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PLANT PROTECTION
Article

Interactive effects of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* on okra cultivars

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ABSTRACT: In the present study, nine okra cultivars were assessed for their resistance levels against both predisposal and simultaneous infections of the root-knot nematode *Meloidogyne incognita* and the wilt-causing fungus *Fusarium oxysporum* f.sp. *vasinfectum*, with the objective to identify cultivars with collective resistance against these two significant pathogens. Okra cultivars displayed varying resistance to *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* based on different inoculation sequences. When nematodes were introduced 15 days before fungus, no high or moderate resistance was observed. Instead, some cultivars showed moderate susceptibility (Pusa Swami, PB Selection, Green Star), susceptibility (Sabz Pari, Neelum, Tulsi), or high susceptibility (Ikra-1, Ikra-2, Arka Anamika) to nematodes. Conversely, nematodes introduced after fungus resulted in some resistance and moderate resistance among certain cultivars, and simultaneous inoculation led to varied responses. In terms of gall formation, eggmasses, and reproductive factors, different cultivars exhibited varying levels of susceptibility. Ikra-2 had the highest, followed by Arka Anamika and Ikra-1, while Pusa Swami had the least, followed by PB Selection and Green Star. Sequentially introducing nematodes before fungus led to the highest gall formation, whereas the reverse sequence resulted in the least on all cultivars. Simultaneous inoculation yielded lower gall formation than nematodes first followed by fungus. Introducing fungus prior to nematodes reduced gall numbers to the lowest level. In summary, resistance of okra cultivars to nematodes and fungus depended on inoculation order. Okra cultivars exhibited varying susceptibility levels, and the timing of inoculation influenced gall formation and reproductive factors.

Key words: *Fusarium* wilt, *Abelmoschus esculentus*, nematode-fungus interaction, cultivar resistance, nematode reproduction factors.

INTRODUCTION

Okra (*Abelmoschus esculentus*) is a widely cultivated vegetable of significant agricultural importance in tropical and subtropical regions of Africa and Asia. This vegetable holds significant popularity as a human food source due to its nutritional composition, encompassing substantial quantities of carbohydrates, crude fibers, minerals, oil, proteins, and vitamins (Dantas et al. 2021). Okra has a global production of approximately 6 million tons per year, making it a highly valuable commodity.

With respect to global production, India stands out as the leading country in terms of okra cultivation, with an impressive area of 509 hectares dedicated to its growth. The annual production in India reaches a staggering 6,094.9 million tons, showcasing a remarkable productivity rate of 12 million tons per hectare (Moulana and Bahadur 2020). In Pakistan, okra is cultivated over a vast area of 15,081 hectares, producing an annual yield of 114,657 tons (Nawaz et al. 2020). In comparison to high-yielding countries, the agricultural productivity of okra per acre in the country is hindered by a combination of abiotic and biotic limitations, including diseases and insect pests (Hussain et al. 2016, Mukhtar and Hussain 2019, Naseer et al. 2023). Among the biotic stresses, the root-knot nematode *Meloidogyne incognita* and the soil-borne fungus *Fusarium oxysporum* f.sp. *vasinfectum* are two major pathogens that cause significant damage to okra plants.

The root-knot nematodes are the most devastating plant pathogens that infect okra (Hussain et al. 2011, 2012, Mukhtar et al. 2013). Root-knot nematodes hold a prominent position among the world's most significant phytopathogens, ranking atop the list of the ten most dangerous and economically important genera of phytopathogenic nematodes globally (Kayani et al. 2013, Saeed et al. 2023, Yaseen et al. 2023). The infestation of root-knot nematodes leads to a sluggish and stunted growth pattern in plants, hinders root development, chlorosis, root galling, and wilting. Severe infestation often results in root destruction, compromised growth, and reduced crop yield (Daramola et al. 2015, Mukhtar et al. 2017). Root-knot nematodes can cause yield losses of up to 91% in crops and vegetables (Pandey and Kalra 2003). In okra, *Meloidogyne* spp. have been reported to cause yield losses of up to 27% (Sikora and Fernandez 2005). In addition to their direct effects, root-knot nematodes can also interact with pathogenic fungi and bacteria, forming disease complexes that exert additional detrimental effects on plant health (Adam et al. 2014, Toju and Tanaka 2019, Shahid et al. 2022, 2023).

Fusarium wilt is a serious disease of okra that can cause significant crop losses. The economic losses caused by *Fusarium* wilt can be significant. In the United States, *Fusarium* wilt has been estimated to cause losses of up to $10 million per year. In other parts of the world, the losses can be even higher (Viljoen et al. 2020). The fungus invades the root system and colonizes the vascular system, blocking water movement and altering normal cell function and can survive in the soil for many years. The fungus causes wilting, yellowing, and stunting of plants, and, in severe cases, the entire plant may die (Bahadur 2021).

Individually, both *M. incognita* and *F. oxysporum* f.sp. vasinfectum pose significant challenges to okra production. However, recent research has indicated that their interactive effects on okra germplasm might exacerbate the severity of disease symptoms, leading to even greater yield losses. The interaction between *M. incognita* and *F. oxysporum* f.sp. vasinfectum on okra plants is a complex phenomenon influenced by various factors, including host genetics, environmental conditions, and the intricate interplay between the two pathogens. Previous studies have focused primarily on individual pathogen interactions, while limited research has been conducted on the interactive effects of these pathogens on okra germplasm. Therefore, understanding the interactive dynamics between these two pathogens is crucial for developing effective management strategies to minimize crop damage and improve overall okra productivity.

The present study aimed to bridge this knowledge gap by providing a comprehensive study on the interactive effect of *M. incognita* and *F. oxysporum* f.sp. vasinfectum on okra germplasm. The primary objective of this research was to assess the resistance levels of nine okra cultivars against both predisposal and simultaneous infections of the root-knot nematode *M. incognita* and the wilt-causing fungus *F. oxysporum* f.sp. vasinfectum, identifying cultivars with collective resistance against these two significant pathogens.

