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FUSARIUM PROLIFERATUM AS A CAUSATIVE AGENT OF FUSARIUM WILT IN SHALLOTS (*ALLIUM CEPA* L.) ON LOMBOK ISLAND, WEST NUSA TENGGARA, INDONESIA

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SUMMARY

Shallot (*Allium cepa* L.) is one of the favorite commodities cultivated by the farming community in West Nusa Tenggara (NTB), Indonesia. However, its potential yield may decline both in quantity and quality due to the fusarium wilt disease and because of the limited information about the wilt disease in shallots. Therefore, the presented research aimed to a) determine the percentage of fusarium wilt disease incidence, b) investigate the new species of pathogen that causes fusarium wilt disease in shallots, and c) identify the distribution of fusarium pathogen species in shallots. The collected shallot samples came from 69 cultivation centers in Lombok Island, identifying the fungus based on morphological characteristics (colony shape, color, hyphae, conidia, and chlamydospores), while applying the ITS (Internal Transcript Spacer) rDNA sequences for molecular examination. The results showed fusarium wilt disease incidence in shallots (*A. cepa* L.) was 45.67%. Various *Fusarium* species as a causative disease on shallot plants were *Fusarium oxysporum, F. solani*, and *F. proliferatum*, distributed in 60 (86.96%), 54 (78.36%), and 43 (60.87%) locations, respectively. Furthermore, a new species (*Fusarium proliferatum*) of causative disease as fusarium wilt on shallots was evident in Lombok Island, West Nusa Tenggara, Indonesia.

Keywords: Shallots (*A. cepa* L.), fusarium wilt disease, pathogen, *Fusarium oxysporum, Fusarium solani*, *Fusarium proliferatum*

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Key findings: The filamentous fungus from *Fusarium proliferatum* was the firstly recognized species causing fusarium wilt disease in shallots (*A. cepa* L.) on Lombok Island, NTB, Indonesia. The fusarium species *Fusarium proliferatum* infestation was prevalent in more than 50% of locations, which confirmed the said species had a remarkable role in shallot yield losses.

INTRODUCTION

A vegetable in the Allium family, shallots (*Allium cepa* L.) are a close relative of onions, garlic, chives, and leeks. They are native to Western and Central Asia and may have been bred from onions rather than growing wild. Shallots are one of the favorite commodities cultivated by farmers in West Nusa Tenggara (NTB), Indonesia. According to the Central Statistics Agency (2020), the NTB province is one of the top four producers of shallots in Indonesia after Central Java, East Java, and West Java. Shallots' production peaked in 2018 at 2,128,849 tons, followed by 1,882,545 tons in 2019 and 1,857,954 tons in 2020. This data showed NTB stands as a vital region and producer of shallots in Indonesia.

Although NTB produced the highest quantities of shallots from 2018 to 2020, its potential to enhance yields is still open to achieve optimal production stability, with a potential yield of 20 tons per hectare for local cultivars. However, achieving this optimal production faces several challenges, including infestation of the fusarium wilt disease. In worse conditions, fusarium wilt infestation can result in yield losses of up to 90%, and in some areas, it can cause a complete crop failure (Mahdy *et al*., 2018). In NTB, the disease intensity can reach to 45%–64.71% (Mariani *et al.,* 2022), potentially destroying half of the shallot's prospective yield. This high disease intensity gets worse by the fusarium's ability to remain dormant for extended periods until it finds a favorable condition and a suitable host plant to germinate and reproduce.

Fusarium wilt reduces the quantity of shallots and deteriorates crop quality (Futane *et al.*, 2018; Nishioka *et al.*, 2019). The decline in production quality begins with the high likelihood of attack, which can affect all parts of the shallot plant, from roots to bulbs and leaves. Fusarium wilt can cause symptoms, such as, necrosis, chlorosis, and leaf twisting, with elongation of the neck and leaf drop in shallots (Herlina *et al.*, 2021). Therefore, identifying fusarium wilt species should be a central focus for preventing significant yield losses in shallots.

In shallots, the fusarium wilt disease results from the fungus *Fusarium* sp. (Futane *et al.,* 2018). According to previous research, the species *Fusarium* sp., which has the potential to attack shallot plants in Indonesia, include *Fusarium oxysporum* in the province of North Sumatera (Afriani and Heviyanti, 2018) and *F. solani* in the province of NTB (Mariani *et al.,* 2022). Different *Fusarium* species cause wilt disease in shallots in several countries, such as, species *F. oxysporum, F. redolens, F. avenaceum,* and *F. solani* in England (Taylor *et al*., 2016). In Iran, *F. oxysporum* and *F. solani* persist*,* and *F. oxysporum, F. proliferatum,* and *F. solani* in Egypt (Mahdy *et al.,* 2018). Meanwhile, *F. oxysporum*, *F. proliferatum,* and *F. redolens* manifest in Finland (Haapalainen *et al.,* 2023). The spreading of fusarium wilt disease worldwide means the fusarium wilt attacks needs serious and immediate attention, especially in NTB, Indonesia being a vital shallot producer.

