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Testing of compatibility among the fusarium isolates from tomato, brinjal and chilli *in vitro*

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Abstract
The study aimed to test the *in vitro* compatibility of Fusarium isolates from tomato, brinjal and chili using Potato Dextrose Agar (PDA) as a growth medium. The experiment, conducted in February 2024 at the Forest College and Research Institute, Mulugu, tested four combinations of Fusarium isolates: tomato + brinjal, tomato + chili, brinjal + chili and tomato + brinjal + chili. Pure cultures were prepared using the single spore technique, and pathogen identification was confirmed by analyzing colony morphology and spore characteristics. The isolates were inoculated in petri plates under aseptic conditions and incubated at 28±1 °C. Growth rates were observed at 2, 5 and 8 days after inoculation. The results showed compatibility between tomato and brinjal isolates, with complete coverage by the 8th day, while distinct mycelial layers indicated incompatibility in combinations involving chili isolates. These findings contribute to understanding Fusarium isolate interactions, particularly the incompatibility reactions of chili isolates with both tomato and brinjal under controlled conditions.

Keywords: *Fusarium oxysporum*, pathogenicity, compatibility, isolates

Introduction
Fusarium wilt caused by the fungal pathogen *Fusarium oxysporum f. sp. lycopersici* (Sacc.) W.C. Snyder and H. N. Hans is known as one of the most devastating diseases of tomato worldwide. Wilting is most likely caused by a combination of pathogen activities such as the accumulation of fungal mycelium and/or toxin production and host defense responses, including production of gels, gums and tyloses and vessel crushing by proliferation of adjacent parenchyma cells *F. oxysporum* isolates are very dynamic and exhibit high variation with respect to their cultural, morphological and pathogenic characters. As in other Fusaria, its identification is generally based on morphological criteria such as shape of micro and macroconidia, structure of the micro conidiophores and the formation and disposition of chlamydospores. (Nirmaladevi and Srinivas, (2012) [8]. *F. oxysporum* produces colorless to pale yellow mycelium that turns pink or purple with age. With the exception of grasses and most tree crops, few of the widely cultivated crops are not hosts to pathogenic form of *F. oxysporum*. (Armstrong and Armstrong J.K., 1981.) [1] Isolates have been divided into more than 120 different formae speciales according to their host range. The aim of the present study was to isolate and study the morphological diversity, growth rate, and compatibility among *Fusarium* isolates from various fields of farmers from central Telangana region.

Materials and Methods
Experimental area
The laboratory experiment was conducted at forest college and research institute, mulugu, in the month of February, 2024. The study employed a complete randomized block design (CRD) with three replications and four treatments.

Experimental materials
The experiment was conducted to test the compatibility of Fusarium isolates using Potato Dextrose Agar (PDA) as the growth medium. The Fusarium isolates were cultured in 100 mm x

15 mm petri plates, with four specific combinations tested: 1) Tomato x Brinjal, 2) Tomato x Chilli, 3) Brinjal x Chilli and 4) Tomato x Brinjal x Chilli. The objective was to observe the interactions between the isolates *in vitro* and determine their compatibility or incompatibility relation.

Collection and identification of disease samples

An extensive field survey was carried out in the major vegetables growing areas of central Telangana region during rabi 2024. The fusarium affected samples of tomato, brinjal and chilli were collected from different farmers fields of vantimamidi and mulugu villages. The stem showing necrosis, dark brown reddish discoloration.

Isolation of pathogen(s)

The wilt-causing fungus was isolated using the standard tissue isolation method under aseptic conditions in November 2023 at the Forest College and Research Institute, Mulugu, Telangana. Infected stems of tomato, brinjal and chili were split open and sections with brown vascular discoloration were cut, washed and surface-sterilized with 0.1% sodium hypochlorite for 15 seconds. (Kamatagi, (2019) [5]. After rinsing with sterile water, the pieces were placed on Potato Dextrose Agar (PDA) plates and incubated at 28±1 °C for 5-7 days in darkness.