MATERIALS AND METHODS

The nematode inoculum

The root-knot nematode *M. incognita*, used in the assessment of okra cultivars for their resistance, was extracted from the okra infested roots. The nematode culture was initiated from a single eggmass on a highly root-knot nematode susceptible cultivar of tomato "Money maker" and identified by making perineal pattern (Taylor and Netscher 1974). The nematode was mass produced on the same variety as described by Mukhtar et al. (2017). Upon completion of the life cycle, eggmasses were collected from the infected roots, and eggs were subsequently extracted (Hussey and Barker 1973). The eggs were then processed through extraction trays, and juveniles were collected (Whitehead and Hemming 1965). The freshly hatched second stage juveniles (J2s) were standardized and concentrated.

Okra germplasm

Seeds of seven okra cultivars, viz. Arka Anamika, Sabz Pari, Tulsi, Neelum, Pusa Swami, Green Star, and P. B. Selection, were collected from the Federal Seed Certification and Registration Department, Islamabad, Pakistan, while the seeds of two cultivars, viz. Ikra-1, and Ikra-2, were obtained from the National Agricultural Research Centre, Islamabad, Pakistan.

Mass culturing of the fungus *Fusarium*

The fungus responsible for wilt disease, *F. oxysporum*, was obtained from the roots of infected okra plants for the purpose of assessing the resistance of different okra cultivars. A small section of the infected root tissue measuring 5-6 mm in length was carefully excised and placed on a Petri dish containing potato dextrose agar supplemented with streptomycin sulfate, an antimicrobial agent. After a period of two–four days, the growth of fungal spores (conidia) on the plate was observed and examined using a microscope. To identify the specific species of *F. oxysporum*, the characteristics of the conidia were examined under a microscope at 40× magnification. To do this, the conidial hyphae were obtained from a 2–4-day-old culture by gently scraping them with a blade and transferring them to a new slide. The hyphae were then fixed using a mixture of water and lactophenol cotton blue stain and examined under the microscope to identify the conidia. Two types of conidia, namely microconidia and macroconidia, were observed.

To propagate *Fusarium*, 500 g of chickpea grains were soaked in a 500-mL flask containing 400 mL of water overnight. The grains were subsequently crushed using a pestle and mortar and left to dry in sunlight. Furthermore, these grains were autoclaved twice at 121°C and 15 psi to sterilize them. Six small plugs of mycelium were taken from a 7-day-old culture of *F. oxysporum* and added to the flask containing the autoclaved grains. The flask was then incubated for a period of two weeks at 25°C. The inoculum of the fungus was quantified using haemocytometer and was used for application in the soil.

Evaluation of okra cultivars for nematode and fungus resistance

The comparative resistance of okra cultivars to the root-knot nematode *M. incognita* and the wilt-causing fungus *F. oxysporum* was assessed using plastic pots with a diameter of 20 cm. Each pot contained 2.5 kg of sterilized soil, which was composed of 70% sand, 22% silt, and 8% clay, with pH = 7.5. Three seeds of each okra cultivar were sown in each pot. Ten days after germination, a single healthy seedling was retained in each pot from each test cultivar. The seedlings of each okra cultivar were then inoculated with 2,500 freshly hatched J2s of *M. incognita* and 4 mL of an aqueous suspension of Fusarium containing 4,000 micro- and macroconidia. The treatments were as followed:

T_1: nematode inoculation 15 days prior to *Fusarium* inoculation;

T_2: *Fusarium* inoculation 15 days prior to nematode inoculation;

T_3: simultaneous inoculation of nematodes and *Fusarium*.

Un-inoculated plants of each cultivar served as controls. Each cultivar was replicated 10 times, and the experiment was repeated once. The pots of all the cultivars were arranged in a completely randomized design under field conditions in an iron cage for seven weeks. The pots were watered as needed.

Data collection

The plants of each okra cultivar were grown for seven weeks and then carefully removed from the pots, and the roots were separated from the shoots. The roots were washed to remove any soil and dried gently. The galls and eggmasses on the roots of each cultivar were counted under a stereomicroscope with a 4× magnification. The eggs were extracted from the roots of individual plants using the method of Hussey and Barker (1973), and the juveniles were extracted from the soil of each pot using the method described by Whitehead and Hemming (1965). The total number of eggs in the roots and the nematodes in the soil constituted the final nematode population. The reproductive factor was determined by dividing this final population by the initial inoculation count of 2,500. The level of resistance or susceptibility was assessed using the rating scale based on number of galls proposed by Taylor and Sasser (1978). The plants were scored for *Fusarium* wilt using a modified version of the 1–6 severity scale by Lebeda and Buczkowski (1986).

Statistical analysis

The experiment was replicated once. All the experimental data were subjected to analysis of variance using the Statistical Package for the Social Sciences (SPSS) software. The homogeneity of variance among samples was assessed using Levene's test at the significance level of $p < 0.05$. The equality of means was evaluated using the Welch's test at the significance level of $p < 0.05$. The interpretation of Levene's test and the Welch's test was conducted using SPSS software. For comparing statistical means, Duncan's multiple range test was employed.

RESULTS

Response of okra cultivars to *Meloidogyne incognita* when inoculated sequentially and concomitantly with the fungus

When inoculated with nematodes 15 days before the inoculation of the fungus (N15 + F), none of the cultivars exhibited high resistance, resistance, or moderate resistance. Instead, three cultivars each showed moderate susceptibility (Pusa Swami, PB Selection, Green Star), susceptibility (Sabz Pari, Neelum, Tulsi), and high susceptibility (Ikra-1, Ikra-2, Arka Anamika) to *M. incognita*. On the other hand, when the nematodes were inoculated 15 days after the fungus (F15 + N), four cultivars (PB Selection, Green Star, Pusa Swami, and Sabz Pari) showed resistance to the nematode, and three cultivars (Neelum, Tulsi, and Arka Anamika) were moderately resistant, while two cultivars (Ikra-1 and Ikra-2) were moderately susceptible. However, when both the nematodes and fungus were inoculated simultaneously, no cultivar was highly resistant or resistant to the nematode. Three cultivars each showed moderate resistance (Pusa Swami, PB Selection, and Green Star), moderate susceptibility (Sabz Pari, Neelum, and Tulsi), and susceptibility (Ikra-1, Ikra-2, and Arka Anamika), as shown in Table 1.