The source of information about the pathogenic fungus *Fusarium* sp. attacks on shallot plants still needs exploration and enhancement in Indonesia. *Fusarium* sp. has a diverse group of fungi with varied species, a vast population, and a broad host range, with classification as the primary soil-borne pathogen (Gordon*,* 2017; Sharma and Cramer, 2023). Similarly, discovering new species aside from *Fusarium oxysporum* and *Fusarium solani,* attacking shallots on Lombok Island, has great potential. The presented study aimed to exhaust identifying various species of pathogens affecting shallot plants on Lombok Island, NTB, Indonesia.

MATERIALS AND METHODS

Plant samples

Shallot (*A. cepa* L.) plant samples' location characteristic is dryland with limited water availability. The soil types are entisol, inceptisol, and ultisol, with sandy and clay soil textures. Samples came from 69 locations across 23 villages, representing key cultivation centers in Lombok Island, Indonesia (Figure 1 and Table 1). A diagonal sampling procedure ran from January 2022 to September 2023 at five points in each location, with 10 diseased plants collected at each point for subsequent isolation and identification processes.

Fungus isolation and morphological identification

The fungus isolation process used the direct plating method as outlined by Haapalainen *et al*. (2016), with some modifications. Additionally, cleaning plant samples with running water continued cutting into ±1 cm × 1 cm pieces from the boundary between healthy and diseased tissues. These plant pieces received sterilization with 70% alcohol for one minute and 5.3% sodium hypochlorite for two minutes before rinsing three times with sterile water, and then, dried on sterile tissue in a laminar airflow. The sterilized plant tissues continued to transfer into selective Fusarium PCNB (Pentachloronitrobenzene) agar, and incubated at room temperature (25 °C–28 °C) in the dark for five days. Transferring the fungus grown on PCNB agar had the hyphae tips collected for culturing on a standard PDA medium. Subsequently, identification of morphological characteristic of *Fusarium* sp. included colony shape, color, hyphae shape, conidia shape and size, as well as, chlamydospores present and their sizes (Leslie and Summerell, 2006).

Molecular identification and phylogenetic analysis

The molecular identification steps included DNA extraction, where suspending the fungus hyphae proceeded in a centrifuge tube containing 100 µl PrepMan Ultra reagent (PrepMan Ultra Protocol, Applied Biosystems, USA) (Putri *et al*., 2022). The suspension sustained vortexing for 30 s, placed in a heat block at 95 °C and 100 °C for 10 min, then left at room temperature for two minutes before centrifuging at a speed of 10,000 rpm for two minutes. Amplification of the 18S rRNA gene succeeded through the polymerase chain reaction (PCR) with the primer sequences from the Internal Transcript Spacer (ITS) 1

Figure 1. Locations of shallot and rhizoplane sampling: East Lombok Regency (8 locations), West Lombok Regency (12 locations), and North Lombok District (3 locations).

Table 1. Percentage of shallot plants affected by Fusarium wilt in Lombok Island, NTB.

Location	Disease (%)
Kediri	58.33
Kediri Selatan	60.67
Montong Are	70.00
Perampuan	30.33
Karang Bongkot	45.67
Merembu	50.00
Badrain	100.00
Tanaq Beak	45.33
Krama Jaya	80.33
Batu Layar	12.50
Senteluk	20.00
Batulayar Barat	35.00
Sembalun	20.33
Sembalun Bumbung	60.67
Sembalun Lawang	31.67
Sem.Timba Gading	43.67
Mamben Daya	45.00
Mamben Baru	22.33
Mamben Lauk	58.33
Keruak	48.00
Bayan	30.67
Anyar	31.67
Santong	50.00
Average	45.67

*Average from three observation points at each village where samples were obtained.

(forward) (5' - TCCGTAGGTGAACCTGCGG-3') and ITS 4 (reverse) (5' - TCCTCCGCTTATTGATATGC - 3') in a Takara PCR thermal cycler personal (Takara Bio, Otsu, Japan). Performing the reaction used the Ex Tag (Takara Bio, Otsu, Japan) under conditions comprising pre-denaturation at 94 °C for four minutes, followed by 35 cycles of denaturation at 94 °C for 35 s, annealing at 52 °C for 55 s, extension at 72 °C for two minutes, and final extension at 72 °C for 10 min. DNA amplification had each PCR microtube filled with 12 µl free water, 5 µl PCR master mix, 1 µl primer ITS5, 1 µl primer ITS4, and 1 µl template DNA, then, thoroughly mixed using a micropipette.

