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Research Article

IMPACT OF SEQUENTIAL AND CONCURRENT INOCULATIONS OF *MELOIDOGYNE INCOGNITA* AND *FUSARIUM OXYSPORUM* F.SP. *VASINFECTUM* ON THE GROWTH PERFORMANCE OF DIVERSE OKRA CULTIVARS

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ABSTRACT

The present study was conducted to assess the effects of inoculation methods viz. concurrent and sequential inoculations of the root-knot nematode, *Meloidogyne incognita*, and the wilt-causing fungus, *Fusarium oxysporum* f.sp. *vasinfectum*, on various growth parameters of nine okra cultivars. The study aimed to identify cultivars that exhibited robust growth parameters in response to these two pathogens. Significant disparities were found in shoot lengths among cultivars and inoculation methods ($F=1033.86$, $P<0.001$), with Arka Anamika, Irka-1, and Irka-2 experiencing the most considerable reductions. In contrast, Pusa Swami and Green Star had the least reduction across all inoculation treatments. Sequential inoculation led to shoot length reductions ranging from 76.00% to 90.25%, averaging 87.07%. Concurrent inoculation resulted in reductions between 82.50% and 95.98%, averaging 90.29%. For nematode inoculation followed by fungus after 15 days, the reductions ranged from 81.74% to 95.34%, with an average of 89.21%. Regarding root length, concurrent inoculation generally had a less detrimental effect on root lengths compared to sequential inoculation. The reduction in root length varied from 0.01% to 24.72%, with concurrent inoculation showing less reduction than sequential methods. Similarly, concurrent inoculation typically resulted in lesser reductions in shoot weights compared to sequential inoculations, with reductions ranging widely among cultivars. Unlike shoot weight, concurrent inoculation did not consistently increase root weight compared to sequential methods. In conclusion, the study suggests that concurrent inoculation of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* is less harmful to the growth parameters of okra cultivars than sequential inoculation, with some variations among specific cultivars.

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INTRODUCTION

Okra (*Abelmoschus esculentus* L.), also known as ladyfinger, belongs to the family *Malvaceae*. This nutrient-rich crop is abundant in vitamins A, B, and C, as well as protein, calcium, potassium, iron, iodine, and other minerals. Additionally, the stem of the okra plant is high in crude fiber and is utilized in the paper industry. Okra is an important summer crop used as a vegetable for cooking, frying, and making soups. Originally from tropical Asia and Africa, okra is now widely cultivated in many parts of the world. The global okra production is estimated to be around 9.96 million tons, with India leading at 6.18 million tons, followed by Nigeria at 1.82 million tons (FAOSTAT, 2020). Pakistan, which ranks fifth globally in okra cultivation, grows okra on 15,529 hectares, yielding a production of 118,986 tons (Anonymous, 2024). However, the productivity in Pakistan is lower compared to other high-yielding countries due to challenges such as diseases and insect pests (Hussain et al., 2016; Mukhtar and Hussain, 2019).

Two major pathogens that affect okra plants are the root-knot nematode (*Meloidogyne incognita*) and the soil-borne fungus *Fusarium oxysporum* f.sp. *vasinfectum*, which cause significant damage to the crops. Root-knot nematodes (*Meloidogyne* spp.) are obligate plant parasites with a global host range exceeding 5,500 plant species, making them among the most pervasive and destructive agricultural pests (Trudgill and Blok, 2001; Chen et al., 2017). These nematodes have been ranked among the top ten plant-parasitic nematodes due to their profound scientific and economic impact (Jones et al., 2013). Their exceptional adaptability and broad host spectrum significantly impact commercial agriculture, causing substantial yield losses and increased production costs (Tariq-Khan et al., 2017, 2020; Mukhtar et al., 2021; Haq et al., 2022; Mejias et al., 2022; Yaseen et al., 2023; Saeed et al., 2021, 2023; Saeed and Mukhtar, 2024).

Root-knot nematodes inflict direct damage to plant roots by inducing the formation of characteristic galls. These galls disrupt the normal function of the roots, impairing water and nutrient uptake, which leads to stunted growth and reduced crop yields. Furthermore, these nematodes exacerbate plant diseases by interacting synergistically with other soil-borne pathogens such as *Thielaviopsis*, *Fusarium*, *Phytophthora*, and *Ralstonia solanacearum*. This synergism intensifies the severity of plant diseases, leading to complex disease syndromes and significant agricultural losses (Kamalnath et al., 2019; Asghar et al., 2020; Khan and Sharma, 2020; Ahmed et al., 2021; Khan et al., 2019, 2022; Aslam and Mukhtar, 2023a, b, 2024; Aziz et al., 2024; Yaseen et al., 2024).