Table 1. Response of okra cultivars to *Meloidogyne incognita* when inoculated sequentially and concomitantly with *Fusarium oxysporum* f.sp. vasinfectum.

Rating scale	No. of galls	Cultivars			Response to *M. incognita*
		N15 + F	Simultaneous treatment	F15 + N	
0	0	-	-	-	Highly resistant
1	1– 2	-	-	PB Selection, Green Star, Pusa Swami, Sabz Pari	Resistant
2	3–10	-	Pusa Swami, PB Selection, Green Star	Neelum, Tulsi, Arka Anamika	Moderately resistant
3	11–30	Pusa Swami, PB Selection, Green Star	Sabz Pari, Neelum, Tulsi	Ikra-1, Ikra-2	Moderately susceptible
4	31–100	Sabz Pari, Neelum, Tulsi	Ikra-1, Ikra-2, Arka Anamika	-	Susceptible
5	< 100	Ikra-1, Ikra-2, Arka Anamika			Highly susceptible

Response of okra cultivars to *Fusarium* wilt when inoculated sequentially and concomitantly with the nematode

Okra cultivars exhibited varying degrees of resistance or susceptibility to *Fusarium* wilt in response to different nematode and fungus inoculations. In the case of sequential inoculations, three cultivars were moderately susceptible (Pusa Swami, Green Star, and PB Selection), three were susceptible (Sabz Pari, Tulsi, and Neelum), and three were highly susceptible (Ikra-1, Ikra-2, and Arka Anamika) when they were inoculated with the fungus 15 days after the nematode. Conversely, when the nematodes were introduced for inoculation 15 days subsequent to the fungus inoculation, four cultivars (Sabz

Pari, Green Star, Pusa Swami, and PB Selection) demonstrated resistance to the fungus, while five cultivars exhibited moderate resistance (Ikra-1, Ikra-2, Tulsi, Arka Anamika, and Neelum). Notably, none of the cultivars showed any level of susceptibility to *Fusarium* wilt in this case. However, in cases in which okra cultivars were simultaneously inoculated, their responses ranged from moderately resistant to susceptible, as detailed in Table 2. The percentages of fungal infection and disease severities for all nine okra cultivars under different inoculation methods are given in Table 2.

Table 2. Response of okra cultivars to *Fusarium* wilt when inoculated sequentially and concomitantly with *Meloidogyne incognita**.

Cultivar	Sequential inoculation						Concomitant inoculation		
	N15 + F			F15 + N					
	Infection %	Disease severity	Response	Infection %	Disease severity	Response	Infection %	Disease severity	Response
PB Selection	15.6 ± 3.36 a	3.45–4.44	MS	4.80 ± 0.83 a	1.45–2.44	R	6.20 ± 0.83a	2.45–3.44	MR
Green Star	16.0 ± 2.91 a	3.45–4.44	MS	4.40 ± 1.14 a	1.45–2.44	R	6.0 ± 1.58a	2.45–3.44	MR
Neelum	41.0 ± 5.43 c	4.45–5.44	S	7.40 ± 1.14 b	2.45–3.44	MR	17.60 ± 1.51c	3.45–4.44	MS
Tulsi	30.6 ± 5.77 b	3.45–4.44	S	7.40 ± 1.14 b	2.45–3.44	MR	14.40 ± 2.07b	3.45–4.44	MS
Arka Anamika	72.0 ± 12.04 d	> 5.45	HS	9.0 ± 1.58 b	2.45–3.44	MR	27.0 ± 3.16d	3.45–4.44	S
Pusa Swami	13.2 ± 1.92 a	3.45–4.44	MS	4.80 ± 1.30 a	1.45–2.44	R	6.0 ± 1.58a	3.45–4.44	MR
Sabz Pari	34.8 ± 8.07 bc	3.45–4.44	S	5.20 ± 1.09 a	1.45–2.44	R	15.80 ± 0.83bc	3.45–4.44	MS
Irka-1	68.8 ± 5.26 d	> 5.45	HS	16.60 ± 1.14 c	3.45–4.44	MR	37.40 ± 4.15d	3.45–4.44	S
Irka-2	76.4 ± 9.34 d	> 5.45	HS	16.60 ± 2.30 c	3.45–4.44	MR	39.0 ± 2.91d	3.45–4.44	S
Analysis of variance	F = 70.76 Df = 8,36 P = 0.000			F = 64.03 Df = 8,36 P = 0.000			F = 64.03 Df = 8,36 P = 0.000		
Levene's statistic	F = 3.35 Df = 8,36 P = 0.006			F = 0.881 Df = 8,36 P = 0.542			F = 0.881 Df = 8,36 P = 0.542		
Welch's test	F = 83.44 Df = 8,14.68 P = 0.000			F = 50.82 Df = 8,14.95 P = 0.000			F = 50.82 Df = 8,14.95 P = 0.000		

*Values (± standard deviation) are means of ten replicates; at $P < 0.05$, Levene's test is significant (variances of statistical data are not equal); at $P < 0.05$, Welch's test (robust test of equality of means) is significant; it rejects the null hypothesis of equality of means; same letter in every column of means indicate that there is no significant difference among means according to Duncan's multiple range test at $P = 0.05$; F: F value; Df: degree of freedom; MR: moderately resistant; MS: moderately susceptible; S: susceptible.

