

Screening and Identification of *Fusarium* sp. Associated with Wilt Disease in Shallot Plants (*Allium cepa* L. var. *aggregatum*)

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Abstract

Background: *Allium cepa* L. var. *aggregatum* is an important horticultural commodity in Indonesia, particularly in East Java. However, shallot production is affected by *Fusarium* wilt, locally known as moler disease. Accurate identification of the associated *Fusarium* is important for supporting appropriate disease management. This study aimed to screen and characterize *Fusarium* sp. associated with wilt symptoms in shallot plants from Probolinggo and Nganjuk, East Java, through isolation, morphological characterization, and PCR amplification of the internal transcribed spacer (ITS) region. **Methodology:** Disease incidence was assessed based on symptomatic plants in five raised beds at each location. Three fungal isolates, P1 from Probolinggo and NG2 and NG3 from Nganjuk, were characterized morphologically and subjected to PCR using ITS1 and ITS4 primers. **Findings:** Disease incidence was 10.88% in Probolinggo and 0.45% in Nganjuk, with Probolinggo showing an approximately 24-fold higher incidence. All three isolates showed white to white-cream cottony colonies, hyaline and septate hyphae, and oval to ellipsoidal microconidia, consistent with *Fusarium*. PCR amplification produced a single band of approximately 750 bp in all isolates. **Contribution:** These findings provide baseline information on *Fusarium* associated with shallot wilt in Probolinggo and Nganjuk and support further sequencing-based identification.

Keywords: *Allium cepa*; *Fusarium oxysporum*; Isolation; Morphology; Pathogen Identification



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INTRODUCTION

Shallots (*Allium cepa* L. var. *aggregatum*) have high economic value for farmers in Indonesia, function as a staple in the kitchen and provide compounds such as allicin and flavonoids. Shallots (*Allium cepa* L. var. *aggregatum*) are a group of onions that have distinctive morphological characteristics, especially in the formation of several saplings in one clump, and are generally propagated vegetatively using bulbs (Irawan et al., 2025). Farmers in East Java grow it over a large area, but the yield they harvest tends to remain below the 20t ha⁻¹ that can be harvested (Masroni et al., 2023). Pests and diseases account for most of the shortage. Then based on data from Badan Pusat Statistik (2025) in 2025 East Java will produce shallots to reach 5,003,012.27 quintals.

One disease behind it is *Fusarium* wilt, which growers call *moler* (Aprilia et al., 2020; Cahyaningsrum et al., 2023). The fungus that causes it, *Fusarium* sp., survives in the soil as chlamydospores for years even when no shallot grows there, so a field that once carried the disease keeps infecting the crops planted after it (Hanif & Zamriyetti, 2023). Inside the plant, the fungus spreads from the roots into the xylem and restricts the flow of water and nutrients (Awal et al., 2025). Leaves turn yellow and twist, and the base of the bulb rots. On unmanaged plots the loss exceeds half the crop and can reach total failure (Sharma et al., 2024).

Growers who spray against the disease without knowing which fungus they face often see little effect, and the chemicals can reach the surrounding soil and water (Wildan et al., 2021). Reported that *Fusarium* wilt was one of the major fungal diseases affecting shallot production in West Sumatra, with mean disease severity and incidence of 26.1% and 27.7%, respectively. However, their study primarily focused on the distribution, incidence, and severity of fungal diseases based on field observations and did not provide detailed morphological and molecular characterization of *Fusarium* isolates. This limitation highlights the need for further identification of *Fusarium* associated with wilt symptoms in other major shallot-producing regions, particularly East Java (Yudha et al., 2023). Therefore, the present study aimed to screen and characterize *Fusarium* associated with wilt symptoms in shallot plants from Nganjuk and Probolinggo using morphological observations and PCR-based molecular analysis (Yanti et al., 2023). Farmers in Nganjuk grow shallot on a large scale, but no one has described *Fusarium* on the area's wilting plants in this detail.