Fusarium wilt, a severe disease affecting okra, is known for its detrimental impact on crop yield and results in substantial economic losses globally. In the United States alone, annual losses of up to $10 million has been reported due to *Fusarium* wilt (Viljoen et al., 2020). The pathogenic fungus infiltrates the root system of okra plants, establishing itself in the vascular system. This colonization disrupts water transport mechanisms and interferes with normal cellular functions. Remarkably, the fungus can persist in the soil for extended periods, posing a persistent threat to future crops. Symptoms of *Fusarium* wilt in okra manifest as wilting, yellowing, and stunted growth, with severe cases leading to plant death (Bahadur, 2021).

Notably, the combined presence of *M. incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* exacerbates the challenges faced by okra cultivation. Recent studies have highlighted the synergistic effects of these pathogens on okra germplasm, intensifying disease symptoms and resulting in heightened yield losses. The interaction between *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* in okra plants is a multifaceted process influenced by factors such as host genetics and environmental conditions. Although past research has predominantly focused on individual pathogen interactions, limited attention has been given to the combined impact of these pathogens on the growth of okra germplasm. Understanding the intricate dynamics between these two pathogens is imperative for devising effective management strategies to mitigate crop damage and enhance overall okra productivity.

The primary objective of this research was to assess the individual and combined effects of the root-knot nematode, *Meloidogyne incognita*, and the wilt-causing fungus, *Fusarium oxysporum* f.sp. *vasinfectum*, on various growth parameters of nine okra cultivars under both predisposing and simultaneous infection conditions. The study aimed to identify cultivars that exhibited robust growth parameters in response to these two significant pathogens.

MATERIALS AND METHODS

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 while the seeds of two cultivars viz. Ikra-1, Ikra-2 were obtained from the National Agricultural Research Centre, Islamabad, Pakistan.

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 maintained in the department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi. The nematode was mass produced on the susceptible cultivar of tomato "Money maker" and the freshly hatched second stage juveniles (J2s) were used in the evaluation as described by Mukhtar et al (2017).

Mass culturing of the fungus *Fusarium*

The fungus *Fusarium oxysporum* f.sp. *vasinfectum* was isolated from the roots of infected okra plants. A small section of the infected root tissue (5-6 mm) was placed on Potato Dextrose Agar with streptomycin sulfate. After 2-4 days, fungal spores (conidia) were observed under a microscope at 40× magnification. The conidial hyphae were scraped from a 2-4-day-old culture, stained with lactophenol cotton blue, and examined to identify microconidia and macroconidia.

To propagate the fungus, 500 g of chickpea grains were soaked in water overnight, crushed, dried, and sterilized by autoclaving twice. Six mycelium plugs from a 7-day-old culture of *F. oxysporum* f.sp. *vasinfectum* were added to the sterilized grains, and the mixture was incubated at 25°C for two weeks. The fungal inoculum was then quantified using a haemocytometer and used for soil application (Yaseen et al., 2023).

Assessment of okra germplasm for growth performance

The growth performance of okra cultivars with varying resistance to the root-knot nematode (*M. incognita*) and the wilt-causing fungus (*F. oxysporum* f.sp. *vasinfectum*) was evaluated using 20 cm diameter plastic pots. Each pot contained 2.5 kg of sterilized soil composed of 70% sand, 22% silt, and 8% clay, with a pH of 7.5. Three seeds of each okra cultivar were sown per pot, and ten days after germination, a single healthy seedling was retained. The seedlings were inoculated with 2,500 freshly hatched J2s of *M. incognita* and 4 ml of an aqueous *Fusarium* suspension containing 4,000 micro- and macroconidia. There were three treatments:

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*.

Uninoculated plants of each cultivar served as controls. Each treatment was replicated 10 times, and the experiment was repeated once. The pots were arranged in a completely randomized design under field conditions within an iron cage for 7 weeks and watered as needed.