Effect of okra cultivars and inoculation methods on nematode infestations

The number of galls, eggmasses, and reproductive factors varied significantly among nine okra cultivars and three methods of inoculation of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum*. The cultivar Ikra-2 produced the maximum galls, eggmasses and reproductive factors followed by Arka Anamika and Ikra-1. On the other hand, the cultivar Pusa Swami produced the minimum galls, eggmasses and reproductive factors followed by PB Selection and Green Star. Among the three methods of inoculation, the sequential inoculation of *M. incognita* 15 days before *F. oxysporum* f.sp. vasinfectum resulted in the maximum number of galls, eggmasses and reproductive factors, while the sequential inoculation of *F. oxysporum* f.sp. vasinfectum 15 days before *M. incognita* resulted in the minimum number of galls, eggmasses and reproductive factors on all the cultivars. The concomitant inoculation of both the pathogens resulted in significantly lower galls, eggmasses and reproductive factors than the sequential inoculation of *M. incognita* 15 days before *F. oxysporum* f.sp. *vasinfectum*. The lowest numbers of galls, eggmasses and reproductive factors were produced on okra cultivars, in which *F. oxysporum* f.sp. *vasinfectum* was inoculated 15 days before *M. incognita*, compared to the other two inoculation methods (Tables 3, 4 and 5).

Table 3. Effect of sequential and concomitant inoculations of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* on number of galls on okra cultivars*.

Cultivar	Number of galls		
	Sequential inoculation		Concomitant inoculation
	N15 + F	F15 + N	
PB Selection	13 ± 0.54 b	1.60 ± 0.54 a	5.6 ± 0.50 ab
Green Star	15.8 ± 0.37 c	2.0 ± 0.0 a	4.6 ± 0.50 a
Neelum	36 ± 0.70 e	5.40 ± 2.07 b	24.6 ± 0.92 d
Tulsi	31.6 ± 0.50 d	8.40 ± 1.14 c	23.2 ± 0.58 d
Arka Anamika	74.8 ± 0.37 fg	7.60 ± 0.54 bc	47.6 ± 0.50 g
Pusa Swami	10.8 ± 0.37 a	1.40 ± 0.54 a	6.8 ± 0.58 b
Sabz pari	35 ± 0.70 e	1.40 ± 0.54 a	17.2 ± 0.37 c
Irka-1	73.4 ± 0.50 f	23.40 ± 2.30 e	40.6 ± 0.50 f
Irka-2	75.6 ± 0.50 g	19.80 ± 5.06 d	38.6 ± 0.50 e
Analysis of variance	F = 2,640.96 Df = 8,36 P = 0.000	F = 81.46 Df = 8,36 P = 0.000	F= 792.0 Df = 8,36 P = 0.000
Levene's statistic	F = 0.58 Df = 8,36 P = 0.783	F= 10.308 Df = 8,36 P = 0.000	F= 1.124 Df = 8,36 P = 0.371
Welch's test	F = 2940.99 Df = 8,14.95 P = 0.000	F = 0 Df = 0 P = 0	F = 756.23 Df = 8,14.96 P = 0.000

*Values (± standard deviation) are means of ten replicates; at $P < 0.05$, Levene's test is significant (variances of statistical data are not equal); at $P < 0.05$, Welch's test (robust test of equality of means) is significant; it rejects the null hypothesis of equality of means; same letter in every column of means indicate that there is no significant difference among means according to Duncan's multiple range test at P = 0.0; F: F value; Df: degree of freedom.

Table 4. Effect of sequential and concomitant inoculations of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* on number of eggmasses on okra cultivars*.

Cultivar	Number of eggmasses		
	Sequential inoculation		Concomitant inoculation
	N15 + F	F15 + N	
PB Selection	10.8 ± 1.64 a	1.20 ± 0.44 a	4.6 ± 2.07 a
Green Star	11.6 ± 1.51 a	1.0 ± 0.0 a	4.2 ± 0.83 a
Neelum	33.4 ± 2.19 c	3.60 ± 2.07 bc	18.2 ± 1.92 c
Tulsi	31.0 ± 1.92 b	3.20 ± 1.30 ab	20 ± 1.58 c
Arka Anamika	71 ± 1.58 d	5.40 ± 1.14 c	39 ± 2.07 e
Pusa Swami	9.8 ±1.3 a	1.20 ± 0.44 a	5.6 ± 1.14 a
Sabz pari	30.6 ± 2.4 b	1.20 ± 0.44 a	14.8 ± 1.92 b
Irka-1	69.4 ± 3.36 d	17.80 ± 3.42 e	37.8 ± 1.92 e
Irka-2	71.4 ± 2.3 d	12.20 ± 1.78 d	32.6 ± 1.14 d
Analysis of variance	F = 782.74 Df = 8,36 P = 0.000	F = 69.07 Df = 8,36 P = 0.000	F = 349.29 Df = 8,36 P = 0.000
Levene's statistic	F = 0.780 Df = 8,36 P = 0.623	F = 12.836 Df = 8,36 P = 0.000	F = 0.987 Df = 8,36 P = 0.462
Welch's test	F = 790.25 Df = 8,14.94 P = 0.000	F = 0 Df = 0 P = 0	F = 375.77 Df = 8,14.87 P = 0.000

*Values (± standard deviation) are means of ten replicates; at $P < 0.05$, Levene's test is significant (variances of statistical data are not equal); at $P < 0.05$, Welch's test (robust test of equality of means) is significant; it rejects the null hypothesis of equality of means; same letter in every column of means indicate that there is no significant difference among means according to Duncan's multiple range test at P = 0.05; F: F values; Df: degree of freedom.

Table 5. Effect of sequential and concomitant inoculations of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* on reproductive factor of the nematode on okra cultivars*.