This study aimed to screen and characterize *Fusarium* associated with wilt symptoms in shallot plants from Nganjuk and Probolinggo through isolation, morphological characterization, and PCR amplification. The observed morphological characteristics were evaluated for consistency with those described for *Fusarium oxysporum*. The findings provide baseline information on *Fusarium* associated with shallot wilt disease in the study areas and support further investigation of its identification and management. The study explains the fungus from symptomatic tissue and examines it macroscopically also microscopically against published descriptions of *Fusarium oxysporum*.

METHOD

Study Area and Sampling Design

The research was carried out from April 2026, on shallot farmers' fields in Nganjuk and Probolinggo Regencies, East Java. Observations were conducted on one field in Probolinggo (Biru Lonjor cultivar) and two fields in Nganjuk (Thailand and Bauji cultivars), which were selected based on the presence of plants showing moler symptoms. The Probolinggo sampling location was recorded at 7°50'14.20" S, 113°14'41.50" E (-7.8372784, 113.2448622), while the Nganjuk sampling location was recorded at 7°34'22.79" S, 111°57'00.56" E (-7.5729960, 111.9501550). The plants were approximately two weeks old at the time of sampling. A total of 1,221 plants per bed in Probolinggo and 2,000 plants per bed in Nganjuk were observed. Isolation and morphological identification were carried out at the Plant Pests and Diseases Laboratory of UPN "Veteran" East Java, while DNA extraction and PCR amplification were performed at the Molecular Genetics Laboratory of UIN Maulana Malik Ibrahim Malang. Sequencing was conducted through an external sequencing service provider.

Instrument

Equipment used in this study included a compound microscope, autoclave, laminar airflow cabinet, Petri dishes, pipettes, Erlenmeyer flasks, an inoculation needle (ose), an analytical balance, a thermal cycler for PCR amplification, and a gel electrophoresis apparatus fitted with a UV transilluminator. Materials consisted of potato dextrose agar (PDA), 70% ethanol, distilled water, sodium hypochlorite (NaOCl), the collected fungal isolates, and PCR reagents (ReadyMix, forward and reverse primers, nuclease-free water).

Field Sampling and Disease Assessment

Observations were made on shallot plants in Nganjuk Regency and Probolinggo Regency which showed symptoms of withered disease (moler). Observations were made on Blue cultivars of Lonjor, Thailand, and Bauji. Symptomatic plants were documented using digital cameras, then samples of symptomatic plants and soil were taken to be used in the fungal isolation stage. Symptoms of disease observed included yellowing leaves, twisted leaves, and decay at the base to stem of the plant. Disease intensity was calculated based on the ratio of the number of plants showing symptoms to the total number of plants in the observation unit For each surveyed plot, disease intensity was calculated using the incidence formula applied in comparable field assessments of *Fusarium*-associated wilt in *Allium* crops (Saputri et al., 2018; Irfandri et al., 2025):

$$IP = \frac{a}{a+b} \dots\dots\dots (1)$$

Where IP is disease intensity (%), a is the number of symptomatic plants, and b is the number of asymptomatic plants within the same observation unit.



Figure 1. Wilt symptoms on Shallot Probolinggo.



Figure 2. Wilt symptoms on Shallot Nganjuk

Field data were collected through direct observation of shallot plots in Probolinggo and Nganjuk. Plants displaying yellowing or curling foliage together with basal bulb rot were identified and photographed in situ. Symptomatic tissue, together with the surrounding rhizosphere soil, was collected from each affected plant for isolation. For every surveyed plot, the number of symptomatic and asymptomatic plants was recorded to allow subsequent calculation of disease intensity.

Isolation of Fungal Isolates

Symptomatic plant tissues collected from the field were surface-sterilized using 1% sodium hypochlorite (NaOCl) for 1 minute and subsequently placed on Potato Dextrose Agar (PDA) under aseptic conditions in a laminar airflow cabinet that had been pre-treated with ultraviolet light for 30 minutes. The plates were sealed and labeled, then incubated at room temperature under light conditions provided by laboratory lamps for 1–2 weeks until fungal colonies emerged. Fungal colonies were subsequently sub-cultured onto fresh PDA to obtain pure isolates for further characterization refers to [Asrul et al., \(2021\)](#).