Data collection

The plants of each okra cultivar were grown for seven weeks before being carefully removed from the pots. The roots were separated from the shoots, washed to remove any adhering soil, and gently blotted dry. Fresh weights and lengths of the shoots and roots were then recorded as described by Yaseen et al. (2023).

Statistical analysis

The experiment was replicated once, and all data were subjected to Analysis of Variance (ANOVA) using SPSS software. Levene's test was used to assess the homogeneity of variance among samples at a significance level of $p < 0.05$. The equality of means was evaluated using the Welch test, also at a significance level of $p < 0.05$. The interpretations of both the Levene's test and the Welch test were conducted with SPSS software. Duncan's Multiple Range Test was employed for comparing statistical means.

RESULTS

Effect of sequential and concurrent inoculations of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* on shoot lengths of okra cultivars

The ANOVA indicated significant differences in shoot lengths among cultivars and inoculation methods ($F=1033.86$, $P=0.000$) (Table 1). The shoot length reductions were highest in Arka Anamika, followed by Irka-1 and Irka-2 while Pusa Swami and Green Star had the lowest shoot length reductions for all three inoculation treatments. Shoot length reductions for the sequential inoculation treatment ranged from 76.00% (Arka Anamika) to 90.25% (Neelum), with an average reduction of 87.07%. Shoot length reductions for the concurrent inoculation treatment ranged from 82.50% (Irka-1) to 95.98% (Pusa Swami), with an average reduction of 90.29%. Similarly, shoot length reductions for the nematode inoculation 15 days prior to fungus inoculation treatment ranged from 81.74% (Irka-1) to 95.34% (PB Selection), with an average reduction of 89.21%. The individual reductions in this parameter in all the okra cultivars are given in Table 1.

Table 1: Effect of sequential and concurrent inoculations of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* on shoot lengths of okra cultivars.

Cultivar	% reductions in shoot lengths		
	Sequential inoculation		Concurrent inoculation of nematodes and fungus
	Nematode inoculation 15 days prior to fungus inoculation	Fungus inoculation 15 days prior to nematode inoculation	
PB Selection	6.49 ± 0.33 c	2.0±0.08 a	2.93 ± 0.73 a
Green Star	5.57 ± 0.24 b	4.39±0.10 b	4.74 ± 0.64 b
Neelum	9.75 ± 0.32 d	8.26±1.53 d	9.52 ± 0.63 d
Tulsi	9.83 ± 0.22 d	6.47±0.24 c	6.71 ± 0.12 c
Arka Anamika	24.00 ± 0.27 h	8.54±0.15 d	15.60 ± 0.20 f
Pusa Swami	3.83 ± 0.19 a	4.02±0.12 b	4.66 ± 0.60 b
Sabz pari	10.98 ± 0.22 e	4.06±0.39 b	9.44 ± 0.28 d
Irka-1	21.81 ± 0.18 f	17.50±0.29 f	18.26 ± 0.40 g
Irka-2	22.63 ± 0.07 g	11.88±0.31 e	12.23 ± 0.50 e
ANOVA	F= 1033.86 df= 8,36 P= 0.000	F= 372.54 df= 8,36 P= 0.000	F= 107.96 df= 8,36 P= 0.000
Levene statistic	F= 1.625 df= 8,36 P= 0.152	F= 4.330 df= 8,36 P= 0.001	F= 1.22 df= 8,36 P= 0.311
Welch test	F= 1527.31 df= 8,14.59 P= 0.000	F= 1939.15 df= 8,14.75 P= 0.000	F= 203.18 df= 8,14.51 P= 0.000

Values (± SD) 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 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.

Effect of sequential and concurrent inoculations of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* on root lengths of okra cultivars

The statistical analysis confirmed significant differences in root lengths among cultivars and inoculation techniques (F=670.03, P=0.000) (Table 2). Overall, the results suggested that concurrent inoculation of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* had a less negative effect on root lengths of okra cultivars compared to sequential inoculation. For all cultivars, concurrent inoculation of nematodes and fungus resulted in less reduction in root length compared to sequential inoculation. The reduction in root length ranged from 0.01% (Pusa Swami) to 24.72% (Arka Anamika). When sequential inoculation (nematode inoculation 15 days prior to fungus inoculation) was compared to concurrent inoculation, the reduction in root length ranged from 1.25% (Pusa Swami) to 18.94% (Neelum). When comparing sequential inoculation (fungus inoculation 15 days prior to nematode inoculation) to concurrent inoculation, the reduction in root length ranged from 1.70% (Pusa Swami) to 19.35% (Tulsi). The individual reductions in root lengths of all the okra cultivars has been given in Table 2.

