*, 2021; Lestiyani *et al.*, 2021; Wibowo *et al.*, 2023). Herlina *et al.* (2021), reported that the the causes of Fusarium basal rot disease on the island of Java in Indonesia are *F. oxysporum, F. verticillioides, F. solani* and *F. proliferatum.*

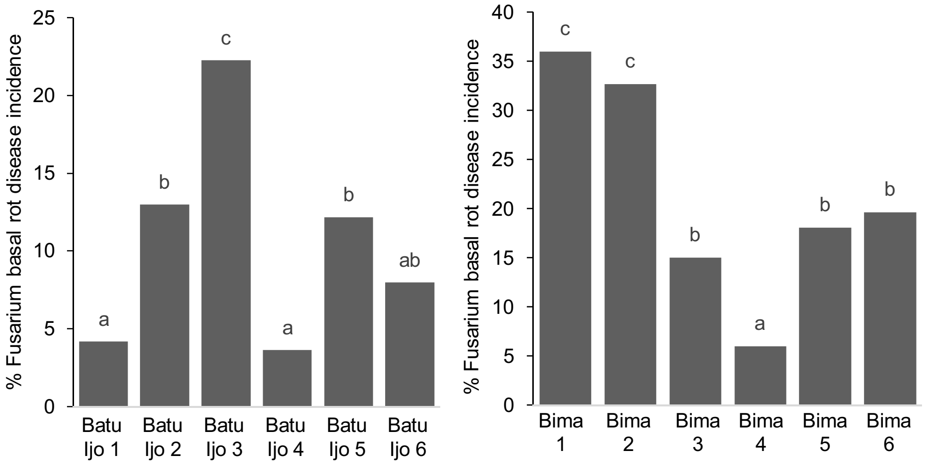

Fig. 2. Incidence of Fusarium-basal-rot disease using the growing on test-method. Var.'Batir Ijo' (right) and Var.-'Bima' (left). The same letter in each column are not significantly different in DMRT test at *P*=0.05

Correlation of Foc infection in shallot bulbs and fusarium basal rot disease incidence: The results of the correlation between infection of Foc in bulbs, which is characterized by necrosis on basal plate, and the incidence of Fusarium basal rot disease of the 'Batu Ijo' variety had a correlation coefficient (r) of 0.470 with a moderate degree of correlation, higher than the 'Bima' variety with a correlation coefficient (r) of 0.407 (Fig. 3). The correlation value (r) between infection of Foc in bulbs and the incidence of Fusarium basal rot disease, indicated that bulbs infected of Foc with symptoms of necrosis on the basal plate, have the potential to produced shallot plants with symptoms of Fusarium basal rot disease. The results of this correlation indicate that the basal plate

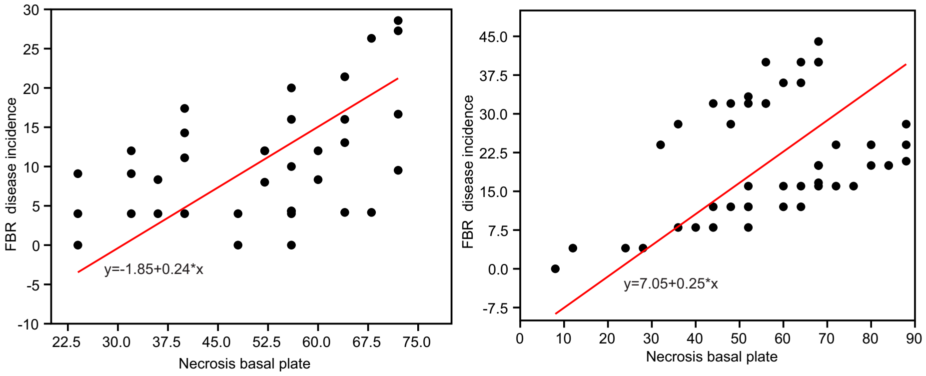

Fig. 3. Correlation between necrosis of basal plate in shallot bulb and Fusarium basal rot disease incidence, on 'Batu Ijo' (left) and 'Bima' (right) varieties

necrosis parameter of bulb shallot can be used as a parameter to estimate the incidence of Fusarium basal rot disease of shallot plants in the field (Fadhilah *et al.*, 2014).

Transmission efficiency of Foc carried by shallot bulbs: The transmission efficiency of Foc of shallot bulbs on plants is seen from the transmission ratio. Transmission ratio is ratio of infection Foc of shallot bulbs and incidence of Fusarium basal rot disease in plants. The lower transmission ratio indicates a higher efficiency of seed-borne pathogen inoculum being transmitted to the plant. Based on transmission ratio of Foc in both varieties, transmission efficiency of Foc in shallot bulbs of 'Batu Ijo' variety was lower than that of 'Bima' variety (Fig. 4).

