

Influence of Inoculation Methods on the Pathogenicity of *Fusarium* Isolates Infecting Susceptible Tomato (*Solanum lycopersicum* L.) Varieties in Northern Nigeria

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ABSTRACT

Fusarium wilt is among the most destructive diseases limiting tomato production worldwide. Accurate pathogenicity assessment is essential for understanding host–pathogen interactions and developing effective disease management strategies. This study investigated the influence of different inoculation methods on the pathogenicity of *Fusarium* isolates infecting two susceptible tomato varieties. Wilted tomato plants were collected from major tomato-producing areas in Northern Nigeria, namely Bomo and Kwarin Bala (Kaduna State), Kadawa (Kano State), and Danja (Katsina State). Fungal isolates obtained from diseased vascular tissues were identified using morphological characteristics and molecular sequencing. Three *Fusarium* isolates were selected for pathogenicity evaluation using soil inoculation, leaf smear, and foliar spraying methods on tomato varieties NHT332 and NHT18 under screen house conditions. Disease severity was assessed fourteen days after inoculation using a five-point rating scale. Significant differences ($P < 0.05$) were observed among inoculation methods, *Fusarium* isolates, and tomato varieties. Soil inoculation resulted in the highest disease severity (3.33), followed by leaf smear (3.00), whereas foliar spraying recorded the lowest severity (1.33). The Danja isolate (FUSDJ) exhibited the greatest virulence, while the Kwarin Bala isolate (FUSKB) was the least virulent. Variety NHT332 showed significantly greater susceptibility than NHT18. Significant interactions among isolate source, inoculation method, and tomato variety were also observed. The findings indicate that soil inoculation most effectively reproduces *Fusarium* wilt symptoms and represents the most suitable method for pathogenicity studies involving tomato and *Fusarium* species.

Keywords: *Fusarium* wilt, pathogenicity, disease severity, tomato, inoculation methods, virulence.

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetable crops worldwide owing to its nutritional, economic, and industrial value. It has a high global economic significance as the second most widely cultivated vegetable (Ma *et al.*, 2023) with an annual production of approximately 115.95 million tons (Hassan, 2020). The crop serves as a major source of vitamins, minerals, dietary fibre, and antioxidant compounds such as lycopene and vitamin C which are of health benefits (Haruna *et al.*, 2024). Global demand for tomato continues to increase due to its importance in fresh consumption and food processing industries.

However, tomato productivity is constrained by biotic and abiotic stresses, among which fungal pathogens constitute a major threat to food security (Tian *et al.*, 2020). Among the fungal pathogens affecting tomato, *Fusarium* wilt remains one of the most destructive and economically significant worldwide. While *Fusarium oxysporum* f. sp. *lycopersici* remains the most widely studied causal agent of *Fusarium* wilt, recent evidence suggests that members of the *Fusarium solani* species complex (FSSC) are emerging as equally important pathogens in many cropping systems (Rubin *et al.*, 2024). These pathogens cause root rot and vascular wilt in economically important crops like tomato leading to upto 90% yield losses in susceptible cultivars around the world (Singh and Kamal, 2012).

The disease has also been reported in Nigeria, with incidence of upto 33.51 (Borisade *et al.*, 2017). The persistence of the pathogen in soil and plant debris for many years further complicates disease management and contributes to recurrent outbreaks (Salisu *et al.*, 2024).

Fusarium species are predominantly soil-borne pathogens that infect plants through root tissues and subsequently colonize the vascular system thereby causing interference in water and nutrient transport within the plant leading to plant death (Buster *et al.*, 2023).

Disease severity caused by *Fusarium* species is influenced by factors such as pathogen virulence, host type genotype and environmental conditions. Pathogen virulence differs with different isolates and this influences the extent of symptoms expression and severity (Wang and Gottwald, 2017). In plant pathology, methods of inoculation play an important role in determining the outcome of host-pathogen interactions as various methods differ in their ability to stimulate infection and establish disease (Okafor *et al.*, 2024).

Therefore, the present study was conducted to evaluate the influence of different inoculation methods on the pathogenicity of *Fusarium* isolates recovered from wilted tomato plants in Northern Nigeria using two susceptible tomato varieties. The findings are expected to contribute to improved methodologies for pathogenicity testing and provide valuable information for future *Fusarium* wilt management and tomato breeding programs.

Materials and Methods

Study Area and Sample Collection

Wilted tomato plants showing typical symptoms of *Fusarium* infection were collected from commercial tomato farms located in Bomo and Kwarin Bala (Kaduna State), and Danja (Katsina State), Nigeria. Samples exhibiting vascular discoloration, chlorosis, and wilting symptoms were transported to the laboratory for fungal isolation.

