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SHORT COMMUNICATION

First report of *Fusarium incarnatum* causing fruit rot of chili pepper in Indonesia

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ABSTRACT

Chili pepper (*Capsicum annuum* L.) is a widely cultivated horticultural crop with significant potential as an export commodity. Fruit rot of chili pepper was recorded in Lubuk Cuik village, North Sumatra Province, Indonesia. One hundred and fifty symptomatic fruits were randomly collected from four locations using purposive random sampling. Fungal colonies were isolated from infected fruits until pure cultures were obtained. Pure colonies observed for macroscopic examination exhibited abundant, dense white aerial mycelia with a brown–yellowish base. Microscopic observations revealed numerous long and slender macroconidia, 3–5 septate, with a curved shape and tapering ends. Microconidia were rare, pyriform to obovate in shape, and mostly 0–1 septate. Chlamydospores were thick-walled, intercalary, globose, and produced singly or in chains. Molecular identification was performed using the internal transcribed spacer (ITS) and translation elongation factor (TEF-1α) regions. The pathogenicity of the eight *Fusarium incarnatum* isolates was tested twice. The morphological characteristics, phylogenetic analysis, and pathogenicity assay confirmed that all isolates were consistent with *Fusarium incarnatum.* This study represents the first confirmed report of *F. incarnatum* causing fruit rot of chili pepper in Indonesia.

Keywords: Chili pepper, first report, fruit rot, *Fusarium incarnatum*

INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is a globally cultivated vegetable and spice crop valued for its nutritional content, culinary applications, and bioactive compounds (Faisal & Mustafa, 2025). In Indonesia, chili pepper is also a superior horticultural product with significant potential as an export commodity. Lubuk Cuik Village, located in the District of Lima Puluh, North Sumatra Province, Indonesia, is one of the largest producers in the country, achieving an annual yield of 12-15 tonnes/ha, which increases to 40-50 tonnes/ha during the peak harvesting period (Sumutprov, 2023).

However, its productivity and quality are often constrained by various fungal diseases, notably those caused by *Fusarium* species. While Fusarium wilt (a vascular disease) has long drawn research attention, Fusarium fruit and stem rot is becoming increasingly recognized as a significant concern (Zakaria, 2023). Fusarium rot in chili typically begins as localized, water-soaked, sunken lesions near the calyx or stem end of the fruit, which later expand, often coalescing and showing white to pinkish mycelial growth, eventually leading to complete fruit decay (Zhu et al., 2021). The quality and shelf life of chili pepper and other *C. annuum* species can be further deteriorated by postharvest fungal rots, including *Alternaria* (Balamurugan & Kumar, 2023), *Aspergillus niger* and *Rhizopus stolonifer* (Fatimoh et al., 2017), as well as *Botrytis cinerea* (Naz et al., 2018).

From a taxonomic and pathogen variability perspective, *Fusarium* is a diverse genus organized into multiple species complexes with high genetic, morphological, and pathogenic diversity. The fungal genus *Fusarium* comprises soil-borne pathogens that are widespread worldwide and occur in all types of soils (Backhouse et al., 2001). *Fusarium* spp. are regarded as one of the top ten plant-pathogenic fungi in the world in terms of both scientific and economic significance. Several *Fusarium* species are associated with plant diseases, including fruit rot of numerous crops (Ramdial et al., 2017). Within chili pepper, *F. solani*, *Fusarium oxysporum* (in some contexts), and other *Fusarium* taxa have been implicated in rot or wilt diseases (Engalycheva et al., 2024; Delai et al., 2024).