Varieties of plants are related to the efficiency of transmission of seed-borne pathogens to plants. In a study by Akem and Melouk (1990), they reported that there was an influence of host genotype resistance on the transmission efficiency of the *Sclerotonia* pathogen carried by peanut seeds to plants. Based on the characteristics of shallot resistance to Fusarium basal rot disease, the 'Bima' variety was grouped into varieties susceptible to Fusarium basal rot disease (Aprilia *et al.*, 2020). The transmission efficiency of Foc in 'Bima' was higher than 'Batu Ijo' variety. These results proved that resistant varieties to Fusarium basal rot have low transmission efficiency and susceptible varieties have high transmission efficiency. Shallot bulbs planted in soil from Samosir had low transmission efficiency compared to soil from Karo and Binjai. This is showed by the three seed lots that did not cause an incidence of Fusarium basal rot disease, and the seed lot that caused the disease incidence had a high transmission ratio, this means that Foc carried by shallot bulbs has low transmission efficiency to plants on soil origin from Samosir (Table 3). These results indicated that the soil from Samosir was suppressive soil to Fusarium basal rot disease compared to the soil from Karo and Binjai.

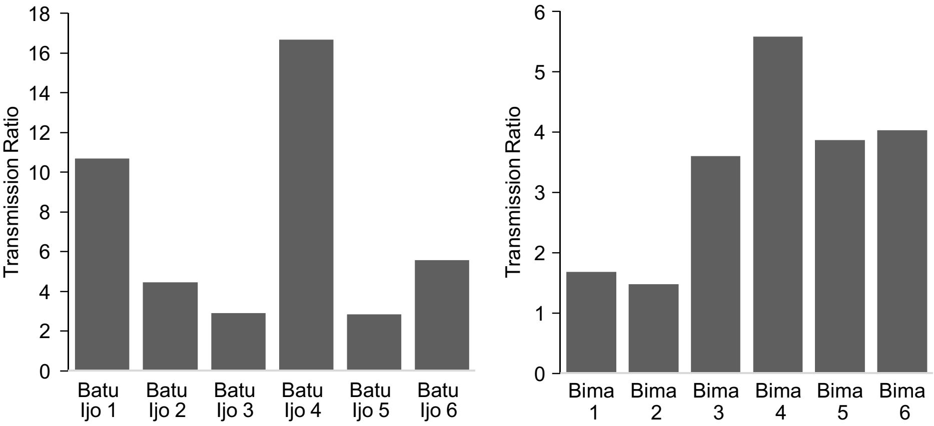

Fig. 4. Transmission ratio of Foc carried by shallot bulbs in sterile sand

The origin of the shallot bulb also influenced the transmission efficiency of Foc in shallot bulb (Table 3). 'Batu Ijo' shallot bulbs from Sumatera island did not have Foc transmission carried by the bulbs in the soil treatment from Samosir and the transmission ratio was higher than shallot bulbs from Java Island which were planted in soil from Karo and Binjai. 'Bima 5' shallot bulb from Sumatera island had a higher transmission ratio than other seed lots.

The transmission efficiency of pathogen inoculum from seeds to plants is associated with several factors including the environment, host plant genotype, and pathogen virulence. Several studies have been conducted to determine factors related to the efficiency of seed pathogen transmission, including soil moisture (Robert 1992), environmental temperature (Wilke *et al.*, 2007), the location of the pathogen inoculum in seeds (Al-Juboory and Juber, 2013), and soil microbial diversity (Rochefort *et al.*, 2021).

Environmental factors influence the seed transmission efficiency of plant pathogens (McGee 1995). Disease suppressiveness of soil from Samosir can be caused by chemical, physical, and biological properties of soil or their interaction. Soil properties are one of the environmental factors that influence the transmission efficiency of seed-borne pathogens. Soil properties that are related to the infection of Foc in shallot bulbs are the biological, chemical, and physical properties of the soil. The results of the analysis of properties of soil in this study showed that the nitrogen and clay content and also the population of microorganisms in the soil from Samosir, which was higher than in the soil from Karo and Binjai, had the increased potential as suppressive soil to Fusarium basal rot disease (Tabel 3).