Effect of sequential and concurrent inoculations of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* on shoot weights of okra cultivars

The ANOVA analysis revealed significant variations in shoot weights across different cultivars and inoculation methods (F=1269.29, P=0.000) (Table 3). Notably, simultaneous inoculations of nematodes and fungus led to a lesser reductions in shoot weights compared to sequential inoculations for all cultivars. The degree of reduction in shoot weight varied widely, ranging from 0.26% (Pusa Swami) to 28.13% (Arka Anamika) among the different cultivars. Specifically, when comparing sequential inoculation (nematode inoculation 15 days prior to fungus inoculation) with simultaneous inoculation, the reduction in shoot weight ranged from 0.54% (Pusa Swami) to 19.42% (Neelum). Similarly, when comparing sequential inoculation (fungus inoculation 15 days prior to nematode inoculation) with simultaneous inoculation, the reduction in shoot weight ranged from 2.28% (Green Star) to 27.76% (Arka Anamika). The individual reductions in shoot weight for each inoculation are provided in Table 3.

Table 2: Effect of sequential and concurrent inoculations of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* on root lengths of okra cultivars.

Cultivar	% reductions in root lengths		
	Sequential inoculation		Concurrent inoculation of nematodes and fungus
	Nematode inoculation 15 days prior to fungus inoculation	Fungus inoculation 15 days prior to nematode inoculation	
PB Selection	7.26 ± 0.33 a	4.02±0.32 a	6.03 ± 1.57 a
Green Star	8.13 ± 0.97 a	5.72±0.11 b	5.57 ± 1.66 a
Neelum	25.53 ± 0.68 d	12.03±0.82 d	13.38 ± 1.18 b
Tulsi	18.04 ± 0.58 b	12.38±0.42 d	13.12 ± 1.19 b
Arka Anamika	41.30 ± 0.50 e	16.58±1.02 e	31.51 ± 0.82 e
Pusa Swami	8.33 ± 0.45 a	8.32±0.22 c	8.79 ± 2.73 a
Sabz pari	20.13 ± 0.79 c	9.11±0.48 c	19.78 ± 0.89 c
Irka-1	41.31 ± 0.51 e	28.05±0.68 g	29.53 ± 0.94d e
Irka-2	48.05 ± 0.38 f	24.85±1.149 f	26.94 ± 0.75 d
ANOVA	F= 670.03 df= 8,36 P= 0.000	F= 755.21 df= 8,36 P= 0.000	F= 49.85 df= 8,36 P= 0.000
Levene statistic	F= 1.98 df= 8,36 P= 0.077	F= 2.079 df= 8,36 P= 0.064	F= 1.30 df= 8,36 P= 0.273
Welch test	F= 976.52 df= 8,14.91 P= 0.000	F= 816.48 df= 8,14.31 P= 0.000	F= 54.82 df= 8,14.91 P= 0.000

Values (± SD) 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 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.

Effect of sequential and concurrent inoculations of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* on root weights of okra cultivars

The ANOVA indicated significant variations in root weights across different cultivars and inoculation methods (F=15.32, P=0.000) as shown in Table 4. Contrary to the trend observed for shoot weight (Table 3), it was observed that simultaneous inoculation with *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* did not consistently result in a marked increase in root weight for the majority of okra cultivars when compared to the sequential inoculation approach. Notably, except for the cultivar Neelum, the increases in root weight due to concurrent inoculation were within the range of those recorded for the two methods of sequential inoculation. Specifically, Neelum was the only cultivar to exhibit a statistically significant increase in root weight (1.40%) following concurrent inoculation versus nematode inoculation occurring 15 days before fungal inoculation. In a similar vein, when concurrent inoculation was measured against fungal inoculation preceding nematode inoculation by 15 days, only Neelum and Arka Anamika demonstrated statistically significant root weight gains of 0.40% and 0.77%, respectively. The individual reductions in root weight for all okra cultivars are provided in Table 4.