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Fusarium shows a high degree of genetic diversity, and numerous morphospecies have been demonstrated to form species complexes. Several species complexes, such as the *F. incarnatum-equiseti* species complex (FIESC), *F. oxysporum* species complex (FOSC), and *F. solani* species complex (FSSC), are ubiquitous (Summerell et al., 2010). Members of FIESC, *F. incarnatum,* the *F. lactis* species complex (FLASC), *F. oxysporum, F. proliferatum, F. equiseti, F. solani, F. fujikuroi, F. subglutinans,* and *F. concentricum* are primary causes of chilli pepper fruit rot (Frans et al., 2017; Mathur & Utkhede, 2004; Ramdial et al., 2016; Ramdial et al., 2017; Tariq et al., 2018; Wang et al., 2013; Wang et al., 2019; Yang et al., 2009; Zhu et al., 2021). *Fusarium incarnatum* is part of the *Fusarium incarnatum-equiseti* species complex, which includes species or lineages with morphological and genetic diversity and variable pathogenicity. Members of this complex have been implicated in diseases of a wide range of hosts (vegetables and fruits) in both field and postharvest settings (Xia et al., 2019). Because many members are morphologically similar but genetically distinct, accurate identification often requires molecular tools (multi-locus sequencing and phylogenetic analyses) in addition to morphological characters (Wang et al., 2019). Key genetic markers such as the internal transcribed spacer (ITS) region, translation elongation factor-1 alpha (EF1-α), and the second largest subunit of RNA polymerase II (RPB2) are commonly used for accurate species identification within the genus (Chen et al., 2025).

F. incarnatum as part of FIESC, has a global distribution in tropical, subtropical, and temperate regions (Ramdial et al., 2016). This species can act as a plant pathogen, causing various diseases including leaf spots, fruit rot, and postharvest decay in vegetables and fruits. *F. incarnatum* has been reported in luffa (*Luffa cylindrica*), causing leaf spot and fruit spot in China (Chen et al., 2025), as well as in corn, causing corn stalk rot disease in Iraq (Jamel et al., 2025). Compared with other Fusarium species, fruit rot symptoms on ripe chili pepper caused by *F. incarnatum* include chlorosis, water-soaked spots, and soft lesions developing on fruit with white to light gray fungal mycelium present inside. Under moist conditions, white or pale pink mycelial growth appears, and the diseased fruit becomes soft and decays at later stages (Zhu et al., 2021). In the genus *Capsicum*, *F. incarnatum* has been reported infecting chili pepper in China (Li et al., 2018; Zhu et al., 2021), bell peppers in Trinidad (Ramdial et al., 2016), chili in Pakistan (Tariq et al., 2018), and in Malaysia (Ishak et al., 2024). Yield loss in affected fields caused by *F. incarnatum* reached up to 15% in China (Zhu et al., 2021) and 11% in Pakistan (Tariq et al., 2018). This species also produces various secondary metabolites (mycotoxins), including trichothecenes, zearalenone, enniatins, beauvericin, apicidin, equisetin, and other compounds (Villani et al., 2019).

In Indonesia, *F. incarnatum* causing fruit rot on mature fruit has not previously been reported in chili pepper. However, Maryani et al. (2019) reported *F. incarnatum* causing fruit rot disease in banana in Indonesia. Fusarium wilt of chili pepper in Indonesia is usually associated with *F. oxysporum*, which attacks both young and mature plants. Diseased young plants show stunting, chlorosis, wilting, and eventual death, while flowers and fruits fail to develop normally in infected mature plants (Ferniah et al., 2014). Since this species has not been documented in chili pepper in the country, information on symptoms and pathogen characteristics remains limited.

This study aims to document the first occurrence of *F. incarnatum* infecting chili pepper in Indonesia. These findings will serve as a reference for future research on chili pepper fruit rot, including management strategies, particularly in Indonesia.

MATERIALS AND METHODS

Research Site. Sampling was conducted between 2022 and 2023 in four locations that serve as primary centers for chili pepper plantation in Lubuk CuikVillage (3^o 16′ 55.2″ N, 99^o 27′ 0″ E), North Sumatra Province, Indonesia. Five chili pepper cultivars were cultivated with the same treatments in Lubuk Cuik Village, including three local cultivars (*Hijau, Putih,* and *Coklat*) and two hybrid cultivars (*Laju* and *Djitu*). No reports of Fusarium wilt disease were recorded in these locations.