The nitrogen content in the soil is related to the suppression of Fusarium wilt disease, increasing nitrogen has been shown to reduce the severity of the disease in plants. Lack of nitrogen will cause

Table 2. Transmission ratio of Foc carried by shallot bulbs in several soil sources

Sample	Bulbs infection (Blotter) (%	Soil from Karo		Soil from Samosir		Soil from Binjai	
		Disease incidence (%)	Tranmission ratio	Disease incidence (%)	Tranmission ratio	Disease incidence (%)	Tranmission ratio
Batu Ijo 1	44.66^{ab}	18.33^{abcde}	2.43	9.36^{abcd}	4.77	18.60^{abcde}	2.40
Batu Ijo 2	58.00^{bcd}	6.67^{abcd}	8.69	6.82^{abcd}	8.50	21.43^{abcde}	2.70
Batu Ijo 3	64.66^{bcd}	13.49^{abcd}	4.79	14.95^{abcd}	4.32	14.72^{abcd}	4.39
Batu Ijo 4	60.66^{bcd}	4.44^{abc}	13.66	0^{a}	-	0^{a}	-
Batu Ijo 5	34.66^{a}	0.0^{a}	-	0^{a}	-	2.22^{ab}	15.61
Batu Ijo 6	44.66^{ab}	2.22^{ab}	20.11	0^{a}	-	13.33^{abcd}	3.35
Bima 1	60.50^{bcd}	48.89^{e}	1.23	37.78^{de}	1.60	23.33^{abcde}	2.59
Bima 2	48.50^{ab}	33.33^{bcde}	1.45	22.22^{abcde}	2.18	24.44^{abcde}	1.98
Bima 3	54.00^{abc}	28.89^{abcde}	1.86	22.22^{abcde}	2.43	24.44^{abcde}	2.21
Bima 4	33.50^{a}	11.11^{abcd}	3.01	4.44^{abc}	7.61	8.88^{abcd}	3.77
Bima 5	70.00^{cd}	26.67^{abcde}	2.62	13.33^{abcd}	5.25	20.00^{abcde}	3.50
Bima 6	79.00^{d}	35.56^{cde}	2.22	28.89^{abcde}	2.73	28.89^{abcde}	2.73

* Numbers followed by the same letter in each column charts are not significantly different in DMRT test at the 5% level (α = 5%)

Table 3. Chemical, physical and biological properties of soil

Parameter	Soil source		
	Karo Regency	Samosir Regency	Binjai Regency
pH H_2O	6.62	6.25	6.72
C (%)	1.58	1.74	3.66
N - Kjehdahl (%)	0.17	0.21	0.17
P - Bray II (mg/kg)	389.28	280.16	420.98
KTK (me/100g)	28.15	9.88	14.62
K (me/100g)	1.59	0.43	1.48
Ca (me/100g)	6.25	3.14	9.05
Mg (me/100g)	3.65	1.23	2.58
Na (me/100g)	0.21	0.16	0.26
Sand (%)	72.60	65.90	71.50
Slity (%)	18.17	20.45	17.75
Clay (%)	9.21	13.62	10.72
Bacterial population (cfu/g)	2.37×10^8	6.97×10^8	4.73×10^8
Fungi population (cfu/g)	3.1×10^5	2.67×10^7	1.9×10^6
Bacterial diversity	1.0	1.0	1.1
Fungi diversity	0.5	0.7	0.7
Population of phosphate solubilizing bacteria (cfu/g)	3.8×10^4	7.1×10^4	3.2×10^4
Population of phosphate solubilizing fungi (cfu/g)	3.5×10^3	2.3×10^4	2×10^4
Potassium solubilizing microbes	(-)	(-)	(-)
Nitrogen fixation Microbes	(+)	(+)	(+)

plant stress so that the plant will be susceptible to disease (Dita *et al.*, 2018). Deltour *et al.* (2017) reported high clay content in soil correlated with suppression of wilt disease in soil infested with Foc. Clay content can suppress disease by changing oxygen diffusion, pH buffering, and nutrient availability (Orr *et al.*, 2018).

Soil from Samosir had a higher population of microorganisms than other soils, both bacteria and fungi (Table 3). Soil microorganisms are thought to influence the transmission of Foc from shallot bulbs to plants. The high population of microorganisms in Samosir soil was associated with the low transmission efficiency of Foc from shallots bulb in soil from Samosir. The population of microorganisms in the soil provides many benefits for plants. The presence of microorganisms in the soil can suppress plant diseases by stimulating the production of phytohormones, competition with pathogens, production of nutrients, or activating microbiota-modulated immunity in plants (Enebe *et al.*, 2019; Vannier *et al.*, 2019).

Infection of Foc in shallot bulbs and incidence of Fusarium basal rot disease of shallot had a correlation. The transmission efficiency of Foc carried of shallot bulbs was related to the shallot variety, physiochemical properties, and microorganisms population of soil. Therefore, the use of resistant varieties of shallots and planting shallots in suppressive soil to Fusarium basal rot disease is recommended for farmers to reduce the efficiency of transmission of Foc carried by shallot bulbs, so that it can prevent the spread of Fusarium basal rot disease carried by bulb shallot.

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