Subsequently, amplification continued in a PCR machine (PTC 100, M.J. Research) commencing with initial denaturation at 95 °C for 90 s. Following this was 35 cycles of denaturation at 95 °C, annealing at 55 °C, extension at 72 °C, and final extension at 72 °C for 90, 30, and 90 seconds, and five minutes, respectively. The results remained at 4 °C, and after DNA purification, electrophoresis ensued to determine the desired target DNA. DNA visualization commenced with the soaking of an agarose gel in 0.12 µg/ml ethidium bromide solution for 15 min before performing electrophoresis for 30 min at a 110 voltage. The resulting DNA's visualization utilized a Digi-Doc Imaging System, with the profiles observed as bright bands between gene loci. The determination of sequencing ITS region and DNA fragments from PCR employed the ABI Prism 3100-Avant Genetic Analyzer, with the sequence edited using ChromasPro version 1.5 software. The sequence data's analysis for homology with sequences in GenBank engaged the BLASTn software (Basic Local Alignment Search Tools for nucleotide). Alignment depended on the ClustalW method. Afterward, applying results as input data developed a phylogenetic tree through the Maximum Likelihood method and Kimura 2-parameter model, with 1000x bootstrap analysis, using Mega 11 software (Tamura *et al*., 2021).

Pathogenicity test

The conduct of pathogenicity test followed the method outlined by Haapalainen *et al*. (2016), with some modifications. The fusarium isolates Lbk01, Lbk02, and Lbk03 were specimens used along with shallot seeds belonging to the shallot cultivar Keta Monca. The test procedure included the reparation of 40 bulbs of healthy shallot seeds and the development of conidial (spores) suspensions for each isolate, with a conidial density of 10^6 conidia mL-1. The preparation of 120 kg sterilized compost soil preceded placing 3 kg of soil in each polybag as shallot planting medium. Ten of the 40 shallot seeds bore soaking in each fusarium spore suspension, as well as, in sterile water (as control), and the soaking process remained for 30 min. Planting ensued with one seed per polybag placed in the greenhouse, followed by observations for 30 days and the identification and isolation of infected plants showing symptoms.

Statistical analyses

The conducted experiment used a randomized complete block design with 10 replications. All recorded data underwent the analysis of variance (ANOVA). In case of significant differences among the treatments and for comparison of the mean values, further analysis employed the Duncan's Multiple Range Test (DMRT) at a 5% probability level.

RESULTS

Pathogen causing fusarium wilt in shallot

Field observations identified symptoms of fusarium wilt in shallot (*A. cepa* L.), characterized by yellowing (chlorosis), starting from the leaf tips and extending to other plant parts. Simultaneously, the leaves showed curling and twisting, while the roots and bulbs had yellow and dark brown discoloration. Infected shallot plants had abnormal growth due to root and bulb damages (rotting), leading to wilting, and ultimate death. Additional observed symptoms included stunted growth in infected plants with failure to attain the supposed full size. Infected plants manifested disease signs, such as, the presence of white fungus hyphae around the affected areas (Figure 2).

In the field, the observed symptoms served as the foundation for determining the percentage of fusarium wilt occurrence in shallot plants. After evaluating 20% of the total population, the occurrence percentage determined across the 69 locations had the value varying from 12.50% to 100% in Lombok Island, NTB. The lowest value appeared in Batu Layar (12.50%), and the highest in Badrain village (100%). However, the average percentage of the disease occurrence was 45.67% in NTB Island (Table 1).

Morphological identification

Obtaining a total of 70 fusarium fungus isolates were successful from the field. Meanwhile, the results of macroscopic and microscopic identification showed considerable variations in phenotypic characteristics, including colony color and shape, mycelium shape, conidia size, and chlamydospore presence (Figure 3). Based on the morphological characteristics, these isolates reached grouping into three clusters, i.e., Cluster I (Fusarium – Lbk01), II (Fusarium – Lbk02), and III (Fusarium – Lbk03).

Cluster I (Fusarium – Lbk01) comprised 30 isolates possessing morphological characteristics, such as, white and a cream-colored colony on PDA media, with a slightly smooth texture. Additionally, the fungus had a thin surface with loosely spaced growth of mycelium, which completely covered the Petri dish for an average of seven days at 27 °C–28 °C. Macroconidia showed a crescent moon shape, including sharp and curved tips, thin cell walls, as well as, a size ranging from 14.84 to 58.3 µm × 2.1 to 4.28 µm, with 2–6 septa. Microconidia formed on conidiophores were round and oval, with curved microconidia possessing a bulbous base and slightly pointed tips, featuring thin cell walls and a size ranging from 4.26 to 9.98 µm × 1.76 to 3.25 µm.

Figure 2. Symptoms of Fusarium wilt in shallot: (A) Yellowing of plants (chlorosis), twisted leaves; (B, C) Yellow-brown discoloration and decay of bulbs; and (D) Disease signs in the form of white fungus mycelium.

