Virulence test of fusarium isolates against local shallot accessions in the highlands of Karo regency

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Abstract. North Sumatra Province still lacks shallots every year. the obstacles that are often encountered and seen in farmers' fields are disturbance of plant pests, generally diseases, especially fusarium basal rot disease. The purpose of this study was to detect the emergence of Fusarium isolates from various sources of infection locations against the increasing attack of fusarium bulbs rot that occurred in 2018 to 2019. Shallots are cultivated on Karo regency in Tongging Village, Rimokayu Village, Tiganderket Village and Payung Village. Statistical analysis showed that the Tongging Fusarium isolate was the most virulent against all the local accession. While Payung Fusarium most virulent against Rimokayu and Payung accession, and Tiganderket Fusarium is the most virulent against Rimokayu and Tiganderket accession. Only two isolates were among the highest virulent, that Tiganderket Fusarium isolate against Payung accession and Tongging accession, and Payung Fusarium isolate against Tiganderket accession and Tongging accession. Batu Bara Fusarium isolate and Deli Serdang Fusarium isolate, was classified as virulence and least virulent against Tongging accession, Payung accession, Tiganderket accession, and Rimokayu accession.

1. Introduction

Shallot (Allium cepa L. var. ascalonicum) is an important and strategic commodity that affects the Indonesian economy. Since 2014 the price of shallots has become a commodity that causes inflation to increase, therefore the Indonesian government since 2018 has intervened in the price of shallots both in traditional and modern markets along with 7 other commodities, namely rice, chicken meat, fresh fish, noodles, eggs, red chilies, cooking oil, and sugar to reduce inflation.

North Sumatra Province still lacks shallots every year, North Sumatra's need for shallots reaches 43.78 tons per year, while the highest production is still around 18 tons per year, or only fulfilled 42% of the people's needs. The productivity of North Sumatra's shallot bulb in 2019 reached 8.05 tonnes per ha, smaller than the national average productivity of 9.93 tonnes per ha, or smaller than the productivity of West Java which has reached 11.04 tonnes per ha [1]

However, the obstacles that are often encountered and seen in farmers' fields in North Sumatra are disturbance of plant pests, generally diseases, especially fusarium basal rot disease. This disease infects the roots and bulb with the symptoms of the leaves rolling into a spiral and turning yellow. The cause of fusarium bulb rot is Fusarium oxysporum f. sp. cepae (Foc) [2], and including one of a Top 10 fungal plant pathogen list for Molecular Plant Pathology [3].

To counter the shortage of shallot production, the Government of North Sumatra has extended shallots to the lowlands including Deli Serdang and Batu Bara districts in addition to the highlands,
the largest of which includes Karo regency, Simalungun regency and Samosir regency. It is suspected that due to this extensification there has been mobilization of the sources of Foc infection through the exchange of tubers for seedlings, resulting in an increase in the incidence of fusarium tuber rot disease. Therefore it is necessary to test the virulence of lowland isolates Fusarium of Deli Serdang, Batu Bara and Karo highland isolates against local accessions of shallots in Karo regency.

The purpose of this study was to detect the emergence of Fusarium isolates from various sources of infection locations against the increasing attack of fusarium bulbs rot that occurred in 2018 to 2019, especially those that occurred in the extensification area of shallot cultivation in Karo district of Sumatera Utara Province.

2. Materials and methods

2.1. Isolation of tuber rot fusarium
Isolation of tuber rot Fusarium (Foc) was carried out in lowland shallot cultivation farmers’ land in Pantai Labu, Deli Serdang regency and Medang Deras, Batu Bara regency. While for the highlands Foc isolation was carried out in shallot farmers’ cultivation land in Tongging, Tiganderket, and Payung respectively in Karo regency.