Isolation and Identification of *Fusarium* Species

Infected vascular tissues were surface sterilized with 96 % ethanol for 1 minute, rinsed three times with sterile distilled water (SDW), plated on potato dextrose agar amended with streptomycin, and incubated at $26 \pm 2^\circ\text{C}$. Emerging fungal colonies were purified using hyphal-tip transfer techniques. Preliminary identification was achieved by comparing colony morphology, pigmentation, and microscopic characteristics including macroconidia, microconidia, septation and chlamydospore to the mycological atlas of common plants pathogenic fungi and pictorial atlas of soil and seed fungi (Ulhan *et al.*, 2006; Watanabe, 2010; Luz *et al.*, 2017).

Preparation of Tomato Seedlings

Seeds of two susceptible tomato varieties (NHT332 and NHT18) obtained from the National Horticultural Research Institute (NIHORT), Nigeria, were surface sterilized with sodium hypochloride for 2 minutes and raised in heat sterilized manure-soil trays for 3 weeks. The seedlings were transplanted into heat sterilized soil contained in 1kg capacity pots and allowed to acclimatize for five days before inoculation.

Pathogenicity Test

Three *Fusarium* isolates designated FUSDJ, FUSBOMO, and FUSKB were evaluated using three inoculation methods: Soil, leaf smear and foliar spray.

Experimental Design

The experiment was arranged in a Completely Randomized Design (CRD) with four replications.

Disease Assessment

Disease severity was assessed fourteen days after inoculation using a five-point scale as described by Shalaby *et al.* (2022) with slight modifications as follows:

- 1 = Healthy plant (0%)
- 2 = 1–25% symptom expression
- 3 = 26–50% symptom expression
- 4 = 51–75% symptom expression
- 5 = 76–100% symptom expression or plant death

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using appropriate statistical software. Treatment means were separated using Student–Newman–Keuls (SNK) test at $P \leq 0.05$. All statistical analyses were carried out using SAS version 9.0.

Results

Identification of *Fusarium* Isolates

Morphological and molecular analyses identified two isolates as members of the *Fusarium solani* species complex (FUSDJ and FUSBOMO), while the third isolate (FUSKB) was identified as *Fusarium* species closely related to members of the *Fusarium incarnatum-equiseti* species complex.

Disease Symptoms

Typical symptoms observed included leaf chlorosis, leaf spot formation, stem browning, and stem rot, wilting, and plant death.

Symptom expression varied according to inoculation method, isolate virulence, and tomato variety (Table 1).

Effect of Inoculation Method, location on Disease Severity of Two Tomato Varieties

Disease severity differed significantly among inoculation methods. Soil inoculation produced the highest mean disease severity (3.33), followed by leaf smear inoculation (3.00), whereas foliar spraying produced the lowest disease severity (1.33). The Danja isolate (FUSDJ) produced significantly higher disease severity than FUSBOMO and FUSKB. Variety NHT332 consistently recorded higher disease severity scores than NHT18, indicating greater susceptibility (Table 2).

Interaction Effects

Significant interactions were observed among isolate source, inoculation method, and tomato variety ($P < 0.01$), demonstrating that disease development was influenced by combined effects of pathogen virulence, inoculation technique, & host genotype (Figures 1- 4).

Table 1: Pathogenicity of *Fusarium* isolates from The Study Locations on Tomato variety 1 (N332) and variety 2 (N18)

<i>Fusarium</i> Isolate	Symptoms							
	Treatment variety	Method of inoculation	Leaf spot	Stem rot	Wilt	Brown lower stem	Chlorosis	Death
FUSDJ	V1	SI	+	+	+	+	+	+
		SP	-	-	-	-	-	-
		LS	+	+	+	+	+	+
	V2	SI	+	-	+	-	+	-
		SP	-	-	-	-	+	-
		LS	-	-	+	+	-	-
FUSBOMO	V1	SI	-	-	-	+	-	-
		SP	+	-	-	+	-	-
		LS	+	-	+	+	+	-
	V2	SI	-	-	-	+	-	-
		SP	-	-	-	-	-	-
		LS	+	+	+	+	+	+

FUSKB	V1	SI	+	-	-	+	-	-
		SP	+	-	-	+	-	-
		LS	-	-	-	+	-	-
	V2	SI	+	+	+	+	+	+
		SP	-	-	-	-	-	-
		LS	-	-	-	-	-	-

Key: FUSDJ=Danja *Fusarium*; FUSBOMO=Bomo *Fusarium*; FUSKB=Kwarin Bala *Fusarium*; SI=Soil inoculation; SP=Spraying; LS=Leaf smear; V1=Variety 1 (NHT332); V2=Variety 2 (NHT18)