Fungal Isolation and Purification. Fifty affected plants were surveyed, and 150 symptomatic plants with mature symptomatic fruits were randomly collected from each location using purposive random sampling. Symptomatic mature fruit samples showing chlorosis, water-soaked spots, and soft lesions were placed in polypropylene plastic bags and stored in a cooler box for transport to the laboratory. Under sterile conditions, stems, roots, and fruits were carefully washed under running tap water, surface-disinfected with 70% ethanol, then cut into pieces (3 mm^2) and plated on potato dextrose agar (PDA) culture medium. The cultures were incubated at room temperature (27 ºC) for seven days (Umesha et al., 2016). After seven days of incubation, fungal colonies were re-isolated onto fresh media using both PDA and synthetic nutrient-deficient agar (SNA) using the single-spore isolation method until pure cultures were obtained.

Macroscopic and Microscopic Characterization. The key characteristics observed included the structure and color of the mycelium; the shape, size, and septation of macroconidia and microconidia; and the presence of chlamydospores, false heads, and conidiophores.

Molecular Characterization. Molecular identification of the fungal mycelium was carried out at the Indonesian Genetic Science Laboratory, Tangerang, Banten, Indonesia, using DNA barcoding. Total fungal DNA was extracted using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, D6005) according to the manufacturer's protocol. Two DNA fragments, the internal transcribed spacer (ITS) and translation elongation factor (TEF-1α), were amplified and sequenced using primer pairs ITS1/ITS4 (White et al., 1990) and EF1/EF2 (Carbone & Kohn, 1999).

PCR conditions started with a initial denaturation at 95 ºC for 1 min, followed by denaturation at 95 ºC for 10 s, annealing at 52 ºC for 15 s, and extension at 72 ºC for 15 s, repeated for 35 times. PCR products were then stored at 4 ºC. DNA concentration and quality were determined by spectrophotometry using a NanoDrop Spectrophotometry (Table 1 and 2) and stored at -20 ºC until use.

The ITS and TEF-1α amplification products were electrophoresed on 1% agarose gel in TBE buffer at 50 volts for 30 min. DNA bands were visualized using a UV transilluminator. Fragment sizes were compared with a 3 kb DNA ladder and 1 kb DNA ladder for ITS and TEF-1α gene products, respectively (Figure 1 and 2).

Two-way sequencing was performed using the Sanger DNA sequencing method with capillary electrophoresis (1st BASE Subcontract Lab Testing). BLAST analysis was conducted using GenBank NCBI. The ITS gene sequences generated for the eight fungal isolates were viewed and checked using ChromasPro 1.5, then assembled into contigs using the CAP3 Sequence Assembly Program (Huang & Madan, 1999). Phylogenetic trees were analyzed using the neighbor-joining method (Saitou & Nei, 1987) implemented in MEGA software version 11.0 (Tamura et al., 2021). Bootstrap analysis with 1000 replicates was performed to test the statistical reliability of the phylogenetic trees.

Pathogenicity Assay. The pathogenicity of the eight isolates of *F. incarnatum* was tested twice on five

Table 1. DNA quantification of ITS amplicons using NanoDrop spectrophotometry

No.	Isolates	Concentration
1	FoLC1	20.8
2	FoLC2	12.2
3	FoLC3	14.8
4	FoLC4	27.9
5	FoLC5	44.7
6	FoLC6	44.1
7	FoLC7	15.7
8	FoLC8	21.0

Table 2. DNA quantification of TEF-1α amplicons using NanoDrop spectrophotometry

No.	Isolates	Concentration
1	FoLC1	4.01
2	FoLC2	7.79
3	FoLC3	13.57
4	FoLC4	16.31
5	FoLC5	15.96
6	FoLC6	4.01
7	FoLC7	7.79
8	FoLC8	21.73

healthy unwounded and five wounded mature chili pepper fruits. After surface disinfection, unwounded fruits were inoculated by spreading 100 µL of a conidial suspension (10^6 conidia/mL) obtained from 7-day-old cultures over the entire fruit surface, including the pedicle and calyx.