Identification of the pathogen(s)

Fungal colonies on Potato Dextrose Agar (PDA) were distinguished by characteristics like colony color, mycelial growth, Identification was further confirmed using a compound microscope. Slides of dark-colored fungi were prepared in lactophenol and the pathogen was identified based on morphological traits, including the shape, size and color of microconidia and macroconidia (Ginting, J. (2021) [4] as well as cultural characteristics like colony color on PDA.

Purification of fusarium isolates

To isolate pure *Fusarium* cultures, the single spore technique was used. The fungus was grown on PDA for 12-15 days and a 5 mm mycelial disc was suspended in 10 ml of sterile water. After diluting the spore suspension, 1 ml was pipetted onto PDA plates and incubated at 28±1 °C for 12-24 hours. Individual germinating spores were picked under a microscope with a sterile needle and transferred to fresh PDA plates (Asif *et al*., 2023) [2] then incubated again until colonies formed. These pure cultures were transferred to new plates for further study.

Morphological variability of spores in culture media

Three different *Fusarium* isolates of tomato, brinjal and chilli were collected from the Central Zone of Telangana, testing their cultural and morphological variations on Potato Dextrose Agar (PDA). Observations include micro conidia, macro conidia and chlamydospores.

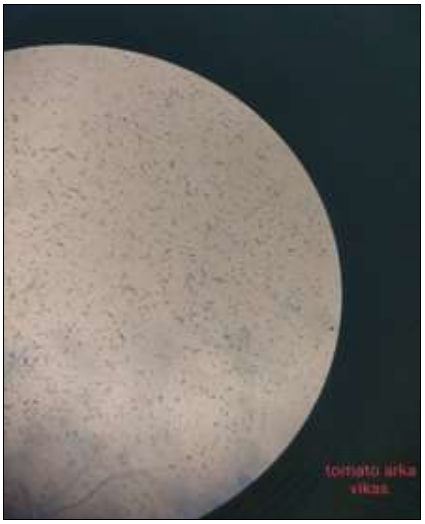


Fig 1: Microscopic observations of micro and macro spores of tomato

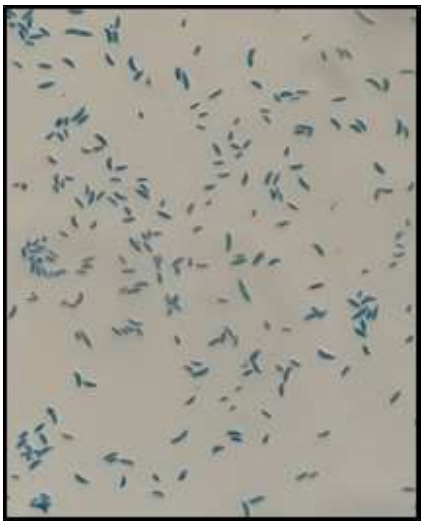


Fig 2: Microscopic observations of micro and macro spores of brinjal

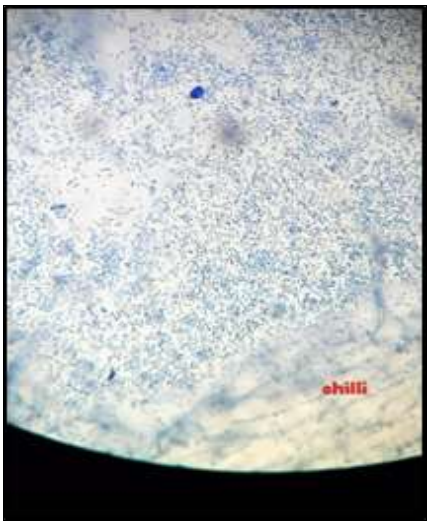


Fig 3: Microscopic observations of micro and macro spores of chilli

Cultural characteristics of fusarium isolates of tomato, brinjal and chill

Fusarium, isolates were cultured the test isolates on PDA (pH 6.5) in 100 mm x 15 mm Petri dishes and then incubated them at 28±1 °C in a B.O.D. incubator. After 7 days of inoculation, observed the growth, mycelium type, pigmentation in mycelium.