Cultivar	Number of reproductive factor		
	Sequential inoculation		Concomitant inoculation
	N15 + F	F15 + N	
PB Selection	1.35 ± 0.38a	0.20 ± 0.07a	0.74 ± 0.26ab
Green Star	1.36 ± 0.36a	0.16 ± 0.03a	0.61 ± 0.42a
Neelum	2.87 ± 0.35c	0.80 ± 0.07a	2.26 ± 0.22d
Tulsi	2.15 ± 0.17b	1.20 ± 0.29a	1.89 ± 0.4cd
Arka Anamika	4.47 ± 0.36d	1.07 ± 0.09a	3.85 ± 0.33e
Pusa Swami	1.28 ± 0.44a	0.20 ± 0.07a	1.13 ± 0.27b
Sabz pari	2.68 ± 0.23c	0.13 ± 0.07a	1.75 ± 0.30c
Irka-1	4.49 ± 0.41d	36.92 ± 7.18a	3.53 ± 0.30e
Irka-2	4.23 ± 0.37d	2.79 ± 0.40a	3.45 ± 0.38e
Analysis of variance	F = 72.11 Df = 8,36 P = 0.000	F = 1.09 Df = 8,36 P = 0.386	F = 68.98 Df = 8,36 P = 0.000
Levene's statistic	F = 0.708 Df = 8,36 P = 0.682	F = 7.084 Df = 8,36 P = 0.000	F = 0.784 Df = 8,36 P = 0.620
Welch's test	F = 49.57 Df = 8,14.86 P = 0.000	F = 86.94 Df = 8,13.69 P = 0.000	F = 54.68 Df = 8,14.95 P = 0.000

*Values (± standard deviation) are means of ten replicates; at $P < 0.05$, Levene's test is significant (variances of statistical data are not equal); at $P < 0.05$, Welch's test (robust test of equality of means) is significant; it rejects the null hypothesis of equality of means; same letter in every column of means indicate that there is no significant difference among means according to Duncan's multiple range test at $P = 0.05$; F: F values; Df: degree of freedom.

DISCUSSION

Root-knot nematodes (*Meloidogyne* spp.) and the wilt-causing fungus *F. oxysporum* are two of the most important pests of plants worldwide. They can cause significant yield losses in a wide range of crops, including vegetables, fruits, and field crops. Various studies have discussed the interaction between the root-knot nematode *M. incognita* and *F. oxysporum* (Meena et al. 2016, Kumar et al. 2017, Agbaglo et al. 2020, Parveen et al. 2020, Regmi et al. 2022, Vigbedor et al. 2022, Wagner et al. 2022). In summary, these studies showed that *M. incognita* and *F. oxysporum* had a synergistic interaction that enhanced their pathogenicity and reduced the growth and yield of various crops. The mechanisms involved in this interaction may include physical damage, nutrient depletion, hormonal imbalance, and altered defense responses caused by the nematode infection, which facilitate the invasion and colonization of the fungus in the host tissues. The management of this disease complex requires an integrated approach that combines cultural, biological, chemical, and genetic methods to reduce the inoculum and the damage of both pathogens. One of the most effective ways to manage these pests is to use resistant cultivars (Afzal et al. 2023, Pathan et al. 2023).

Previously, researchers have conducted screenings of a wide variety of germplasm, commercial cultivars, and accessions of different crops to identify resistant sources against root-knot nematodes and the wilt-causing fungus (Boyhan et al. 2003, Ulloa et al. 2016, Khan and Sharma 2020). These screenings aimed to identify plant varieties that exhibit resistance to these pathogens, which can help in developing strategies for disease management and crop improvement (Mustafa et al. 2023, Zainab et al. 2023). Preliminary findings have shown that many wilt-resistant rootstocks are highly susceptible to root-knot nematodes and other plant parasitic nematodes. This suggests that resistance to one pathogen does not necessarily confer resistance to another. In the case of cotton, root-knot nematodes, specifically *M. incognita*, can lower the threshold of

F. oxysporum f.sp. *vasinfectum* required to elicit wilt symptoms (Khan and Sharma 2020). This indicates that the presence of nematodes can exacerbate the severity of wilt disease caused by the fungus.

The results of the present study showed that the interaction between root-knot nematode and *Fusarium* wilt in okra cultivars depends on the timing and sequence of inoculation. The results suggested that root-knot nematodes infection predisposed the plants to *Fusarium* wilt infection, but *Fusarium* wilt infection did not enhance the susceptibility to root-knot nematode infection. The results also indicated that there was variation in the resistance or susceptibility of different okra cultivars to the disease complex. The results are consistent with previous studies that reported a synergistic effect of root-knot nematodes and *Fusarium* wilt on various crops, such as tomato, cotton, watermelon, and chickpea (McLeod et al. 1983, Boyhan et al. 2003, Singh et al. 2012, Khan and Sharma 2020). These studies found that root-knot nematodes infection increases the colonization and sporulation of Fusarium in the roots and stems of the infected plants, and also disrupts the vascular system and the defense responses of the plants. However, *Fusarium* wilt infection does not affect the population or reproduction of root-knot nematodes in the roots.

In the present study, okra cultivars exhibited different levels of resistance or susceptibility to *Fusarium* wilt depending on whether they were inoculated with the nematode before, after, or simultaneously with the fungus. This indicated that there was an interaction between *M. incognita* and *F. oxysporum* f.sp. vasinfectum on okra cultivars that influenced their response to *Fusarium* wilt. The sequential inoculation of *M. incognita* 15 days before F. oxysporum f.sp. vasinfectum resulted in the highest susceptibility of okra cultivars to *Fusarium* wilt, as none of the cultivars showed resistance and most of them showed moderate or high susceptibility. This suggested that *M. incognita* infection increased the vulnerability of okra cultivars to *F. oxysporum* f.sp. *vasinfectum* infection, possibly by creating wounds for fungal entry, altering plant physiology and defense mechanisms, and enhancing fungal growth and sporulation by nematode secretions.