For wounded fruits, inoculation was conducted at three different points, i.e., apex, middle, and calyx. Twenty microliters of macroconidial suspension were applied to each wound using a sterile needle to a depth of approximately 3 mm, creating wounds of approximately 2 mm. Fruits treated with sterile distilled water served as negative controls.

All treated fruits were placed on moist cotton in a moist chamber at 25 ± 2 ºC with a 16-h light/8-h dark photoperiod. Symptom development was continuously monitored. Fruit rot symptoms appeared as soft rot on the fruit surface of inoculated mature chili peppers.

RESULTS AND DISCUSSION

Macroscopic and Microscopic Characterization. The isolates consistently exhibited abundant, dense white aerial mycelia with a brown–yellowish pigmentation at the colony base (Figure 3). This pigmentation pattern is a diagnostically relevant trait within the *F. incarnatum–equiseti species complex* (FIESC) and has been used to distinguish *F. incarnatum* from closely related taxa such as *F. subglutinans* sensu lato and *F. sporotrichoides* (Leslie & Summerell, 2006). However, colony pigmentation alone is insufficient for definitive identification due to the well-documented phenotypic plasticity within the genus *Fusarium*. This limitation underscores the need to interpret macroscopic traits cautiously and in conjunction with microscopic and molecular data.

Microscopically, the isolates produced

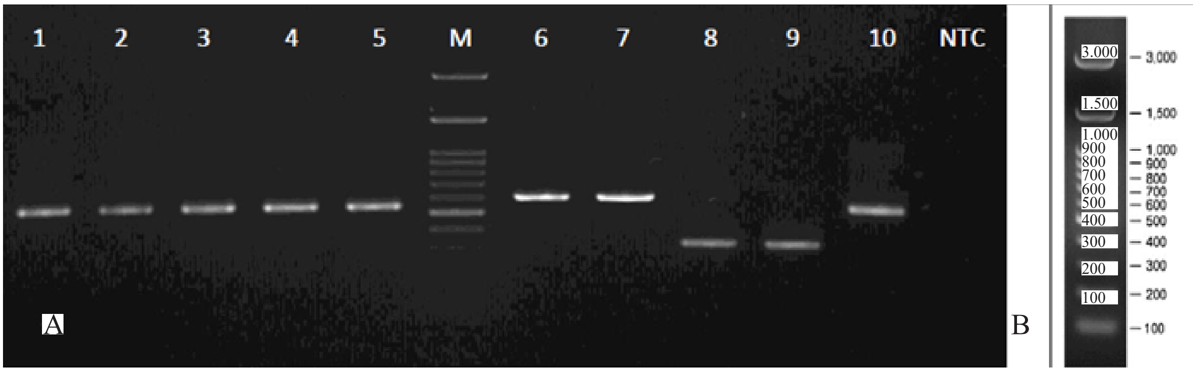

Figure 1. Agarose gel electrophoresis of ITS gene amplification products of *Fusarium* isolates. A. Electrophoresis results of the ITS gene amplification products. 1. FoLC1 isolate; 2. FoLC2 isolate; 3. FoLC3 isolate; 4. FoLC4 isolate; 5. FoLC5 isolate; 6. FoLC6 isolate; 7. FoLC7 isolate; 8. FoLC8 isolate (not *F. incarnatum*); 9. FoLC9 isolate (not *F. incarnatum*); 10. FoLC10 isolate; B. Marker 3 kb DNA ladder (Geneaid).