Table 3: Effect of sequential and concurrent inoculations of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* on shoot weights of okra cultivars

Cultivar	% reductions in shoot weights		
	Sequential inoculation		Concurrent inoculation of nematodes and fungus
	Nematode inoculation 15 days prior to fungus inoculation	Fungus inoculation 15 days prior to nematode inoculation	
PB Selection	8.96 ± 0.39 b	5.57±0.74 a	3.68±0.19 a
Green Star	8.71 ± 0.32 b	6.13±0.17 a	8.67±0.45 c
Neelum	21.35 ± 0.11 e	12.22±0.30 d	12.09±0.30 e
Tulsi	16.85 ± 0.38 c	9.47±0.22 b	8.93±0.44 c
Arka Anamika	38.54 ± 0.36 g	10.41±0.49 c	25.03±0.12 h
Pusa Swami	5.55 ± 0.33 a	5.29±0.21 a	5.01±0.34 b
Sabz pari	18.71 ± 0.17 d	6.06±0.41 a	10.78±0.60 d
Irka-1	39.80 ± 0.55 h	23.71±0.72 e	18.31±0.31 f
Irka-2	37.33 ± 0.55 f	24.33±1.32 e	21.07±0.47 g
ANOVA	F= 1269.29 df= 8,36 P= 0.000	F= 732.15 df= 8,36 P= 0.000	F= 354.37 df= 8,36 P= 0.000
Levene statistic	F= 2.35 df= 8,36 P= 0.038	F= 2.522 df= 8,36 P= 0.027	F= 2.18 df= 8,36 P= 0.053
Welch test	F= 841.98 df= 8,14.6 P= 0.000	F= 559.512 df= 8,14.79 P= 0.000	F= 1081.0 df= 8,14.63 P= 0.000

Values (± SD) 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 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.

DISCUSSION

The present study examined the effects of sequential and concomitant inoculations of *Meloidogyne incognita* and *Fusarium oxysporum* f.sp. *vasinfectum* on the shoot and root lengths and weights of various okra cultivars. Results varied across cultivars and inoculation methods. Notably, PB Selection showed a 6.49% reduction in shoot length with sequential inoculation and 2.0% with concurrent inoculation. Irka-1 and Irka-2 had the most significant reductions in shoot lengths and weights. Arka Anamika exhibited the highest root length decreases, while Irka-2 had the highest root weight reductions under both inoculation methods.

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; Vigbedor et al., 2022; Regmi 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.

Table 4: Effect of sequential and concurrent inoculations of *M. incognita* and *F. oxysporum* f.sp. *vasinfectum* on root weights of okra cultivars.

Cultivar	% Increases in root weights		
	Sequential inoculation		Concurrent inoculation of nematodes and fungus
	Nematode inoculation 15 days prior to fungus inoculation	Fungus inoculation 15 days prior to nematode inoculation	
PB Selection	3.04 ± 0.22 a	1.38 ±0.18 a	2.29 ± 1.72 ab
Green Star	4.10 ± 0.65 ab	1.98 ±0.09 ab	1.69 ± 0.37 a
Neelum	5.85 ± 0.36 bc	4.89±5.26 d	4.45 ± 0.92 ab
Tulsi	6.82 ± 0.52 cd	5.23±0.18 abc	2.81 ± 0.82 ab
Arka Anamika	8.98 ± 1.22 d	4.18 ±0.43 abc	4.95 ± 1.59 ab
Pusa Swami	3.59 ± 0.35 a	1.76897±2.16 abc	2.19 ± 0.58 ab
Sabz pari	6.50 ± 0.46 c	1.77 ±0.12 ab	3.61 ± 1.33 ab
Irka-1	7.13 ± 1.05 cd	5.43 ±0.45 abc	5.70 ± 1.61 b
Irka-2	12.03 ± 0.92 e	5.95±0.57 cd	5.30 ± 0.95 ab
ANOVA	F= 15.32 df= 8,36 P= 0.000	F= 2.14 df= 8,36 P= 0.000	F= 1.55 df= 8,36 P= 0.172
Levene statistic	F= 1.79 df= 8,36 P= 0.111	F= 5.934 df= 8,36 P= 0.111	F= 1.66 df= 8,36 P= 0.141
Welch test	F= 17.68 df= 8,14.74 P= 0.000	F= 74.317 df= 8,14.62 P= 0.000	F= 2.35 df= 8,14.14 P= 0.074

Values (± SD) 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 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.