Morphological Characterization and Identification

Pure fungal isolates were characterized based on macroscopic and microscopic observations. Macroscopic characteristics were examined after seven days of incubation on Potato Dextrose Agar (PDA), including colony color, mycelial texture, and growth pattern. Microscopic observations were performed using wet-mount preparations under a compound light microscope to examine hyphal and conidial characteristics, including microconidia and macroconidia. The observed morphological characteristics were compared with published descriptions of *Fusarium oxysporum* to evaluate their correspondence with the reference species ([Hanif & Zamriyetti, 2023](#); [Marianah et al., 2024](#)).

Molecular Identification

Genomic DNA was extracted from pure isolates using the Quick-DNA™ Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) according to the kit protocol. A total of 50 - 100 mg of mycelium was used as the extraction material and the DNA was stored at –20 °C until used for PCR. The single-band PCR product was then sent for Sanger sequencing using ITS1 and ITS4 primers. The quality of the product was checked using the Agarose Quantification Report, while the sequencing results were analyzed using Sequence Scanner Software 2 and compared to the GenBank/NCBI database.

PCR Amplification and Electrophoresis

PCR amplification of the ITS region was performed using the universal fungal primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). The PCR reaction had a total volume of 12 µL, consisting of 6 µL ReadyMix, 1 µL of each primer, 1 µL of template DNA, and 3 µL of distilled water. PCR amplification was performed with an initial denaturation at 95 °C for 5 min, followed by 34 cycles of denaturation at 95 °C for 1 min, annealing at an optimized temperature range of 52.7–61°C for 1 min, and extension at 72 °C for 2 min. A final extension was performed at 72 °C for 10 min. The PCR products were separated by electrophoresis on a 0.8% agarose gel prepared in 1× TBE buffer, using a 1-kb DNA ladder as a molecular-size marker. Electrophoresis was performed at 50 V for 50 min, and the DNA bands were visualized using a UV transilluminator.

Data analysis

Data were analyzed descriptively and qualitatively. Macroscopic and microscopic characters of the isolated fungi were compared against published reference descriptions of *F. oxysporum*, while disease intensity values were summarized to sSscharacterize the distribution of symptomatic plants across the surveyed plots in Probolinggo and Nganjuk.

RESULT AND DISCUSSION

Disease Intensity on Surveyed Plots

Disease incidence varied substantially between the two sampling locations. In Probolinggo, disease incidence across the five raised beds ranged from 3.93% to 19.98%, with an overall incidence of 10.88%. In Nganjuk, disease incidence ranged from 0.15% to 1.00%, with an overall incidence of 0.45%. Thus, the overall disease incidence in Probolinggo was approximately 24-fold higher than that in Nganjuk. This substantial difference indicates that disease occurrence varied considerably between the two locations. However, the difference should be interpreted cautiously, as factors such as pathogen inoculum density, environmental conditions, crop growth stage, and field management were not directly measured in this study.

Table 1. Shallot Disease Incidence in Probolinggo and Nganjuk

Location	Raised Bed	Symptomatic	Total	Incidence (%)	Mean (%)
Probolinggo	Bed 1	48	1,221	3.93	10.88
	Bed 2	142	1,221	11.63	
	Bed 3	244	1,221	19.98	
	Bed 4	180	1,221	14.74	
	Bed 5	50	1,221	4.09	
Nganjuk	Bed 1	6	2,000	0.30	0.45
	Bed 2	10	2,000	0.50	
	Bed 3	3	2,000	0.15	
	Bed 4	20	2,000	1.00	
	Bed 5	6*	2,000	0.30	

Interpreted in epidemiological terms, this contrast carries two distinct implications. First, the magnitude gap between Probolinggo and Nganjuk suggests that the two fields differ substantially in cumulative inoculum exposure and not in some uniform background risk shared by all shallot-growing areas in East Java. Second, the considerable bed-to-bed variability recorded within Probolinggo itself, where intensity in Bed 3 was roughly five times that of Bed 1, indicates that disease pressure is not homogeneously distributed even within a single farming unit. Such patchiness is a recognized feature of soil-borne pathogens, whose propagules disperse through localized mechanisms such as irrigation water, contaminated soil particles, and field tools instead of through wind-borne spores capable of producing a more even distribution across a planting area (Jayadi et al., 2025; Degani et al., 2022).