Clumps of shallot plants with symptoms of Foc infection are removed and cleaned with clean water, packed in transparent plastic bags and stored in ice boxes to be taken to the plant disease laboratory of the Fakultas Pertanian Universitas Sumatera Utara. In the laboratory, the sample of shallots, the source of the isolate Foc, was cleaned with running water while removing the rotten leaf and rot of tuber layer, then after that it was dried at room temperature. After drying, the tuber layer that still shows signs of infection is peeled off and cut to the size of 1 x 1 x 2 cm. This sample is then disinfected by soaking in 70% alcohol for 60-90 seconds, washed with sterile distilled water and then dried with sterile filter paper before culturing on sterile Potato Dextrose Agar (PDA) medium in a 9 cm diameter Petri dish. This preparation was then incubated at room temperature and observed every day. The mycelium that grows and develops on PDA media is then subculture to another PDA medium in a Petri dish and incubated at room temperature until a fungal culture is obtained with a uniform colony shape and colour, these colonies are then observed with a light compound microscope to characterize Fusarium fungi as pure culture of Foc of each isolate source targeted.

2.2. Microscopic characterization of Foc isolates
The fungi were identified according to cultural character described by Barnett and Hunter [4], Nelson et al. [5] and Summerell et al., [6]. The isolated fungi were identified to the genus level and species which was possible on the basis of micro-morphological and macro-morphological characteristic. Pure cultures of the isolates were subculture and transferred onto potato dextrose agar (PDA) for species identification using macro and micromorphological characteristics. All the plates were grown in triplicates for the complete isolation and purification of plant pathogenic fungi the microscopic photo plate was tabulated [7]

2.3. Survey of local shallots accession sources
Karo Regency, North Sumatra Province is the centre of the development of shallots in addition of Java island to meet the consumption needs of shallots in Indonesia. Among the areas where shallots are cultivated on Karo regency are Tongging Village, Rimokayu Village, Tiganderket Village and Payung Village. In this study, the four villages were used as sources of local shallot seeds as genetic material for the development of local accessions that were resistant to Fusarium oxysporum infection. From the four shallot cultivation locations, collected shallot seed tubers that meet the criteria as seeds, including bulbs harvested from old plants, stored for 2-3 months, tuber diameter 1.5-2 cm, weight 5-10 grams, shiny dark red colour, single bulb, and not defective.
2.4. Virulence test of Foc isolates
Foc virulence test was carried out by inoculating the spores into the soil in a polybag before planting shallot seed tubers. An isolate of Foc is grown on PDA for six days, after which three agar plugs containing the isolate are transferred to potato dextrose broth (PDB) liquid medium and incubated on a shaker rotating at 120 rpm under continuous fluorescent cool white light for six days at 23 to 25°C. The inoculum is incubated for 1 week, and the conidial suspension strained through a double layer of cheesecloth. The conidial suspension is diluted with sterilized water to a concentration of 6-7 x 10³ conidial/ml water as determined by hemocytometer. The conidial suspension was evenly sprayed onto the soil surface as much as 30 ml per polybag measuring 17 x 21 cm. Soil is sterilized with metam sodium as a fumigant before use. The inoculum is diluted with sterilized water to a concentration of 6-7 x 10³ conidial/ml water as determined by hemocytometer. The conidial suspension was evenly sprayed onto the soil surface as much as 30 ml per polybag measuring 17 x 21 cm. Soil is sterilized with metam sodium as a fumigant before use. The inoculum is incorporated to a depth of 5 cm with garden little fork by hand. The experiment was arranged in a completely randomized design with a combination of treatment of 5 isolates, that's Deli Serdang Foc, Batu Bara Foc, Tongging Foc, Tiganderket Foc, and Rimokayu Foc, while 4 local shallots accessions were Tongging accessions, Tiganderket accessions, Payung accessions, and Rimokayu accessions. Each treatment combination was repeated three times with 5 duplicate plants for each replication. Thus, 300 sample plants were needed to run the virulence test of Foc isolates against local shallot accessions. This virulence test was carried out in a plastic house in Berastagi, Karo regency with an altitude of 1380 m above sea level, from April to June 2018.