Growth of the pathogens

Growth rate of the isolates, a 5mm disc was cut out from a 4- 5 day old culture using a sterilized cork borer (Mutua, P. M. 2014) [7] and placed in center of petri plates of a 100 mm x 15 mm Petri dish containing solidified PDA medium. Three plates were inoculated for each isolate. The inoculated plates were then kept in a B.O.D incubator at 28±1 °C. After 7 days, the growth rate was measured in terms of colony diameter.

Mycelium type

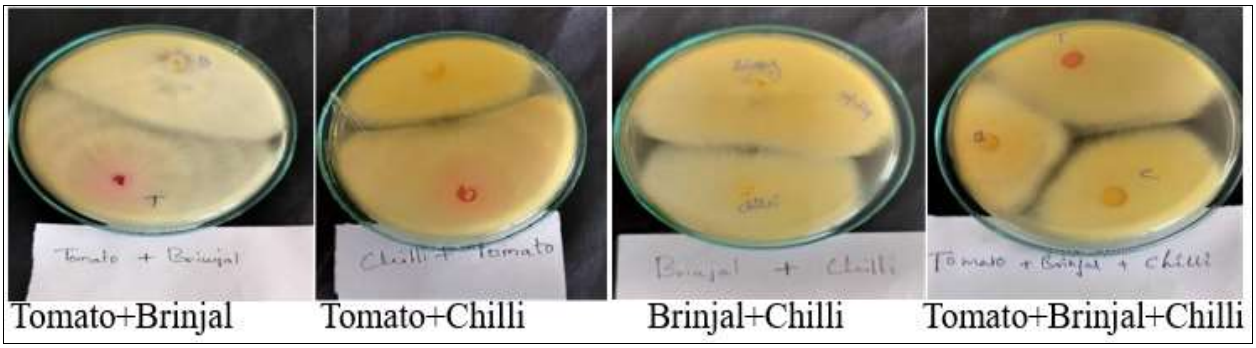
Based on the colony morphology, the mycelium type of isolates was classified as fluffy, moderately fluffy, fibrous and scanty. (Mania *et al*., 2017) [6].

Results and Discussion

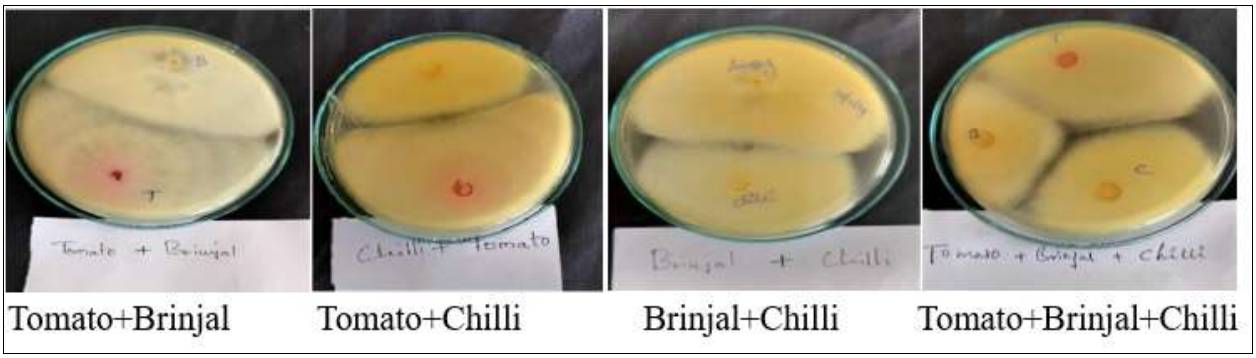
For testing of compatibility among the fusarium isolates of tomato, brinjal and chilli, experiment was conducted *in vitro* at Plant Pathology laboratory, FCRI, Mulugu. The pure cultures prepared earlier for respective pathogens were used in different combinations (Tomato + brinjal, Tomato + chilli, Brinjal + chilli and Tomato + brinjal + chilli). These cultures were inoculated with uniform rate in the petri plates (100 mm x 15 mm) containing PDA media at equal distance from periphery under fully aseptic conditions. The inoculated petri plates were kept in BOD incubator at 28±1˚C. Isolates of fusarium sps of tomato, brinjal and chilli growth rate were observed at different days of interval on percent area covered in the petri plates of replicated thrice for each combination and average value was calculated.