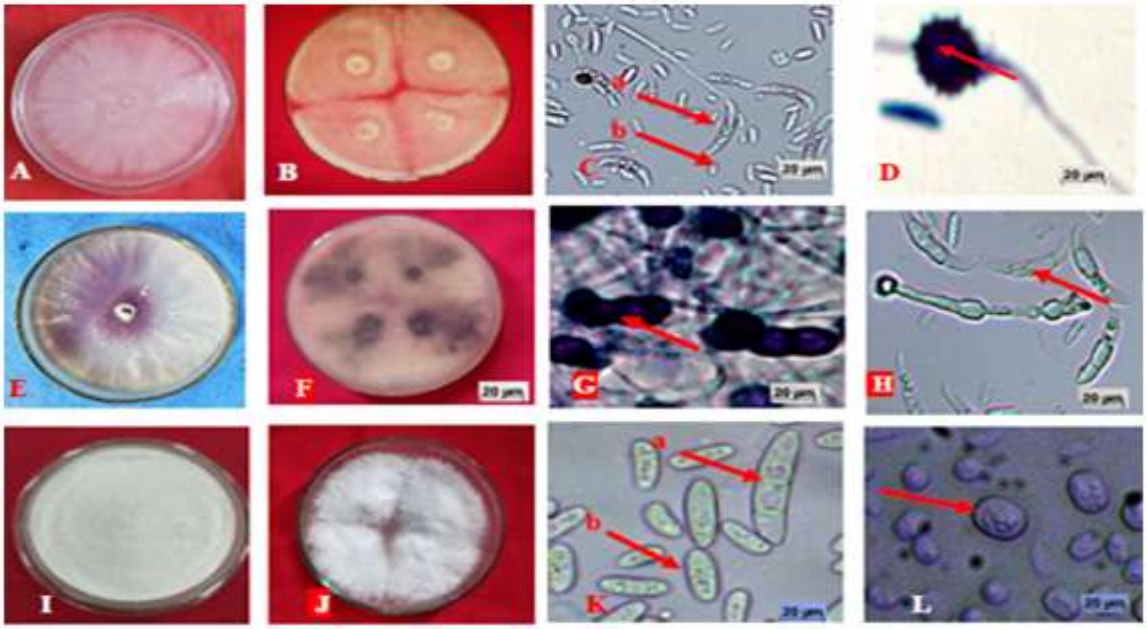

Figure 3. Morphological characteristics of *Fusarium* sp. isolates Lbk01, Lbk02, and Lbk03 causing fusarium wilt in shallot: Fusarium Lbk01: (A, B) Colony, (C) a. Macroconidia, b. Microconidia, (D) Chlamydospore; Fusarium Lbk02: (E, F) Colony, (G) Microconidia, (H) Macroconidia; and Fusarium Lbk03: (I, J) Colony, (K) a. Macroconidia, b. Microconidia, (L) Chlamydospore.

Chlamydospores with a diameter ranging from 8.38 to 9.88 µm also occurred in the isolates (Figure 3).

Cluster II (Fusarium – Lbk02), comprising 15 isolates, showed purple on PDA media. The isolate age also influenced the varied color, transitioning from purple mixed with white in early colony growth to light purple, and finally, to dark purple. Fungus mycelium texture was fine and thin, covering the Petri dish over the average of eight days at a temperature of 27 °C–28 °C. Additionally, the macroconidia and microconidia found in isolates in Cluster II were similar to those observed in Cluster I. Macroconidia were long and slender, curved to form a crescent shape, with slightly pointed and curved tips at the end, blunt at the base, featuring 2–6 septa,

and the size ranging from 13.57 to 47.56 µm – 2.1-4.38 µm with thin cell walls. Microconidia formed on hyphae and varied from 4.25 to 16.01 µm × 1.95-4.66 µm without septa. The isolates lacked chlamydospores, however, produced numerous microconidia arranged closely to create long chains (Figure 3).

Cluster III (Fusarium – Lbk03) included 25 isolates showing a white color colony resembling cotton on PDA media, with a slightly coarse and thick texture and dense mycelium growth. Additionally, fungus mycelium covered the Petri dish on average of seven days at a temperature of 27 °C–28 °C. Curved macroconidia emerged, while some appeared straight with blunt tips, as well as, wide and elongated shapes, featuring thicker cell walls. In Cluster-III isolates, abundant round and oval microconidia with thick cell walls appeared along with macroconidia, which were larger than the same types identified in Clusters I and II. Macroconidia size ranged from 152.5 to 608.2 µm × 55.8-92.2 µm, while microconidia ranged from 112.6 to 126.2 µm × 43.4 to 72.8 µm. These isolates produced chlamydospores with a wide, round shape, thick cell walls, and diameters varying from 60.5 to 148.0 µm (Figure 3).

Molecular identification and phylogenetic analysis

PCR amplification results of *Fusarium* sp. DNA with ITS1 and ITS4 primers showed the DNA fragments ranging from 550 to 600 bp. DNA amplified from isolates Lbk01, Lbk02, and Lbk03 had fragment sizes of 550, 550, and 600 bp, respectively (Figure 4). The purified and sequenced DNA fragments served as a basis for identifying the level of similarity with other fungus species sequences deposited in the GenBank. The usage of BLASTn on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm.nih.gov) helped the said analysis. The comparison results of the percentage similarity of fusarium clusters Lbk01, Lbk02, and Lbk03 with various isolates in GenBank appear in Table 2.