Fusarium sp. as a Soil-Borne Pathogen

The mean disease intensity of 10.88% recorded at Probolinggo positions the site within the range documented for *Fusarium* wilt outbreaks at East Java shallot production centers, where [Sholeh & Nurcahyanti, \(2023\)](#) reported progressive increases in moler incidence across successive growing seasons in Probolinggo Regency. The capacity of *Fusarium* sp. to sustain infection at this level, even under field management practices, can be attributed to the persistence of chlamydospores in soil. [Hanif & Zamriyetti \(2023\)](#) established that these thick-walled resting structures allow the pathogen to remain viable in the absence of a host for multiple years, rendering previously infected plots a continuous source of primary inoculum. The findings at Probolinggo are consistent with this mechanism: the relatively higher disease intensity at this site compared with Nganjuk is most plausibly explained by a longer accumulated inoculum history in the soil profile rather than by any single environmental event at the time of survey.

The pathogen's infection pathway also accounts for the characteristic symptoms observed at both locations. Symptomatic plants at Probolinggo and Nganjuk displayed yellowing and twisting of foliage, alongside basal bulb rot, a symptom complex that results from the fungus colonizing root tissue, advancing into the xylem, and blocking water and nutrient translocation throughout the plant ([Maulana et al., 2025](#); [Sakane et al., 2023](#)).

[Marianah et al., \(2024\)](#) documented the same progression in shallot fields in Brebes, Central Java, where the causal complex included *Fusarium oxysporum* f.sp. *cepae*, *F. solani*, and *F. proliferatum*, each producing basal rot and vascular discoloration on infected plants. The similarity in symptom expression across these geographically distinct reports ([Kalman et al., 2020](#); [Diabankana et al., 2024](#)) strengthens the inference that the field observations in this study reflect genuine *Fusarium* infection, a presumption that the isolation and characterization work below was designed to test directly.

Macroscopic Characterization of *Fusarium* Isolates on PDA

Isolation from symptomatic tissue at both sites yielded three pure fungal cultures: P1, recovered from Probolinggo, and NG2 and NG3, both recovered from Nganjuk. Grown on potato dextrose agar, all three isolates developed as cottony, floccose colonies with a white to white-cream coloration; mycelial growth in isolate P1 had, by the time of observation, spread densely enough to cover nearly the entire plate, while NG2 and NG3 still presented several discrete, radially expanding colonies with a comparatively lower degree of coalescence. No pink, purple, or orange pigmentation was detected on the colony surface or in the surrounding medium in any of the three isolates (Table 2, Figure 2).

This coloration and cottony texture correspond closely to the young-culture phenotype described for *Fusarium oxysporum* by [Wakhidah et al., \(2021\)](#) and [Kumalasari et al., \(2021\)](#), both of whom noted that violet or reddish pigmentation on the reverse of the colony develops progressively as the culture ages rather than appearing from the outset; its absence at this stage of growth therefore does not exclude *Fusarium oxysporum* identity. The difference between P1, on one hand, and NG2 and NG3, on the other, lies mainly in the extent of colony coalescence rather than in colour or texture, and more

plausibly reflects a difference in the stage of purification each isolate had reached at the time of examination than a distinct taxonomic identity.