Compatibility assessment among the tomato, brinjal and chilli fusarium *in vitro* at 2nd, 5th and 8th day after inoculation

2nd Days after inoculation



5th Days after inoculation



8th Days after inoculation

Overall all view of the experiment

Table 1: Percent mycelia coverage of different fusarium isolates under PDA media *in vitro*

Fusarium isolates combinations		Different days intervals after inoculation in PDA media		
		2nd DAI	5th DAI	8th DAI
		Percent mycelia coverage		
Tomato + Brinjal	Tomato	7	20	60
	Brinjal	9	33	40
	Total coverage	16	53	100
Tomato + Chilli	Tomato	12	30	53
	Chilli	8	15	30
	Total coverage	20	45	83
Brinjal + Chilli	Brinjal	12	28	48
	Chilli	10	13	37
	Total coverage	22	41	85
Tomato + Brinjal + Chilli	Tomato	6	25	35
	Brinjal	10	17	28
	Chilli	11	12	17
	Total coverage	27	54	80

DAI: Days After Inoculation, PDA: Potato Dextrose Agar

Table 2: Compatibility assessment of different fusarium isolates under PDA media *in vitro*

Fusarium isolates	Tomato isolate	Brinaj isolate	Chilli isolate
Tomato isolate	-	✓	×
Brinjal isolate	✓	-	×
Chilli isolate	×	×	-

✓: Compatibility, x: Incompatibility

Growth rate at 2nd day after inoculation

The data pertaining to compatible growth among the isolates was observed that maximum growth covered in combination of Tomato + brinjal + chilli fusarium isolates (27 percent), followed by Brinjal + chilli fusarium isolates (22 percent), Tomato + chilli fusarium isolates (20 percent) and minimum coverage was recorded in Tomato + brinjal fusarium isolates (16 percent).

Growth rate at 5th day after inoculation

Here also the maximum growth coverage was observed in tomato + brinjal + chilli fusarium isolates (54 percent), followed by tomato + brinjal fusarium isolates (53 percent), tomato + chilli fusarium isolates (45 percent) and the minimum growth was recorded in brinjal + chilli fusarium isolates (41 percent).

Growth rate at 8th day after inoculation

At this stage almost maximum growth coverage was noticed among the various combinations of fusarium isolates. The results reveled that maximum growth was observed in combination between tomato + brinjal fusarium isolates (100 percent), followed by brinjal + chilli fusarium isolates (85 percent), tomato + chilli fusarium isolates (83 percent) and the minimum growth was recorded in tomato + brinjal + chilli fusarium isolates (80 percent).

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Conclusion

In Tomato + brinjal fusarium isolate combination, almost similar growth rate (60% + 40%) was noticed and also covered total petri plate without leaving any differentiating layer at 8th day after inoculation. It demonstrated compatibility between these two isolates under *in vitro*. However, distinct mycelial appearances were observed: brinjal culture samples displayed a

fluffy cottony growth mycelium appearance while the tomato samples exhibited a rhizomorphic appearance with pinkish pigmentation.

In other combinations (Tomato + chilli, brinjal + chilli and tomato + brinjal + chilli) displayed comparatively more variations in growth at different intervals. At 8th day after inoculation in tomato + chilli (83%) combination reported 53 and 30 percent growth coverage and in brinjal + chilli (85%) reported 48 and 37 percent growth coverage while, tomato + brinjal + chilli (80%) recorded 35, 28 and 17 percent growth coverage, respectively. It was also clearly evident that, there was a distinct layer was formed between tomato and chilli fusarium isolates as well as brinjal and chilli fusarium isolates. Which may be an indicative of incompatibility reaction of chilli fusarium isolate with brinjal and tomato fusarium isolates under *in vitro* conditions.

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