The homology levels' comparison of the fusarium Lbk01, Lbk02, and Lbk03 sequencing results with species data in the GenBank using NCBI BLASTn are available in Table 2. These three isolates belonged to the *Fusarium* sp. genus with different species determined based on the percentage similarity values of homologous species, total score, and E Value for each. Furthermore, fusarium Lbk01, Lbk02, and Lbk03 had the highest similarity percentage (99.81%) with the fungus species *Fusarium oxysporum* isolate FUS2, *Fusarium proliferatum* strain AH15 (100%), and *Fusarium solani* isolate 174 (99.82%). They indicated a total score of 979, 1037, and 1046, as well as, an E value of 0.0, respectively. The relationship determination between the nucleotide sequences of fusarium pathogen isolates Lbk01, Lbk02, and Lbk03 with nucleotide sequences in the database ensued by conducting a phylogenetic analysis (Figure 5).

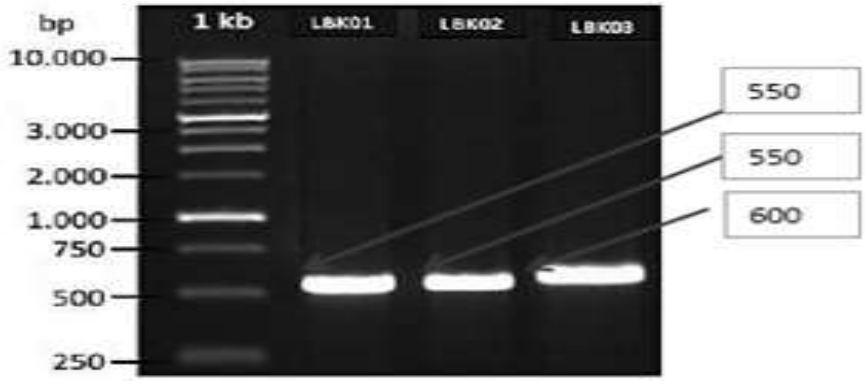

Figure 4. Agarose gel electrophoresis of the 18S rRNA gene of *Fusarium* sp. isolates Lbk 01, Lbk 02, and Lbk 03 gene ladder marker 100, 0.1-2 kbp (Thermo Scientific, K9021).

Table 2. Analysis sequence of Fusarium isolates Lbk01, Lbk02, and Lbk03 with several homologous sequences in the GenBank.

Homologous Species	Isolate/Strain Code	Similarity (%)	Total Score	E-Value	Accessions
Fusarium isolate - Lbk 01					
F. Oxysporum	Isolate FUS2	99.81	979	0.0	KP965006.1
Fusarium oxysporum	Isolate R36	99.63	979	0.0	MT420633.1
Fusarium oxysporum	Isolate R29	99.63	979	0.0	MT420627.1
Fusarium oxysporum	Isolate R25	99.63	979	0.0	MT420624.1
Fusariumoxysporum	Isolate R12	99.63	979	0.0	MT420611.1
Fusarium sp.	Voucher HQU PR06	99.63	979	0.0	MK640560.1
Fusarium isolate - Lbk02					
Fusarium proliferatum	Strain AH15	100	1037	0.0	MH712165.1
Fusarium proliferatum	Isolate XP3S-1ee	99.47	1018	0.0	ON927093.1
Fusarium proliferatum	Isolate DWBM-2-2-1	99.29	1014	0.0	ON527497.1
Fusarium proliferatum	Strain F8	99.29	1014	0.0	HQ380763.1
Fusarium proliferatum	Isolate C2	99.46	1014	0.0	ON126211.1
Fusarium fujikuroi	Isolate F13	99.46	1013	0.0	MW314766.1
Fusarium isolate - Lbk 03					
Fusarium solani	Isolate 174	99.82	1046	0.0	KY697901.1
Fusarium solani	Isolate DCF7	99.82	1046	0.0	OM065830.1
N. haematococca	-	99.82	1046	0.0	AY310442.1
Fusarium solani	Strain WZ-128	99.82	1044	0.0	MN856274.1
Fusarium phaseoli	Isolate PR1	99.82	1044	0.0	KF717534.1