Table 2: Effect of location and method of inoculation on disease severity of *Fusarium* on two variety of tomato *in vitro*)

Method of Inoculation (M)	Disease severity (Location of commercial tomato farm and tomato varieties)					
	Bomo		Kwarin Bala		Danja	
	NHT 332 (V1)	NHT18 (V2)	NHT 332 (V1)	NHT18 (V2)	NHT 332 (V1)	NHT18 (V2)
Leaf smear (LS)	4.00 ^a	5.00 ^a	2.00 ^b	0.00 ^b	5.00 ^a	2.00 ^b
Soil inoculation (SI)	2.00 ^b	1.00 ^b	4.00 ^a	5.00 ^a	5.00 ^a	3.00 ^a
Spray(SP)	2.00 ^b	0.00 ^c	2.00 ^b	0.00 ^b	2.00 ^b	2.00 ^b
SE±	0.00	0.00	0.00	0.00	0.00	0.00

Note: Means followed by same superscripts along the same treatment column are not different statistically at $P=0.05$ level of probability using SNK; **= F-value significant at ($P\leq 0.01$).

Table 3: Effect of location and method of inoculation on disease severity of *Fusarium* on two variety of tomato *in vitro* with regards to their interactions

Treatment	Disease Severity
Location (L)	
Bomo	2.33 ^b
Danja	3.17 ^a
Kwarin Bala	2.17 ^c
SE±	0.00
Variety (V)	
NHT 332 (V1)	3.11 ^a
NHT18 (V2)	2.00 ^b
SE±	0.00
Method of Inoculation (M)	
Leaf smear (LS)	3.00 ^b
Soil inoculation (SI)	3.33 ^a
Spray(SP)	1.33 ^c
SE±	0.00

Treatment Interaction	Disease Severity
L*V	<.0001 **
L*M	<.0001 **
V*M	<.0001 **
L*V*M	<.0001 **

Note: Means followed by same superscripts along same treatment column are not different statistically at $P=0.05$ level of probability using SNK; **= F-value significant at ($P\leq 0.01$).

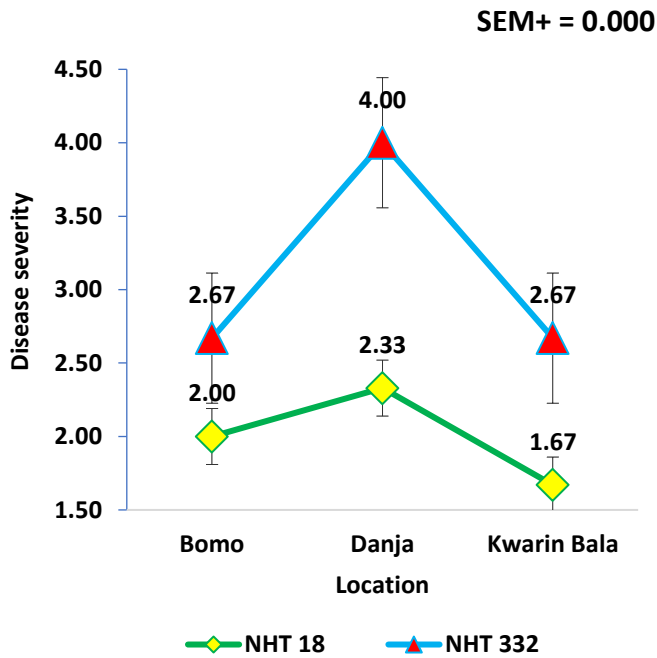


Figure 1: Interaction of location and variety on disease severity of *Fusarium* isolates on two tomato varieties *in vivo*

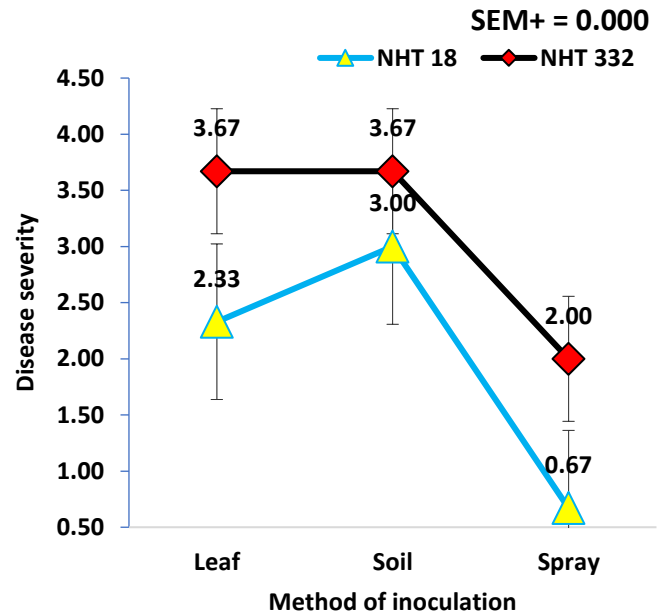


Figure 3: Interaction of method of inoculation and variety on disease severity of *Fusarium* isolates on two tomato varieties *in vivo*