2.5. Disease severity assessment
Fusarium bulb rot incidence was assessed 5 times at a 7-day interval beginning from first appearance of the disease in the plots. Disease severity was recorded on a 0-4 scale [8], where:
0 = no symptom of Foc attacked
1 = up to 10% of the number or part of leaves per clump shows symptoms of Foc infection,
2 = more than 10 to 30% of the number or part of leaves per clump shows symptoms of Foc infection,
3 = more than 30 to 40% of the number or part of leaves per clump shows symptoms of Foc infection,
4 = more than 40 % number or part of leaves per clump showing symptoms of Foc infection.

Foc severity score were converted into percent severity index (PSI) according to the formula:

\[
\text{PSI} = \frac{\text{(sum of numerical rating} \times 100)}{\text{(number of plants scored} \times \text{maximum score on scale})}
\]

The data were analyzed with F test at 95% of level confidence and followed by Duncans’s Multiple Range Test (DMRT). Criteria for resistance level are Immune (0% disease severity), Resistant (1-20% disease severity), Moderate (21-40% disease severity), Susceptible (41-60% disease severity), and Very Susceptible (disease severity >60%) [9;10].

3. Results and discussion

3.1. Microscopic morphological characteristic of Foc isolates
Based on the colouring obtained on PDA medium, five isolates from different areas, namely Deli Serdang isolate, Batu Bara isolate from low land areas, and Tongging isolate, Tiganderket isolate, and Payung isolate from the highland of Karo regency areas were suspected as pathogenic isolates and were identified as F. oxysporum because the isolates had white colony from front view and brown or cream from reverse view on PDA medium. Microconidia isolates of Batu Bara are shorter than others isolate, even macroconidia too, and the size of Tongging isolates microconidia is larger than other isolates. Chlamydospore of all isolates close to oval to globose, while chlamydospore of Deli Serdang and Batu Bara were smaller than Karo high land isolates. Some of the morphological characteristics of isolates are shown in Table 1.
Table 1. Microscopic of morphological characteristics of Foc isolates collected from shallots growing areas (µm)

| Sourcing of isolate areas | Microconidia (µm) | Macroconidia (µm) | Chlamydospores (µm) |
|--------------------------|-------------------|-------------------|---------------------|
|                          | Length | Wide  | Length | Wide  | Length | Wide  |
| Deli Serdang             | 10.15 ± 2.18  | 2.72 ± 0.58  | 39.24 ± 3.08  | 5.57 ± 0.46  | 6.97 ± 1.56  | 6.22 ± 1.09  |
| Batu Bara                | 9.28 ± 1.90  | 1.42 ± 1.25  | 35.64 ± 2.42  | 5.73 ± 0.86  | 6.58 ± 0.72  | 5.57 ± 0.57  |
| Tongging                 | 11.12 ± 2.35  | 2.95 ± 0.75  | 48.72 ± 3.65  | 6.22 ± 1.22  | 8.26 ± 1.26  | 7.56 ± 0.92  |
| Tiganderket              | 10.80 ± 1.60  | 2.40 ± 0.40  | 42.32 ± 3.62  | 5.83 ± 0.72  | 8.42 ± 1.23  | 7.87 ± 0.72  |
| Payung                   | 10.75 ± 2.30  | 2.67 ± 0.62  | 41.84 ± 3.92  | 5.43 ± 0.47  | 8.83 ± 0.86  | 7.73 ± 0.52  |

3.2. Disease severity assessment

Mehdi [11] divided the Foc virulence level based on the percentage of disease severity into four levels based on statistical tests, namely the "most virulent" isolate and the "least virulent" isolate. Other isolates have also been classified into statistically distinct groups as the intermediate group between the most virulent and least virulent isolates, namely the "highest virulent" isolates and the "virulent" isolates. The Foc virulence to shallots and resistance levels of shallots to Foc are shown in Table 2 below.