On the contrary, the sequential inoculation of *F. oxysporum* f.sp. *vasinfectum* 15 days before *M. incognita* resulted in the lowest susceptibility of okra cultivars to *M. incognita*, as all of the cultivars showed resistance or moderate resistance and none of them showed susceptibility. This implied that *F. oxysporum* f.sp. *vasinfectum* infection reduced the vulnerability of okra cultivars to *M. incognita* infection, possibly by inducing plant resistance, competing with nematodes for nutrients and space, and producing toxic metabolites that inhibited nematode development.

Likewise, the concomitant inoculation of both pathogens resulted in intermediate susceptibility of okra cultivars to *Fusarium* wilt, as some of the cultivars showed moderate resistance, some showed moderate susceptibility, and some showed susceptibility. This indicated that neither pathogen had a dominant effect on the other when inoculated simultaneously, possibly due to a balance between plant defense and pathogen virulence, a lack of physical or chemical interactions between nematodes and fungi, and an adaptation of okra cultivars to both pathogens when exposed at the same time.

The results of the present study also demonstrated that some okra cultivars have resistance or tolerance to *Fusarium* wilt or root-knot nematodes, or both, under different inoculation methods. This suggests that there are genetic factors that confer resistance or tolerance to the pathogens, and that these factors may have different modes of action or expression depending on the inoculation method. For example, some cultivars may have resistance genes that prevent or limit the infection of *Fusarium* in the roots, while others may have tolerance genes that reduce the symptoms or damage caused by *Fusarium* in the shoots. Similarly, some cultivars may have resistance genes that inhibit or reduce the penetration or feeding of root-knot nematodes in the roots, while others may have tolerance genes that mitigate the effects or consequences of root-knot nematodes infection in the shoots.

The results of this study also indicated that the interaction between *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* on okra cultivars is influenced by the cultivar resistance and the inoculation method. The cultivar Ikra-2 was the most susceptible to both pathogens, as it produced the highest number of galls, eggmasses and reproductive factors. This suggests that this cultivar has a low level of resistance or tolerance to *M. incognita* and *F. oxysporum* f.sp. *vasinfectum*. The cultivar Pusa Swami was the most resistant to both pathogens, as it produced the lowest number of galls, eggmasses and reproductive factors. This indicates that this cultivar has a high level of resistance or tolerance to *M. incognita* and *F. oxysporum* f.sp. *vasinfectum*. The other cultivars showed varying degrees of susceptibility or resistance to both pathogens, depending on their genetic makeup and physiological characteristics. The sequential inoculation of *M. incognita* 15 days before *F. oxysporum* f.sp. vasinfectum resulted in the highest number of galls, eggmasses and reproductive factors on all

the cultivars, as compared to the other two inoculation methods. This implied that *M. incognita* infection predisposed the okra plants to *F. oxysporum* f.sp. vasinfectum infection, by creating wounds and stress on the roots that facilitated the entry and colonization of the fungus. This is consistent with previous studies that reported that nematode infection increases the susceptibility of plants to fungal pathogens. On the other hand, the sequential inoculation of *F. oxysporum* f.sp. *vasinfectum* 15 days before *M. incognita* resulted in the lowest number of galls, eggmasses and reproductive factors on all the cultivars, as compared to the other two inoculation methods. This suggested that *F. oxysporum* f.sp. vasinfectum infection reduced the susceptibility of okra plants to *M. incognita* infection, by inducing systemic resistance or inhibiting nematode penetration and reproduction. This is in agreement with previous studies that reported that fungal infection decreases the susceptibility of plants to nematode pathogens.

The concomitant inoculation of both pathogens resulted in intermediate number of galls, eggmasses and reproductive factors on all the cultivars, as compared to the other two inoculation methods. This indicated that there is a complex interaction between *M. incognita* and *F. oxysporum* f.sp. vasinfectum when they infected okra plants simultaneously, involving synergistic or antagonistic effects depending on the cultivar and the environmental conditions. This is in line with previous studies that reported that simultaneous infection of plants by nematode and fungal pathogens can result in additive, synergistic or antagonistic effects.

CONCLUSION

This study demonstrated that the timing and sequence of inoculations by *M. incognita* and *F. oxysporum* f.sp. vasinfectum affect the resistance or susceptibility of okra cultivars to *Fusarium* wilt and root-knot nematodes. The findings offer valuable information for devising integrated management strategies to control root-knot *Fusarium* complex in okra.

CONFLITCT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Yaseen, I. and Mukhtar, T.; **Methodology:** Yaseen, I., Mukhtar, T. and Arshad, B.; **Investigation:** Yaseen, I., Mukhtar, T. and Arshad, B.; **Writing – Original Draft:** Yaseen, I. and Mukhtar, T.; **Writing – Review and Editing:** Mukhtar, T. and Kim, H. T.; **Resources:** Yaseen, I. and Kim, H. T.; **Supervision:** Mukhtar ,T.; **Final Approval:** Yaseen, I. and Mukhtar, T.

DATA AVAILABILITY STATEMENT

Data will be made available on request.

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REFERENCES

Adam, M., Heuer, H. and Hallmann, J. (2014). Bacterial antagonists of fungal pathogens also control root-knot nematodes by induced systemic resistance of tomato plants. PloS One, 9, e90402. https://doi.org/10.1371/journal.pone.0090402

Afzal, A., Ahmad, A., Hassaan, M. A., Mushtaq, S. and Abbas, A. (2023). Enhancing agricultural sustainability in Pakistan: addressing challenges and seizing opportunities through effective plant disease management. Plant Protection, 7, 341-350. https://doi.org/10.33804/pp.007.02.4595

Agbaglo, S. Y., Nyaku, S. T., Vigbedor, H. D. and Cornelius, E. W. (2020). Pathogenicity of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *vasinfectum* on growth and yield of two okra varieties cultivatedin Ghana. International Journal of Agronomy, 8824165. https://doi.org/10.1155/2020/8824165

Bahadur, A. (2021). Current Status of *Fusarium* and their management strategies. In: S. M. Mirmajlessi (Ed.). *Fusarium*: An Overview of the Genus. IntechOpen.