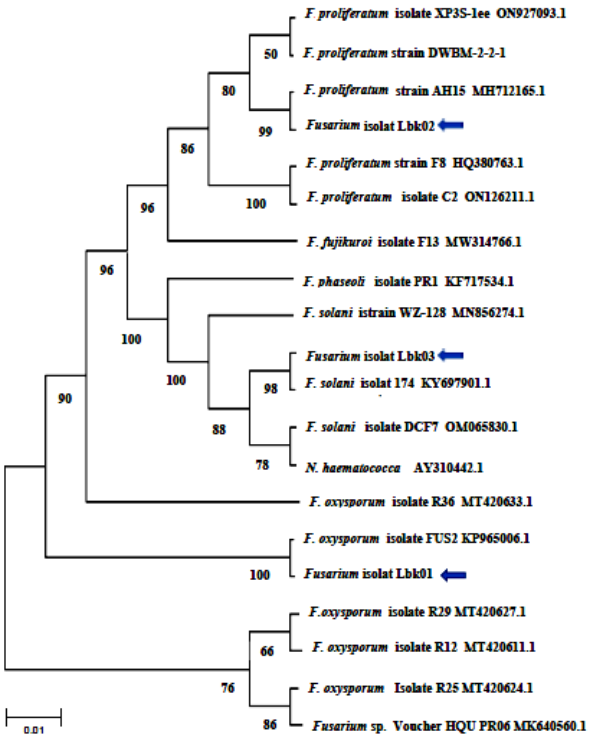

Figure 5. Phylogenetic tree of Fusarium isolates Lbk01, Lbk02, and Lbk03 from shallot using the neighbor-joining method and bootstrap analysis (1,000 replicates) with the Kimura 2-parameter model in MEGA 11 software.

Table 3. The number and distribution of *Fusarium* sp. attacking shallot plants in Lombok Island, NTB.

Pathogen	Number of isolates	Percentage	Distribution at the attacked locations	Percentage
Fusarium oxysporum	33	47.14	60	86.96
Fusarium proliferatum	15	21.43	43	60.87
Fusarium solani	22	31.43	54	78.26

Table 4. Impact of the pathogenic isolates Lbk01, Lbk02, and Lbk03 on the incubation period of Fusarium wilt in shallot plants.

PLK	Incubation period
Fusarium Lbk01	5.87±4.50a*
Fusarium Lbk03	7.45±6.34ab
Fusarium Lbk02	9.93±9.63b
Control	30.00±0.00c

*Mean values followed by the same letter in the same column show no significant difference based on the DMRT 5% test.

Based on macroscopic and microscopic identification results, as well as, molecular analysis, three species of fungus attacking shallot plants were distinctive. These species included 33, 22, and 15 isolates of *Fusarium oxysporum* (Lbk01), *Fusarium proliferatum* (Lbk02), and *Fusarium solani* (Lbk03) distributed across 60 (86.96%), 54 (78.26%), and 43 (60.87%) locations, respectively, in Lombok Island, NTB (Table 3).

Pathogenicity test

Regarding the observations and analysis of variance, the tested pathogenic isolates appeared to significantly influence the incubation period. Furthermore, fusarium Lbk01 had the fastest average incubation period of 5.87 (≈6) days, followed by fusarium Lbk03 with 7.45 (≈7) days. Fusarium Lbk02 had the longest average incubation period, reaching 9.93 (≈10) days. However, the control treatment did not show any disease symptoms until the completion of the observations (Table 4). The observation results of the pathogenicity test showed disease symptoms and signs similar to those found in the field. Macroscopic and microscopic identification of the pathogen detected colony shape, hyphae, and conidia resembling findings visible in the field (Figure 6).

DISCUSSION

Five species of fusarium, including *Fusarium oxysporum, F. solani, F. proliferatum, F. redolens,* and *F. culmorum* typically attack the shallot plants (Gordon, 2017; Gálvez and Palmero, 2022), and *F. solani* (Mariani *et al*., 2022) are prevalent in West Nusa Tenggara (NTB). Meanwhile, only a few reports mention other species attacking the shallot plants in this region. In the pertinent research, the identification results of 70 isolates obtained from 69 locations revealed a new pathogen species, namely, *Fusarium proliferatum*, causing the fusarium wilt disease in shallot. This fungus species infected the shallot plants in 43 locations (60.87%) of the different cultivation centers in Lombok Island, NTB, with a distribution lower than the species *F. oxysporum* and *F. solani*.

The lower distribution of *Fusarium proliferatum* is due to its lack of chlamydospore production, a morphological structure present in the isolates of *F. oxysporum* and *F. solani.* Additionally, conidia and chlamydospores are essential parts of the fungus, functioning as tools for infecting the host plants, where higher initial inoculum led to a greater and faster tendency of pathogen infection (Srinivas *et al*., 2019; Ahmed *et al.,* 2022). Chlamydospores also occurred to be more resistant, surviving