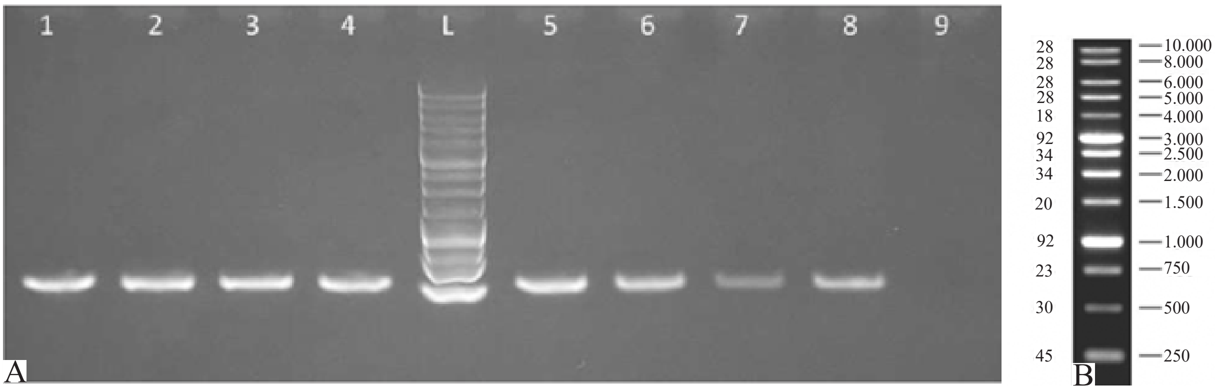

Figure 2. Agarose gel electrophoresis of TEF-1α gene amplification products of *Fusarium* isolates. A. Electrophoresis results of the TEF-1α gene amplification products. 1. FoLC1 isolate; 2. FoLC2 isolate; 3. FoLC3 isolate; 4. FoLC4 isolate; 5. FoLC5 isolate; 6. FoLC6 isolate; 7. FoLC7 isolate; 8. FoLC10 isolate; B. Marker 1 kb DNA ladder (Geneaid).

abundant, slender, 3–5 septate macroconidia with characteristic curvature, tapered apices, and foot-shaped basal cells. These features are consistent with canonical descriptions of FIESC members. The rarity of microconidia and their production on mono- and polyphialides forming false heads further supports their placement within *F. incarnatum*. The presence of thick-walled, intercalary chlamydospores, formed singly or in chains, indicates an adaptive survival strategy under adverse environmental conditions, which may contribute to persistence in soil and plant debris (Figure 4).

Importantly, the observed morphological traits align not only with standard taxonomic descriptions (Leslie & Summerell, 2006) but also with reports of *Fusarium* species associated with chili in Indonesia,

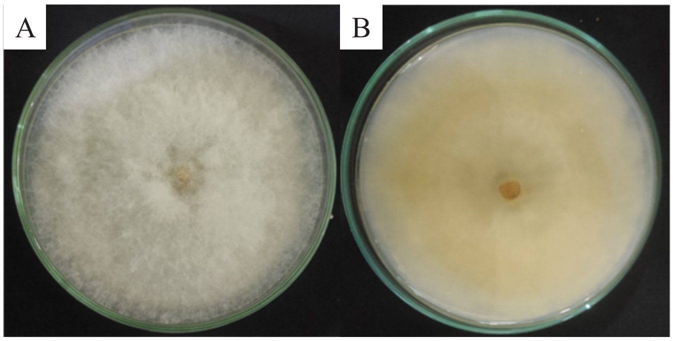

Figure 3. Colony morphology of *F. incarnatum* isolate FoLC3 on PDA after seven days of incubation at 27 ºC. A. Front view; B. Reverse view.