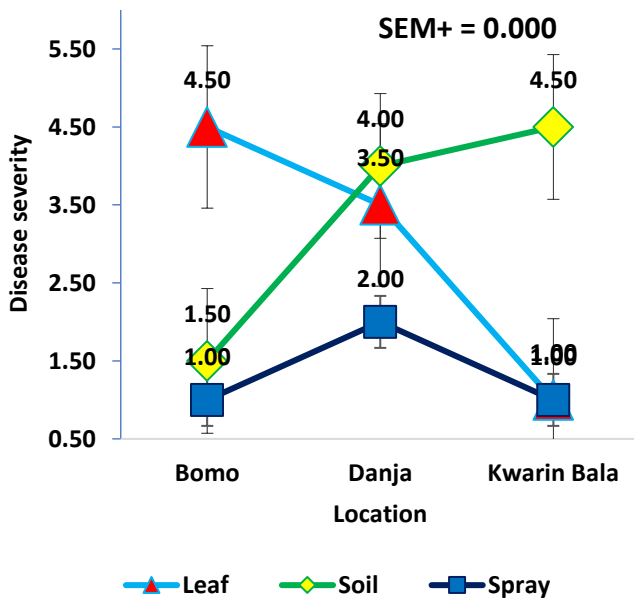


Figure 2: Interaction of location and method of inoculation on disease severity of *Fusarium* isolates on two tomato varieties *in vivo*

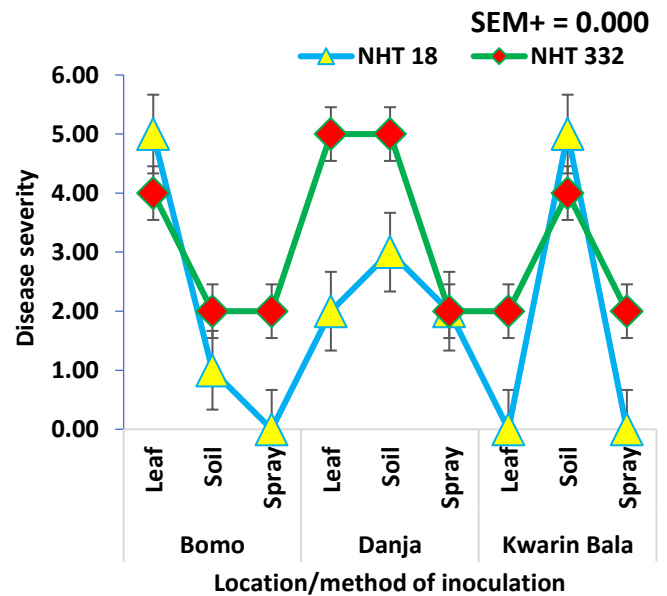


Figure 4: Interaction of location, method of inoculation and variety on disease severity of *Fusarium* isolates on two tomato varieties *in vivo*

Discussion

The present study demonstrated that inoculation method significantly influenced the pathogenicity of *Fusarium* isolates infecting tomato plants, with soil inoculation producing the highest disease severity compared with leaf smear and foliar spraying methods. This finding is consistent with the biology of *Fusarium* species, which are predominantly soil-borne pathogens that naturally infect host plants through root tissues before colonizing the vascular system. Direct introduction of inoculum into the soil increases propagule-root contact, thereby facilitating pathogen penetration and establishment within the xylem tissues. Similar observations have been reported in recent studies where soil infestation and root-zone inoculation resulted in greater disease incidence and symptom expression than foliar inoculation methods because they more closely mimic natural infection pathways (Salisu et al., 2024). The relatively low disease severity observed under foliar spraying may be attributed to the inability of *Fusarium* species to effectively penetrate intact leaf tissues.