Boyhan, G. E., Langston, D. B., Granberry, D. M., Lewis, P. M. and Linton, D. O. (2003). Resistance to *Fusarium* wilt and root-knot nematode in watermelon germplasm. Cucurbit Genetics Cooperative Report, 26, 18-25.

Dantas, T. L., Alonso Buriti, F. C. and Florentino, E. R. (2021). Okra (*Abelmoschus esculentus* L.) as a potential functional food source of mucilage and bioactive compounds with technological applications and health benefits. Plants, 10, 1683. https://doi.org/10.3390/plants10081683

Daramola, F. Y., Popoola J. O., Eni, A. O. and Sulaiman, O. (2015). Characterization of root-knot nematodes (*Meloidogyne* spp.) associated with *Abelmoschus esculentus*, *Celosia argentea* and *Corchorus olitorius*. Asian Journal of Biological Sciences, 8, 42-50.

Hussain, M. A., Mukhtar, T. and Kayani, M. Z. (2011). Assessment of the damage caused by *Meloidogyne incognita* on okra. Journal of Animal and Plant Sciences, 21, 857-861.

Hussain, M. A., Mukhtar, T. and Kayani, M. Z. (2016). Reproduction of *Meloidogyne incognita* on resistant and susceptible okra cultivars. Pakistan Journal of Agricultural Sciences, 53, 371-375. https://doi.org/10.21162/PAKJAS/16.4175

Hussain, M. A., Mukhtar, T., Kayani, M. Z., Aslam, M. N. and Haque, M. I. (2012). A survey of okra (*Abelmoschus esculentus*) in the Punjab province of Pakistan for the determination of prevalence, incidence and severity of root-knot disease caused by *Meloidogyne* spp. Pakistan Journal of Botany, 44, 2071-2075.

Hussey, R. S. and Barker, K. R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter, 57, 1025-1028.

Kayani, M. Z., Mukhtar, T., Hussain, M. A. and Haque, M. I. (2013). Infestation assessment of root-knot nematodes (Meloidogyne spp.) associated with cucumber in the Pothowar region of Pakistan. Crop Protection, 47, 49-54. https://doi.org/10.1016/j.cropro.2013.01.005

Khan, M. R. and Sharma, R. K. (2020). *Fusarium*-nematode wilt disease complexes, etiology and mechanism of development. Indian Phytopathology, 73, 615-628. https://doi.org/10.1007/s42360-020-00240-z

Kumar, N., Bhatt, J. and Sharma, R. L. (2017). Interaction between Meloidogyne incognita with *Fusarium oxysporum* f. sp. *lycpersici* on Tomato. International Journal of Current Microbiology and Applied Sciences, 6, 1770-1776.

Lebeda, A. and Buczkowski, J. (1986). *Fusarium oxysporum*, *Fusarium solani*: Tube test. In A. Lebeda (Ed.). Methods of testing vegetable crops for resistance to plant pathogens. Czech Republic: VHJ Sempra, VŠÚZ Olomouc. p. 247-249.

McLeod, J. M., Witcher, W., Epps, W. M. and Robbins, M. L. (1983). Resistance of okra plant introductions to root knot nematode and Fusarium wilt. HortScience, 18, 249-250. https://doi.org/10.21273/HORTSCI.18.2.249

Meena, K. S., Ramyabharathi, S. A., Raguchander, T. and Jonathan, E. I. (2016). Interaction of *Meloidogyne incognita* and *Fusarium oxysporum* in carnation and physiological changes induced in plants due to the interaction. SAARC Journal of Agriculture, 14, 59-69. https://doi.org/10.3329/sja.v14i1.29576

Moulana, S. and Bahadur, V. P. V. (2020). Effect of different levels of cycocel (CCC) on two different cultivars of okra under Prayagraj Agro climatic conditions (*Abelmoschus esculantus* L.). International Journal of Cardiovascular Sciences, 8, 133-136. https://doi.org/10.22271/chemi.2020.v8.i4b.9680

Mukhtar, T. and Hussain, M. A. (2019). Pathogenic potential of Javanese root-knot nematode on susceptible and resistant okra cultivars. Pakistan Journal of Zoology, 51, 1891-1897. https://doi.org/10.17582/journal.pjz/2019.51.5.1891.1897

Mukhtar, T., Hussain, M. A. and Kayani, M. Z. (2017). Yield responses of 12 okra cultivars to southern root-knot nematode (*Meloidogyne incognita*). Bragantia, 76, 108-112. https://doi.org/10.1590/1678-4499.005

Mukhtar, T., Kayani, M. Z. and Hussain, M. A. (2013). Response of selected cucumber cultivars to *Meloidogyne incognita*. Crop Protection, 44, 13-17. https://doi.org/10.1016/j.cropro.2012.10.015

Mustafa, A., Naseer, S., Ahmad, S., Aatif, H. M., Khan, A. A., Hassan, Z., Hanif, C. M. S., Saeed, S., Shah, J. A. and Ali, Y. (2023). Assessing the resistance of different chili genotypes to chili leaf curl virus (CHILCV) and evaluating insecticides for controlling its vector, *Bemisia tabaci*. Plant Protection, 7, 163-171. https://doi.org/10.33804/pp.007.02.4661

Naseer, S., Mustafa, A., Akhtar, S., Ahmad, S., Shahzad, U., Yousaf, M. J., Zardari, M. A., Arif, S. M., Saeed, S., Khakwani, K., Ali, Y. and Jahan, M. S. (2023). Evaluation of okra (*Abelmoschus esculentus* L.) pest control strategies and cost-benefit analysis. Plant Protection, 7, 385-393. https://doi.org/10.33804/pp.007.03.4749

Nawaz, A., Ali, H., Sufyan, M., Gogi, M. D., Arif, M. J., Ali, A. and Ghramh, H. A. (2020). *In-vitro* assessment of food consumption, utilization indices and losses promises of leafworm, *Spodoptera litura* (Fab.), on okra crop. Journal of Asia-Pacific Entomology, 23, 60-66. https://doi.org/10.1016/j.aspen.2019.10.015

Pandey, R. and Kalra, A. (2003). Root-knot disease of ashwagandha *Withania somnifera* and its ecofriendly cost-effective management. Journal of Mycology and Plant Pathology, 33, 240-245.