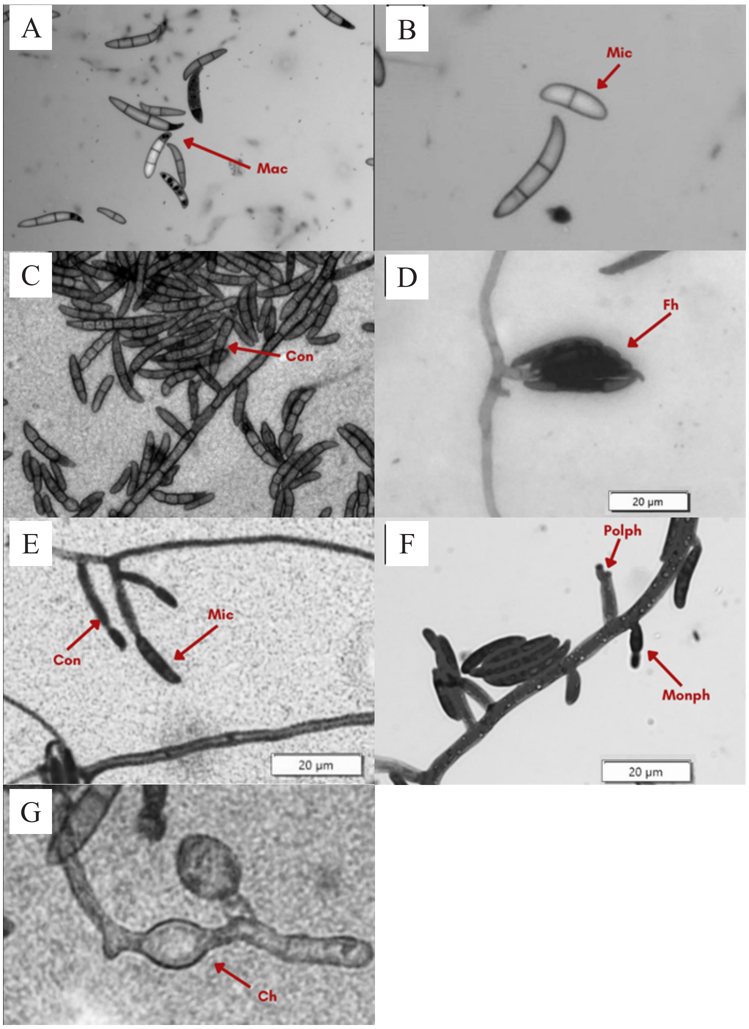

Figure 4. Microscopic characteristics of conidia of *F. incarnatum* on PDA. A. Macroconidia (MaC); B. Microconidia (MiC); C. Conidiophores (Con); D. False heads (Fh); E. Conidiophore with microconidia (Con; Mic); F. Polyphialide (Polph) and Monophialide (Monph); G. Chlamydospores (Ch). Bar= 20 µm.

suggesting that these features are conserved across ecological contexts (Hutauruk et al., 2016). Nevertheless, given the morphological overlap among species within FIESC, reliance on morphology alone would likely lead to misidentification. Therefore, the integration of molecular approaches is essential to resolve species boundaries within this complex.

Molecular Characterization. The ITS and TEF-1α sequence analyses provided robust support for species-level identification. The high sequence similarity (99–100%) with reference *F. incarnatum* isolates, combined with phylogenetic clustering into a well-supported and distinct clade, confirms the taxonomic placement of all eight isolates. Notably, the separation of these isolates from *F. oxysporum* f.sp. *capsici* highlights the importance of molecular tools in distinguishing phylogenetically related but biologically distinct taxa (Figure 5 and 6).