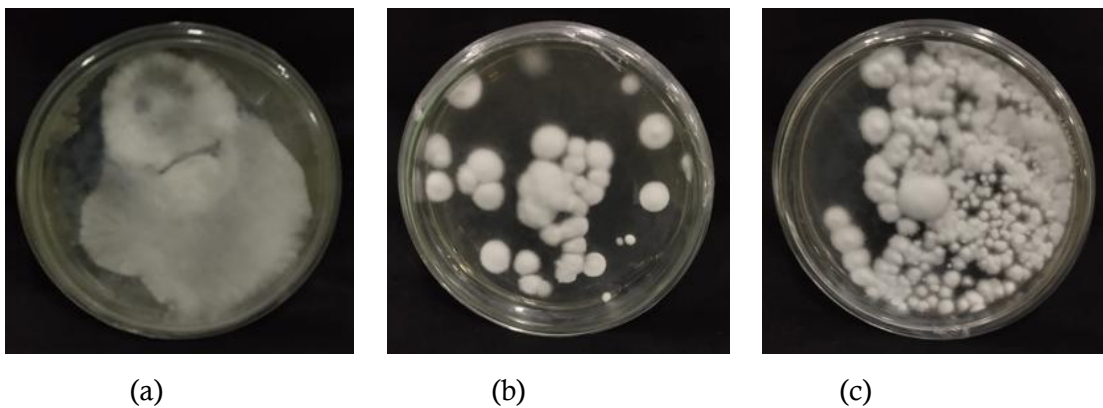


Figure 2. Colony morphology of fungal isolates grown on Potato Dextrose Agar (PDA): (a) P1, (b) NG2, and (c) NG3. The diameter size of Petri dish (90 mm).

Microscopic Characterization of *Fusarium* Isolates on PDA

Microscopic slide preparations of the three isolates revealed hyaline, branched, septate hyphae in every case, a character diagnostic of the hyalohyphomycetes, the group of septate fungi that do not deposit melanin in the hyphal wall (Marianah et al., 2024). Abundant microconidia, oval to elliptical in shape, were observed arranged along the conidiophores and at the tips of short phialides in all three isolates, a pattern of production that Kumalasari et al., (2021) list among the principal microscopic features distinguishing *F. oxysporum* (Table 2, Figure 3). Macroconidia, the sickle-shaped, multiseptate spores that constitute the most reliable diagnostic structure at the species level within *Fusarium*, were not unambiguously identified in any of the three isolates; a small number of elongated, faintly septate conidia observed in isolate P1 may represent immature macroconidia, but their curvature had not developed sufficiently to confirm the characteristic falcate shape reported for the species. Chlamydospores were similarly absent from all three preparations.

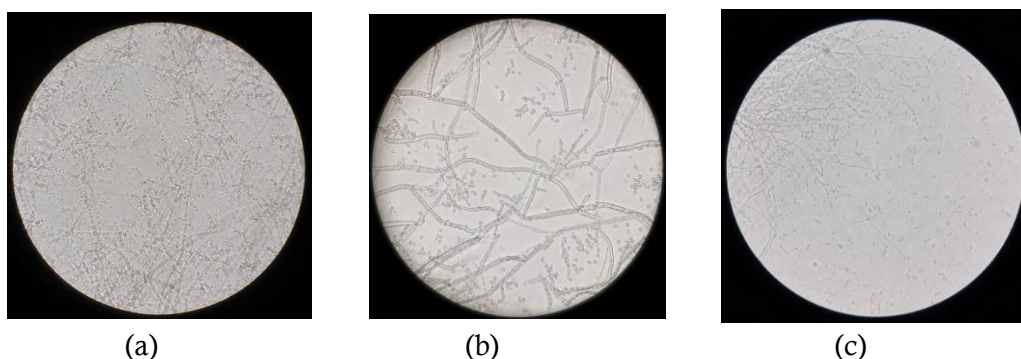


Figure 3. Hyphae and Microconidia of Isolates (a) P1, (b) NG2, and (c) NG3 Under a Compound Microscope (400x Magnification).