Table 2. Combined of twenty of disease severity index (%) of Fusarium tuber rot disease from five different origin of Fusarium isolate aginst four local shallot accessions of Tongging, Payung, Tiganderket, and Rimokayu

| No | Origin of Foc | Local Accessions of | Disease Severity Index (%) a | Virulence of Foc to Local shallot Accession b | Resistance Levels of Local Shallots c |
|----|---------------|---------------------|-------------------------------|---------------------------------------------|--------------------------------------|
| 1  | Payung        | Payung              | 54.77 a                       | Most virulent                              | Susceptible                          |
| 2  | Tongging      | Rimokayu            | 54.13 ab                      | Most virulent                              | Susceptible                          |
| 3  | Tiganderket   | Tiganderket         | 53.60 ab                      | Most virulent                              | Susceptible                          |
| 4  | Tongging      | Tongging            | 53.53 ab                      | Most virulent                              | Susceptible                          |
| 5  | Tiganderket   | Rimokayu            | 53.33 ab                      | Most virulent                              | Susceptible                          |
| 6  | Tongging      | Payung              | 53.10 ab                      | Most virulent                              | Susceptible                          |
| 7  | Payung        | Rimokayu            | 52.90 bc                      | Most virulent                              | Susceptible                          |
| 8  | Tongging      | Tiganderket         | 52.57 c                       | Most virulent                              | Susceptible                          |
| 9  | Tiganderket   | Payung              | 50.93 de                      | Highest virulent                           | Susceptible                          |
| 10 | Payung        | Tiganderket         | 50.87 de                      | Highest virulent                           | Susceptible                          |
| 11 | Tiganderket   | Tongging            | 50.50 de                      | Highest virulent                           | Susceptible                          |
| 12 | Payung        | Tongging            | 49.60 de                      | Highest virulent                           | Susceptible                          |
| 13 | Batu Bara     | Tongging            | 47.90 f                       | Virulent                                   | Susceptible                          |
| 14 | Deli Serdang  | Tiganderket         | 47.67 fg                      | Virulent                                   | Susceptible                          |
| 15 | Batu Bara     | Payung              | 47.37 fgh                     | Virulent                                   | Susceptible                          |
| 16 | Deli Serdang  | Tongging            | 46.83 fghi                    | Virulent                                   | Susceptible                          |
| 17 | Deli Serdang  | Rimokayu            | 46.30 hij                     | Virulent                                   | Susceptible                          |
| 18 | Deli Serdang  | Payung              | 45.53 jk                      | Least virulent                             | Susceptible                          |
| 19 | Batu Bara     | Rimokayu            | 44.97 k                       | Least virulent                             | Susceptible                          |
| 20 | Batu Bara     | Tiganderket         | 44.77 k                       | Least virulent                             | Susceptible                          |

Note. a Value followes by the same letter in the column did differ significantly (0.05 level) in Duncan’s multiple range test. b. According to Mehdi [11]. c. According to Cahyaningrum [9] and Patil [10].
Other isolates have also been classified into statistically distinct groups as the intermediate group between the most virulent and least virulent isolates, namely the "highest virulent" isolates and the "virulent" isolates.

Statistical analysis showed that the Tongging Foc isolate was the most virulent against the Tongging accession, Payung accession, Rimokayu accession, and Tiganderket accession. While Payung foc most virulent against Rimokayu and Payung accession, and Tiganderket foc is the most virulent too against Rimokayu and Tiganderket accession.

Only two isolates were among the highest virulent, that Tiganderket foc isolate against Payung accession and Tongging accession, and Payung foc isolate against Tiganderket accession and Tongging accession. Batu Bara foc isolate and Deli Serdang foc isolate, was classified as virulence and least virulent against Tongging accession, Payung accession, Tiganderket accession, and Rimokayu accession.

4. Conclusions

The test results showed that the virulence of Fusarium oxysporum cepae (Foc) was influenced by the origin of the isolate and the origin of the accession. The Tongging Foc isolate was the most virulent to Tongging accession, Payung accession, Rimokayu accession, and Tiganderket accession. Meanwhile, Payung foc was the most virulent against Rimokayu and Tiganderket accessions, and Tiganderket foc was the most virulent against Rimokayu and Tiganderket accessions. Two isolates had the highest virulence, thats are foc Tiganderket isolates against Payung and Tongging accessions, and foc Payung was the most virulent against Rimokayu and Tiganderket accession. Meanwhile, other isolates have also been classified into statistically different groups as the intermediate group between the most virulent and least virulent isolates, namely the "highest virulent" isolates and the "virulent" isolates.

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