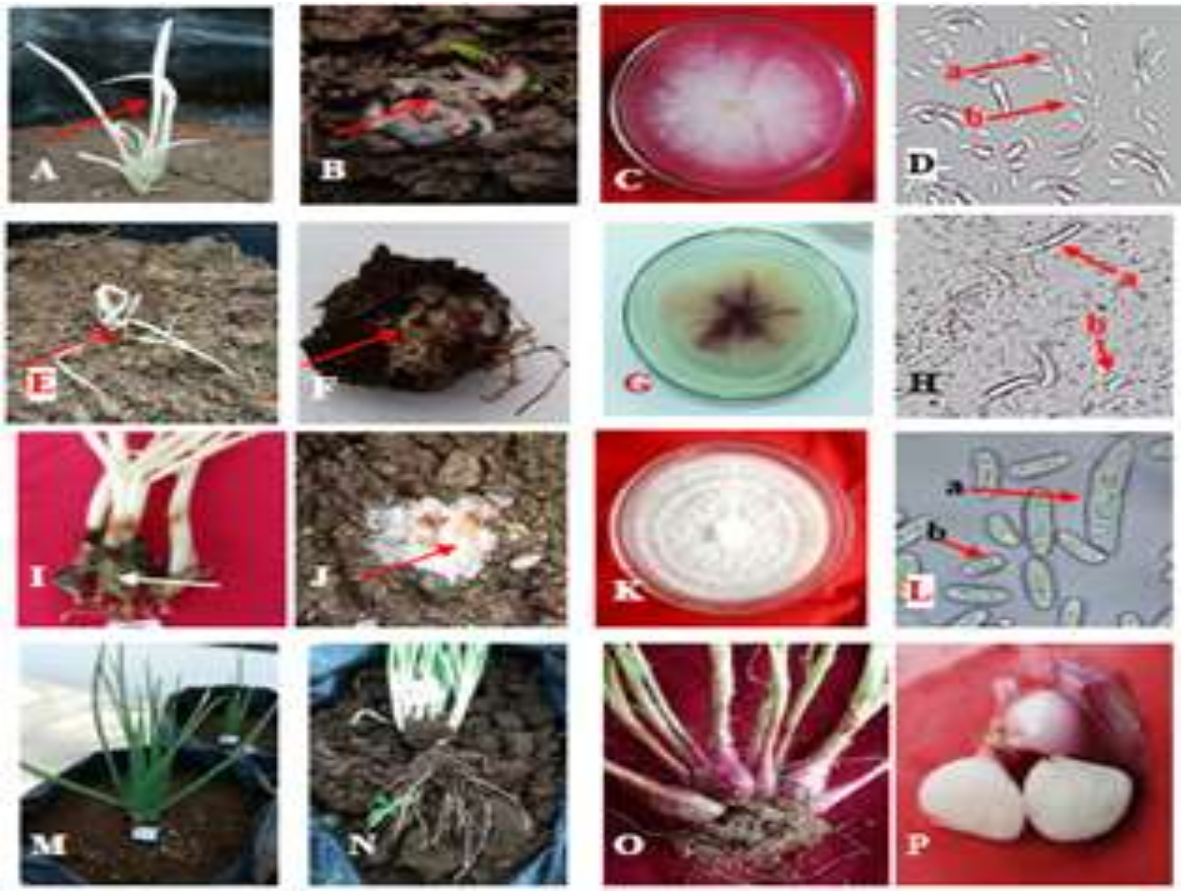

Figure 6. Fusarium isolate Lbk01: (A) Disease symptoms, (B) Disease signs, (C) Colony, (D) a. macroconidia, b. microconidia; Fusarium isolate Lbk02: (E) Disease symptoms, (F) Disease signs, (G) Colony, (H) a. macroconidia, b. microconidia; Fusarium isolate Lbk03: (I) Disease symptoms, (J) Disease signs, (K) Colony (L) a. macroconidia, b. microconidia; Control: (M) Healthy plants without symptoms, (N) Healthy roots and bulbs without symptoms, (O) Bulbs growing normally, (P) Healthy bulbs without disease symptoms.

over a long period in the soil, even in absence of host plants and under extreme weather conditions, compared to other conidia. Chlamydospores around plant rhizosphere caused easier infection, extending to others through the "plant-to-plant spread" mechanism, and the disease spread through root systems (Dita *et al.*, 2018).

The infectivity of *Fusarium* sp. to host plants was also an effect of external factors, such as, environmental conditions. Variations in agroclimatic conditions can also affect the fungus characteristics and trigger the onset of new, more virulent, and resistant species (Shekhar and Singh, 2022). Moreover, environmental factors also alter the speed of pathogen development and the ability to infect host plants. In the infected area, the availability of nutrients also influences the pathogen virulence level, with higher nutrient availability leading to faster pathogen development and host plant infection (Pulkkinen *et al*., 2018). Climate change modifies the fungus biodiversity and the ability to produce toxins, as global temperature variations influence adaptation to the environment and host plants, leading to an increased production of *Fusarium* sp. mycotoxins (Kos *et al*., 2023).