The limitations of ITS as a universal barcode

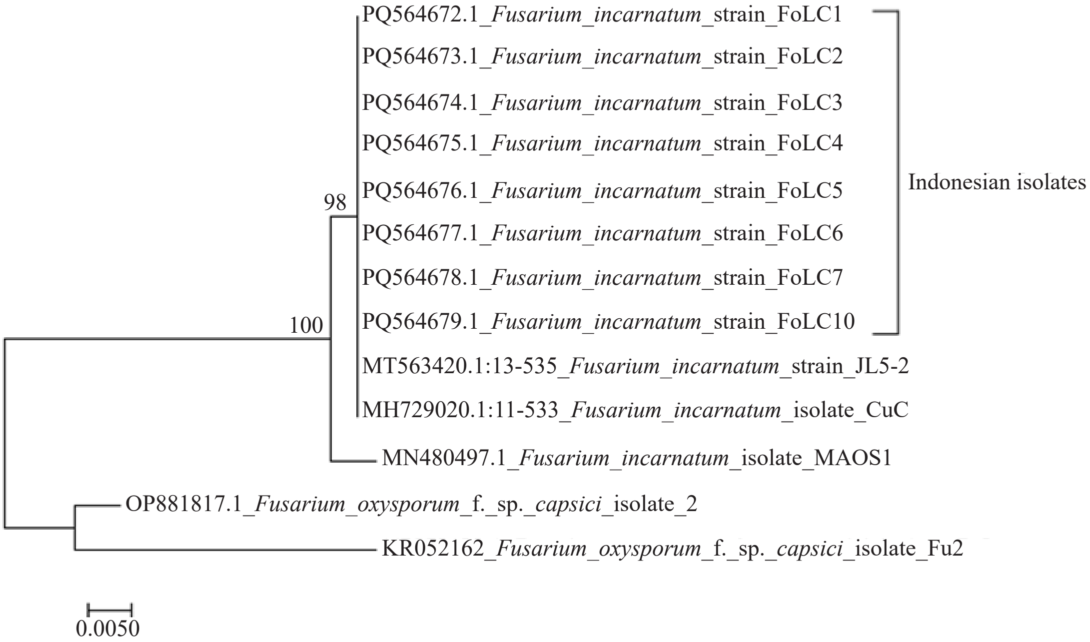

Figure 5. Phylogenetic tree of *F. incarnatum* based on ITS-rDNA sequences, including Indonesian isolates (FoLC1, FoLC2, FoLC3, FoLC4, FoLC5, FoLC6, FoLC7, FoLC10), constructed using the neighbour-joining method in MEGA11 (Tamura et al., 2021). Bootstrap values, expressed as percentages from 1000 replicates, are indicated at branch nodes.

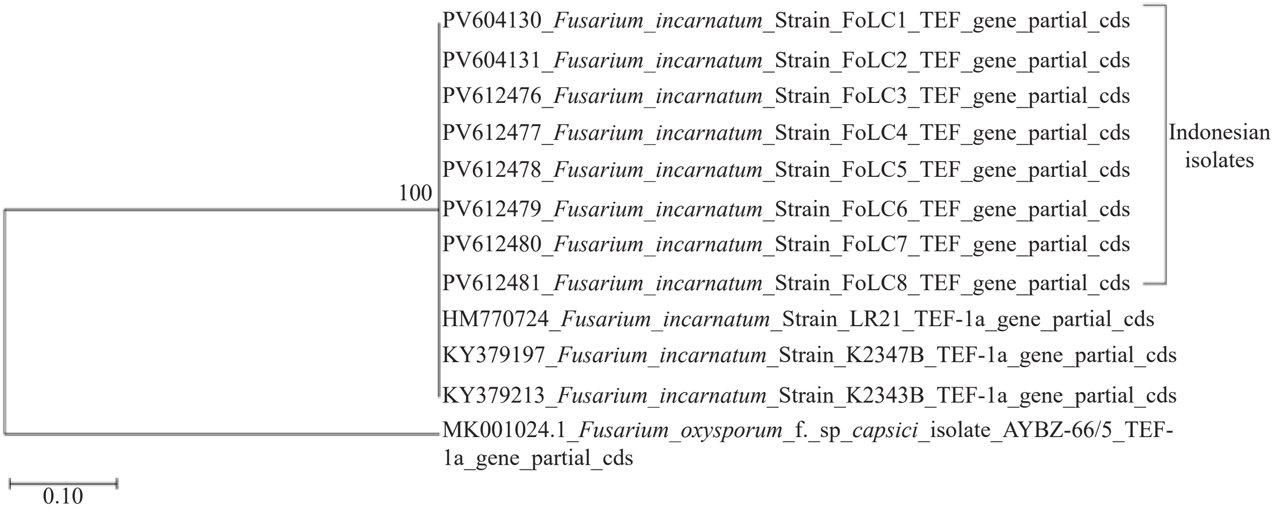

Figure 6. Phylogenetic tree of *Fusarium incarnatum* based on translation elongation factor 1-alpha (TEF-1α) gene sequences, including Indonesian isolates (FoLC1, FoLC2, FoLC3, FoLC4, FoLC5, FoLC6, FoLC7, FoLC10), constructed using the neighbour-joining method in MEGA11 (Tamura et al., 2021). Bootstrap values, expressed as percentages from 1000 replicates, are indicated at branch nodes.

for *Fusarium* identification are well recognized, particularly within species complexes such as FIESC. In this study, the inclusion of TEF-1α significantly improved resolution, supporting previous findings that multi-locus approaches are required for accurate delineation of closely related species (O'Donnell et al., 2009; Xia et al., 2019). This is particularly relevant given the historical taxonomic ambiguity surrounding *F. incarnatum*, which was previously treated as synonymous with *F. semitectum.*

Thus, the combined morphological and molecular evidence provides a high level of confidence in the identification of the isolates as *F. incarnatum*, reinforcing the necessity of integrative taxonomy in studies involving species complexes with high genetic diversity.