In sequential (N15+F) inoculation, nematodes predispose the host, enhance disease severity rapidly, and provide an optimal substrate for secondary pathogens by altering the metabolic and biochemical activities of the hosts (Anwar and Khan, 2002; Bhabesh et al., 2007; Malhotra et al., 2011). Root-knot nematodes (*M. incognita*) and other wilt-causing fungi interact to form disease complexes, increasing wilt incidence and severity, which often confuses farmers regarding the exact disease etiology (Begum et al., 2012). These disease complexes involving root rot fungi (*Rhizoctonia solani* and *F. oxysporum*) and *M. incognita* result in greater crop losses due to their predisposing (N15+F) attacks (Abuzar, 2013; Rivera and Aballay, 2008).

A previous study found that the shorter plant heights, smaller plant girths, and lower chlorophyll contents in inoculated "Essoumtem" and Clemson spineless okra plants were due to root damage in both pot and field experiments. This root damage impaired the ability of plants to absorb water and minerals from the soil, thereby hindering photosynthesis (Agbaglo et al., 2020).

Inadequate supply of water, minerals, energy, and photosynthates affects the growth and development of leaf tissue and chlorophyll content (Safiuddin and Shahab, 2012). Root damage in okra plants impedes the absorption of water and minerals from the soil, leading to stunted growth. Wilting and stunting are further exacerbated by nematode infestations (William and Robert, 2007). Agbaglo et al. (2020) found that sequential treatments (NF7, NF14, and NF21) significantly reduced plant growth parameters, including fresh and dry shoot and root weights, pod weights and yields in "Essoumtem" and Clemson spineless okra varieties in both pot and field experiments. Similarly, a previous glasshouse study showed that *M. incognita* and *F. solani* isolates caused severe reductions in shoot length, root length, and fresh and dry weights of shoots and roots in tomato plants compared to uninoculated plots (Ganaie and Khan, 2011).

Nematodes are a serious pest for okra plants, damaging plant stands and delaying okra pod production by nearly 80% (Bolles and Johnson, 2012; Hussain et al., 2012; Kayani et al., 2018; Kayani and Mukhtar, 2018; Hussain and Mukhtar, 2019; Azeem et al., 2021; Afzal and Mukhtar, 2024). Infection by *Meloidogyne* spp. causes severe growth impairment and significant yield losses in crops (Hussain et al., 2011; Kayani et al., 2013; Mukhtar et al., 2013; Barros et al., 2014; Mukhtar, 2018; Nazir et al., 2019; Mukhtar and Kayani, 2019, 2020). Similarly, Agbaglo et al. (2020) found that nematodes significantly reduced pod production in okra plants, especially in fields with high nematode populations. Poor leaf growth consequently leads to decreased yield (Hussain et al., 2016; Kayani et al., 2017).

The severity of root rot caused by *F. oxysporum* increased in the presence of *M. incognita*, particularly when *M. incognita* inoculation preceded *F. oxysporum* by three weeks in "Essoumtem" and Clemson spineless okra varieties in both pot and field experiments. Severe plant damage was observed when both pathogens were inoculated simultaneously or sequentially (Agbaglo et al., 2020). Specific fungi and other plant pathogens interact to form disease complexes (Begum et al., 2012). The presence of root-knot nematode exacerbated *Rhizoctonia* root rot, and the root-rot disease complex caused by *R. solani* and *M. incognita* has been reported in okra and chili (Bhagawati et al., 2007; Abuzar, 2013). Agbaglo et al. (2020) in his study also showed that concomitant and sequential infections of *M. incognita* and *F. oxysporum* resulted in greater damage than individual infections in both "Essoumtem" and Clemson spineless okra varieties.

CONCLUSION

This study highlights the significant impact of inoculation methods on the growth of okra cultivars. The results indicate that sequential inoculations, particularly those involving fungus inoculations before nematode inoculation, significantly reduced shoot lengths and weights across all cultivars. In contrast, concurrent inoculation generally had a less detrimental effect on root lengths and shoot weights. The findings suggest that the timing and order of inoculation can have substantial effects on the growth of okra plants.

AUTHORS' CONTRIBUTION

IY and TM conceived and designed the experiments, performed the experiments, analyzed and interpreted the data, wrote the paper; TM supervised the work and proofread the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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