Wakhidah et al., (2021) attribute the delayed appearance of *macroconidia* and *chlamydospores* in *Fusarium* cultures to culture age, incubation conditions, and medium composition rather than to their genuine absence from a given isolate, an explanation consistent with the relatively young cultures examined here. On the strength of the hyphal and *microconidial* characters recorded, the three isolates can be assigned with reasonable confidence to the genus *Fusarium* and are morphologically consistent with *F. oxysporum*, although the absence of clearly formed *macroconidia* means that this species-level assignment remains provisional and requires molecular confirmation.

Tabel 2. Macroscopic and Microscopic Characteristics of Isolates P1, NG2, and NG3

No	Character	P1	NG2	NG3
1	Colony Colour	White	White to white-cream	White
2	Texture	Floccose, thick mycelium	Cottony, moderate	Cottony, dense, coalescent
3	Pigmentation	None observed	None observed	None observed
4	Hyphae	Hyaline, branched, Septate	Hyaline, branched, Septate	Hyaline, branched, Septate
5	Microconidia	Present (round to oval)	Abundant (oval-round)	Abundant (oval-elliptical)
6	Macroconidia	Not clearly confirmed	Not observed	Not observed
7	Chlamydospore	Not observed	Not observed	Not observed
8	Genus-level	<i>Fusarium</i> sp.	<i>Fusarium</i> sp.	<i>Fusarium</i> sp.

PCR Amplification and Gel Electrophoresis

Molecular confirmation was pursued by extracting genomic DNA from the three purified isolates and subjecting it to PCR amplification under the conditions described in the Method section. Agarose gel electrophoresis of the amplification products, followed by quantification against a 1 kb DNA ladder on a 0.8% agarose gel, showed a single, well-resolved band of approximately 750 bp in each of the three lanes, with no secondary bands or smearing (Figure 4, Table 3). All three amplicons passed quality quantification and were recommended for gel extraction prior to purification and sequencing, indicating that the PCR reaction generated a specific product of consistent size across isolates from both survey locations.

Gel electrophoresis visualization revealed amplicon sizes within this range, falling within the size window typically reported for the gene regions used to resolve closely related *Fusarium* species, including ITS. This is consistent with the successful, specific amplification reported by Vetrova et al., (2024) and Jayadi et al., (2025) when confirming *Fusarium* identity in onion and shallot tissue, respectively, using comparable primer targets. The consistency of band position and quality across all three isolates supports the conclusion that the amplification performed reliably regardless of the isolate's site of origin, and that the resulting amplicons are of adequate quality to proceed to sequencing. Because sequence data are still pending, however, the present result

confirms the specificity and quality of amplification rather than the identity of the amplified species; definitive assignment to *Fusarium oxysporum* or another closely related species such as *Fusarium proliferatum*, which Jayadi et al. (2025) caution can share overlapping morphology with *Fusarium oxysporum* under standard culture conditions, must await comparison of the sequencing output against reference entries deposited in GenBank.

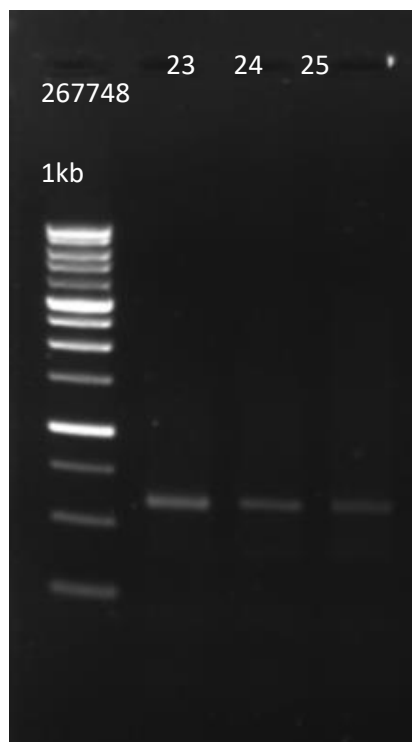


Figure 4. Agarose gel electrophoresis of PCR products amplified using the ITS1/ITS4 primer pair. Lane M: 1-kb DNA ladder; lane 23: isolate P1 from Probolinggo; lane 24: isolate NG2 from Nganjuk; lane 25: isolate NG3 from Nganjuk. The amplified fragments were approximately 750 bp in size.