Global climate change upsets the pathogen distribution, as alterations in Earth climate influence the life of plant-disease-

causing microorganisms. Current climate changes concerning temperature, water availability, quality, and quantity of sunlight, and drought stress are crucial factors affecting the ability of fungus to infect plants, survive, and produce toxins. The ability to produce mycotoxins and other defense components results from an adaptation to global climate change, allowing fungus distribution to various regions with diverse geographical conditions (Zingales *et al*., 2022). *Fusarium* species have a high tolerance for variations in temperature and pH conditions in the environment. The capacity to produce structures, such as, chlamydospores and abundant spores, along with toxins, strengthen the fungus defense mechanism to survive in diverse environmental conditions (Panwar *et al*., 2016; Zingales *et al*., 2022).

Fusarium proliferatum, as identified in this research as a new species, affecting shallot plants cultivated in NTB, Indonesia, had no previous reports of its pathogenicity in this region. Initially referred to as *Fusarium moniliforme* when isolated from shallot plants in 1976; however, it later underwent reclassification, being named *Fusarium proliferatum*. Furthermore, *Fusarium proliferatum* (Matsush.) Nirenberg, formerly described as Gerlach and Nirenberg 1982, has a worldwide distribution and association with various diseases in cereals, legumes, vegetables (including shallots), and fruits, such as bananas, and other crop plants (Stępień *et al*., 2015; Ren *et al*., 2015; Zakaria *et al*., 2016; Lalak-Kańczugowska *et al*., 2023).

Fusarium sp. genus is a group of filamentous fungus, comprising more than 330 species (Yilmaz *et al.,* 2021), while *Fusarium proliferatum* is a member of the Liseola group in this genus. *Fusarium* species belonging to the Liseola group include *Fusarium fujikuroi, F. proliferatum, F. andiyazi, F. verticillioides*, and *F. sacchari*. The teleomorphic form of *Fusarium proliferatum* is *Gibberella intermedia*, which is part of the *Fusarium fujikuroi* species complex (FSSC) (Sun *et al.,* 2018). The morphological form of *Fusarium proliferatum* is challenging to distinguish from *Fusarium fujikuroi*; however, both can reach differentiation through DNA sequencing or sexual cross-fertility tests (Gálvez *et al*., 2017).

Fusarium proliferatum has morphological characteristics similar to *Fusarium solani*, however, unable to produce chlamydospores (Sun *et al*., 2018; Gálvez and Palmero, 2022). This fungus possesses relatively high virulence, with numerous sources reporting it as the main pathogen infecting red and white shallot plants in several countries (Haapalainen *et al*., 2023). *Fusarium proliferatum* produces various toxin compounds, such as, fumonisins (FUMs), which are harmful to host plants. FUMs are mycotoxins capable of causing damage to plants growing in various agroclimatic conditions (Chhaya *et al*., 2022).

The absence of chlamydospores in *Fusarium proliferatum* does not prevent it from producing thick-walled spores capable of long-term survival. Although, some isolates can form sclerotia, this feature is not a taxonomic characteristic for a higher-level sexual reproduction (Gálvez *et al*., 2017). *Fusarium proliferatum* hyphae can thicken, allowing the fungus to survive without host plants and act as a saprophytic organism (Gálvez and Palmero, 2022).

The species *Fusarium proliferatum* and *Fusarium oxysporum* are two groups of fungus widely distributed in various locations, with a broad host range (Gasser *et al*., 2023). Specifically, *Fusarium proliferatum* is polyphagous, with a wide range of hosts, known to produce secondary metabolites toxic to plants (Perincherry *et al*., 2019; Lalak-Ka´nczugowska *et al*., 2023). This fungus attacks the host plants during cultivation and even after harvest (storage) (Gálvez and Palmero, 2022), where the initiated infection can reduce the quality of shallot, causing stunted growth and even death (Alberti *et al*., 2018).

Fusarium proliferatum can exist as an endophytic fungus in plant tissues or as a saprophyte outside plant tissues after the host's death. It spreads through seeds, plant residues, water flow, and even air (Stępień, 2015; Ekwomadu and Mwanza, 2023). Inoculum of *Fusarium proliferatum* has been

evident in soil, shallot seeds, plant residues, grass, or other plants around shallot crops. Moreover, pathogenic propagules have frequent detection in irrigation water, air, rainwater, and atmospheric dust (Martínez *et al*., 2021; Bahri *et al*., 2024; Maulidha *et al*., 2024). After the primary host dies, *Fusarium proliferatum* shifts to alternative hosts, causing infection in other shallot plants during subsequent planting periods (Gou *et al*., 2017; Alberti *et al*., 2018; Nishioka *et al*., 2019).

CONCLUSIONS

The percentage of fusarium wilt disease incidence in shallots (*A. cepa* L.) was 45.67%. Meanwhile, the various fusarium wilt, as a causative disease on shallot plants, were *Fusarium oxysporum, F. solani*, and *F. proliferatum*, distributed in 60 (86.96%), 54 (78.36%), and 43 (60.87%) locations, respectively. Furthermore, a new species of causative disease as fusarium wilt on shallots in Lombok Island, West Nusa Tenggara, Indonesia was the *Fusarium proliferatum*.

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