Parveen, G., Urooj, F., Moin, S., Farhat, H., Fahim, M. F. and Ehteshamul-Haque, S. (2020). Estimation of losses caused by root rotting fungi and root knot nematodes infecting some important crops in lower Sindh and hub, Balochistan of Pakistan. Pakistan Journal of Botany, 52, 673-678. https://doi.org/10.30848/PJB2020-2(15)

Pathan, S. N., Solangi, A. W., Marri, J. M., Lanjar, A. G. and Bukero, A. (2023). Eco-friendly approaches against chili thrips *Scirtothrips dorsalis* Hood (Thysanoptera: Thripidae). Plant Protection, 7, 545-553. https://doi.org/10.33804/pp.007.03.4905

Regmi, H., Vallad, G. E., Hutton, S. F. and Desaeger, J. (2022). Interaction studies between *Meloidogyne Javanica* and *Fusarium* Oxysporum f. Sp. *lycopersici* (Fol) race 3 on different isolines of tomato cv. Tasti Lee. Journal of Nematology, 54. https://doi.org/10.2478/jofnem-2022-0018

Saeed, M., Mukhtar, T., Ahmed, R., Ahmad, T. and Iqbal, M. A. (2023). Suppression of *Meloidogyne javanica* infection in peach (*Prunus persica* (L.) Batsch) using fungal biocontrol agents. Sustainability, 15, 13833. https://doi.org/10.3390/su151813833

Shahid, M., Gowen, S. R. and Burhan, M. (2022). Studies on the possible role of plant host on the development of root-knot nematode, *Meloidogyne javanica* and *Pasteuria penetrans* as affected by different harvesting dates. Plant Protection, 6, 133-141. https://doi.org/10.33804/pp.006.02.4207

Shahid, M., Gowen, S. R., Burhan, M., Niaz, Z. and Haq, A. (2023). Studies on the efficacy of heterogeneously produced *Pasteuria penetrans* (PP3) isolate over individual *Pasteuria* isolates in the spore attachment, and pathogenic potential on three *Meloidogyne* species. Plant Protection, 7, 9-16. https://doi.org/10.33804/pp.007.01.4529

Sikora, R. A. and Fernandez, E. (2005). Nematode Parasites of Vegetables. In R. A. Sikora, D. L. Coyne, J. Hallmann and P. Timper (Eds.). Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (p. 319-392). CABI Publishing.

Singh, M., Khan, Z., Kumar, K., Dutta, M., Pathania, A., Dahiya, O. and Kumar, J. (2012). Sources of resistance to *Fusarium* wilt and root-knot nematode in indigenous chickpea germplasm. Plant Genetic Resources, 10, 258-260. https://doi.org/10.1017/S1479262112000263

Taylor, A. L. and Sasser, J. N. (1978). Biology, Identification and Control of Root-knot Nematodes (*Meloidogyne* spp.). A cooperative publication of North Carolina State University. Raleigh: North Carolina State University.

Taylor, D. P. and Netscher, C. (1974). An improved technique for preparing perineal pattern of *Meloidogyne* species. Nematologica, 20, 268-269.

Toju, H. and Tanaka, Y. (2019). Consortia of anti-nematode fungi and bacteria in the rhizosphere of soybean plants attacked by root-knot nematodes. Royal Society Open Science, 6, 181693. https://doi.org/10.1098%2Frsos.181693

Ulloa, M., Wang, C., Saha, S., Hutmacher, R. B., Stelly, D. M., Jenkins, J. N., Burke, J. and Roberts, P. A. (2016). Analysis of root-knot nematode and Fusarium wilt resistance in cotton (*Gossypium* spp.) using chromosome substitution lines from two alien species. Genetica, 144, 167-179. https://doi.org/10.1007/s10709-016-9887-0

Vigbedor, H. D., Nyaku, S. T. and Cornelius, E. W. (2022). Effect of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *lycopersici* on tomato varietal growth under Ghanaian field conditions. African Crop Science Journal, 30, 155-166. https://doi.org/10.4314/acsj.v30i2.4

Viljoen, A., Ma, L. J. and Molina, A. B. (2020). Fusarium wilt (Panama disease) and monoculture banana production: resurgence of a century-old disease (p. 159-184). In J. B. Ristaino and A. Records (Eds.). Emerging Plant Diseases and Global Food Security. APS. https://doi.org/10.1094/9780890546383.008

Wagner, T. A., Duke, S. E., Davie, S. M., Magill, C. and Liu, J. (2022). Interaction of fusarium wilt race 4 with root-knot nematode increases disease severity in cotton. Plant Disease, 106, 2558-2562. https://doi.org/10.1094/pdis-12-21-2725-sc

Whitehead, A. G. and Hemming, J. R. (1965). A comparison of some quantitative methods of extracting small vermiform nematodes from soil. Annals of Applied Biology, 55, 25-38. https://doi.org/10.1111/j.1744-7348.1965.tb07864.x

Yaseen, I., Mukhtar, T., Kim, H. T. and Arshad, B. (2023). Quantification of resistance to *Melidogyne incognita* in okra cultivars using linear and nonlinear analyses of growth parameters and nematode infestations. Bragantia, 82, e20230114. https://doi.org/10.1590/1678-4499.20230114

Zainab, B., Ali, S., Zeshan, M. A., Sahi, G. M., Iftikhar, Y., Binyamin, R., Ghani, M. U. and Ahmad, A. (2023). Evaluation of chili germplasm against leaf spot and its management by nutrients and fungicides. Plant Protection, 7, 509-518. https://doi.org/10.33804/pp.007.03.4862