Comparison with Previous Studies on Moler Disease

The contrast between the two survey locations remains substantial when placed against earlier reports on moler disease. Nganjuk's mean disease intensity of 0.49% indicates that symptomatic plants were sparse and broadly scattered, with the highest single-plot value reaching only 1.00% in Bed 4. This low intensity does not, however, indicate the absence of infection risk. Irfandri et al., (2025) demonstrated that moler incidence can escalate rapidly under favorable soil moisture conditions and where inoculum is introduced through infected planting material; in Riau Province, disease intensity in newly developed shallot areas increased sharply within a single growing season following the use of non-certified seed bulbs. Nganjuk's status as an established production center means that the pathogen is likely already present in field soil, and the low current intensity may partly reflect the observation period, the specific growth stage of the crop at the time of survey, or localized drainage management that limited disease expression at that point.

Wildan et al., (2021) specified the environmental conditions that support *Fusarium* sp. development on shallot: soil temperature between 25 and 30 °C, soil moisture at 80 – 100%, relative humidity of 70 – 90%, and soil pH between 5.5 and 6.5. These parameters are broadly consistent with conditions typical of East Java's lowland shallot areas during the growing season (Widono et al., 2022). The fact that Probolinggo showed higher disease intensity than Nganjuk does not necessarily mean that conditions at the former site were more favorable in absolute terms during the observation period; rather, the difference more plausibly reflects accumulated inoculum load in the soil, since Probolinggo has a documented history of moler as noted by Suhartiningsih et al., (2023). The Nganjuk data, while recording low current intensity, should be interpreted cautiously given that Supyani et al., (2021) recorded increasing moler severity and associated yield losses at Brebes across three consecutive growing seasons, a trend that could equally emerge in Nganjuk if inoculum builds up over successive plantings.

The yield loss potential associated with the disease levels observed at Probolinggo warrants specific attention. Asrul et al., (2021) cited field losses of up to 50% and storage losses of approximately 30% where *Fusarium* infection went unmanaged; severe outbreaks can result in total crop failure. At a mean disease intensity of 10.88%, the Probolinggo site falls within the range where economic losses are meaningful but where targeted intervention could still limit further spread within the growing season. Supyani et al., (2021) documented that without active management, the trend in affected shallot areas runs toward increasing intensity in successive seasons, which makes early and accurate pathogen identification, now anchored by the morphological data and confirmed sequencing results of *Fusarium oxysporum* obtained in this study, the logical first step toward designing control strategies suited to the specific *Fusarium* species present at each location (Hadiwiyono et al., 2024; Wijayanti et al., 2024).

CONCLUSION

Moler disease was found in shallot plants in Probolinggo and Nganjuk Regencies, with symptoms in the form of yellowing leaves, twisted leaves, and decay at the base of the tubers. The incidence rate of disease differed substantially between the two locations, with an overall disease incidence of 10.88% in Probolinggo and 0.45% in Nganjuk. Three fungal isolates, namely P1 from Probolinggo and NG2 and NG3 from Nganjuk, showed morphological characteristics consistent with the genus *Fusarium*, characterized by white colonies with cotton-like textures, hyaline and septa-like textures, and oval to ellipsoid-shaped microconidia. Amplification of the ITS region using ITS1 and ITS4 primers produced a single band of approximately 750 bp in all three isolates, so that PCR products can be used for further sequencing analysis. The results of this study provide basic information about the existence of *Fusarium* which is associated with the symptoms of molar disease in shallots in Probolinggo and Nganjuk Regencies. However, identification at the species level and *forma specialis* still needs to be treated as an initial identification until the sequencing results are fully validated, while the

causal relationship with moler disease needs to be confirmed through pathogenicity tests.

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