Pathogenicity Assay. The pathogenicity assays clearly demonstrated that all isolates were capable of inducing disease symptoms on both wounded and unwounded chili fruits, fulfilling Koch's postulates. The ability to infect unwounded fruits is particularly noteworthy, as it suggests that *F. incarnatum* possesses mechanisms for direct penetration or exploitation of natural openings, thereby increasing its epidemiological significance under field conditions.

All fruit treatments were placed on moist cotton in a humid chamber at 25 ± 2 ºC under a 16-h light/8-h dark photoperiod. All inoculated fruits, both wounded and unwounded, developed water-soaked necrotic lesions, predominantly around the calyx. As the disease progressed, mild internal rot accompanied by white mycelial growth at the inoculation sites was observed within 4–6 days, whereas control fruits remained symptomless (Figure 7).

Symptom development followed a consistent progression from water-soaked lesions to necrotic tissue and internal rot accompanied by mycelial growth. This pattern is consistent with previous reports of *F. incarnatum* infection in chili and other hosts (Zhu et al., 2021; Ramdial et al., 2016; Ishak et al., 2024). The similarity between symptoms observed under experimental and field conditions strengthens the ecological relevance of the findings and confirms the role of this pathogen in fruit rot disease.

The successful re-isolation of the pathogen, coupled with the absence of other microorganisms, provides strong evidence that the observed symptoms were solely attributable to *F. incarnatum*. These findings are consistent with general infection mechanisms of *Fusarium* spp., which involve colonization of host tissues followed by enzymatic degradation and toxin-mediated damage (Soesanto et al., 2021).

A key factor underlying pathogenicity in *F. incarnatum* is its ability to produce a diverse array of secondary metabolites, including mycotoxins such as trichothecenes, zearalenone, enniatins, and beauvericin (Villani et al., 2019). These compounds not only contribute to host tissue necrosis but may also enhance competitive fitness against other microorganisms, thereby facilitating disease establishment and progression. This biochemical capacity suggests that *F. incarnatum* is not merely an opportunistic pathogen but a highly adapted species with multiple virulence strategies.

This study provides the first confirmed report of *F. incarnatum* causing fruit rot in chili pepper in Indonesia, thereby expanding the known distribution of this pathogen. The ability of the pathogen to infect both wounded and unwounded fruits highlights its potential epidemiological significance and risk for postharvest losses. Furthermore, the known capacity of *F. incarnatum* to produce mycotoxins suggests possible implications for food safety, which warrants

Figure 7. Symptoms of pathogenicity test on chili pepper fruits after six days of incubation. A. Control; B. Wounded inoculation; C. Unwounded inoculation.

further investigation. Overall, these findings provide a basis for future studies on disease management and risk assessment in chili production systems.

CONCLUSION

The present study provides the first report of *F. incarnatum* causing fruit rot disease in chili peppers in Indonesia. These findings serve as a reference for future research on chili pepper fruit rot and provide valuable information for developing management strategies for this disease.

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AUTHORS' CONTRIBUTIONS

IS conceived and designed the experiment. NA and KL conducted the survey and field sampling of chili pepper plants in Lubuk Cuik Village, North Sumatra, Indonesia. ES and NAIMZ performed the laboratory work for morphological characterization. LS, WA, and IS analyzed the molecular identification data. All authors provided feedback on the research design, contributed to manuscript preparation, and read and approved the final version of the manuscript.

COMPETING INTEREST

The authors declare that we have no conflicts of interest related to the publication of this manuscript.

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