Unlike foliar pathogens that possess specialized infection structures for leaf colonization, *Fusarium* wilt pathogens depend primarily on root infection and vascular colonization. Consequently, inoculum deposited on leaf surfaces may fail to establish successful infections unless favorable environmental conditions or wounds are present. Similar reductions in disease expression following foliar inoculation have been reported for *Fusarium* wilt pathogens in tomato, cucumber, and pepper, where root-associated inoculation methods consistently produced greater disease severity than aerial applications (Shalaby et al., 2022; Sun et al., 2025).

The significant differences in disease severity among *Fusarium* isolates indicate considerable variability in pathogenic aggressiveness. The Danja isolate (FUSDJ) consistently produced higher disease severity than the Bomo (FUSBOMO) and Kwarin Bala (FUSKB) isolates, suggesting isolate-dependent variation in virulence. Such variation is common within *Fusarium* populations and is often associated with differences in pathogenicity genes, production of cell wall-degrading enzymes, toxin biosynthesis, host adaptation, and ecological fitness.

Members of the *Fusarium solani* species complex are known to exhibit substantial genetic diversity and varying degrees of aggressiveness depending on geographical origin and host association. Recent studies have similarly reported significant differences in virulence among *Fusarium* isolates infecting soybean, maize, wheat, and tomato, demonstrating that pathogenicity is frequently isolate-specific rather than species-specific (Sanna et al., 2023; Wu et al., 2024; Sun et al., 2025).

The greater pathogenicity observed in FUSDJ may also reflect adaptation to local environmental conditions that favored the evolution of more aggressive strains. Environmental factors such as temperature, moisture regime, soil properties, cropping history, and host availability can exert selective pressure on pathogen populations, leading to the emergence of isolates with enhanced infective capacity. Previous studies have shown that geographical origin can significantly influence pathogen aggressiveness due to local adaptation and differential selection pressure operating within agricultural ecosystems (Laraba et al., 2023; Swalarsk-Parry-et al., 2024).

The higher disease severity recorded on tomato variety NHT332 compared with NHT18 indicates differences in host susceptibility and suggests the presence of genotype-dependent resistance mechanisms. Host genotype plays a fundamental role in determining the outcome of plant-pathogen interactions because disease development is influenced by the ability of plants to recognize invading pathogens and activate effective defense responses. Resistant or tolerant cultivars often exhibit rapid activation of defense-related enzymes, increased accumulation of phenolic compounds, enhanced lignification, and stronger oxidative stress management systems that restrict pathogen spread within vascular tissues. Similar genotype-dependent responses have been reported in tomato cultivars challenged with *Fusarium* pathogens, where significant differences in wilt severity were associated with variation in host defense capacity (Okocha et al., 2023; Wu et al., 2025).

The susceptibility of NHT332 may be associated with weaker structural and biochemical defense mechanisms compared with NHT18.

Studies have shown that resistant tomato genotypes frequently accumulate higher concentrations of phenolic compounds, flavonoids, lignin, peroxidases, polyphenol oxidases, and pathogenesis-related proteins following *Fusarium* infection. These defense responses contribute to reinforcement of cell walls, inhibition of pathogen growth, and limitation of vascular colonization. Consequently, cultivars with reduced capacity to activate these defense pathways often exhibit greater symptom severity and disease progression (Chen *et al.*, 2021; Soltabayeva *et al.*, 2021).

The significant interaction among inoculation method, *Fusarium* isolates, and tomato variety further demonstrates the complexity of host-pathogen-environment relationships. Disease severity was not determined solely by pathogen virulence or host susceptibility but by the combined influence of pathogen genotype, host genotype, and infection pathway. Such interactions are widely recognized in plant pathology and form the basis of the disease triangle concept, which proposes that disease development depends on the interaction between a susceptible host, a virulent pathogen, and a conducive environment. The present findings support this concept by demonstrating that changes in inoculation technique can substantially alter disease outcomes even when the same host and pathogen are involved (Okafor *et al.*, 2024).

The occurrence of diverse disease symptoms including chlorosis, stem browning, stem rot, leaf spots, wilting, and plant death further reflects differences in host responses and pathogen colonization patterns. Recent studies have shown that symptom variation among *Fusarium*-infected plants may result from differences in pathogen species, isolate aggressiveness, host physiology, and environmental conditions affecting disease progression (Sanna *et al.*, 2023).

Conclusion

This study demonstrated that inoculation method significantly influences the pathogenicity of *Fusarium* isolates infecting tomato. Soil inoculation was the most effective method for inducing disease symptoms and produced the highest disease severity. The Danja isolate exhibited the greatest virulence, while tomato

variety NHT332 was more susceptible than NHT18. These findings provide valuable information for standardizing pathogenicity protocols and improving future screening programs for *Fusarium* wilt resistance in tomato.

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