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RESEARCH ARTICLE

Assessment of citrus rootstocks against dry root rot induced by *Fusarium solani*

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Abstract

Citrus rootstocks play a vital role in imparting tolerance or resistance to diseases. Dry root rot induced by *Fusarium solani* is one of most destructive soil-borne diseases of citrus. The present study was conducted under the All India Coordinated Research Project (AICRP) on Fruits scheme at Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, to evaluate the tolerance of different citrus rootstocks against *F. solani*. A total of 8 rootstocks (NRCC-1, NRCC-2, NRCC-3, NRCC-4, CRH-12, Rangpur lime, Alemow and Jambheri) were evaluated using different screening methods, including the seedling inoculation method, leaf inoculation with injury, leaf inoculation without injury and application of culture filtrate on wounded leaf segments. All the examined rootstocks exhibited variable levels of susceptibility to dry root rot. In the seedling inoculation method, rootstock mortality ranged from 20.00 % in Rangpur lime to 60.67 % in Jambheri. Leaf lesion lengths following injury ranged from 14.45 mm (Rangpur lime) to 24.43 mm (Jambheri), whereas without injury lesions ranged from 11.95 mm (Rangpur lime) to 20.43 mm (NRCC-4). Culture filtrate bioassays produced lesion areas ranging from 1.01 cm^2 (Rangpur lime) to 1.69 cm^2 (NRCC-1). Among the evaluated rootstocks, Rangpur lime exhibited comparatively higher tolerance to *F. solani*. Leaf inoculation with injury was found to be a rapid and reliable method for screening citrus rootstocks against dry root rot.

Keywords: citrus; dry root rot; evaluation; *Fusarium solani*; rootstocks; screening methods; tolerance

Introduction

Citrus is native to a wide geographical area, extending from the Himalayan foothills of northeast India to north-central China, the Philippines in the east and Burma, Thailand, Indonesia and New Caledonia in the southeast (1). The world's leading producers of citrus include Brazil, the United States, Spain, Italy, Egypt, Mexico, China and India. In India, citrus ranks third among fruit crops in terms of land area after banana and mango (2). The commercial cultivars of mandarins grown in India include Nagpur mandarin (Central India), Kinnow mandarin (North-West India), Coorg mandarin (South India) and Khasi mandarin (North-East India). Mandarin orange (*Citrus reticulata* Blanco) is the most widely grown citrus fruit in India, accounting for nearly 40 % of the total area under citrus cultivation (3). It is grown across the country, however, the major producing states include Madhya Pradesh, Punjab, Maharashtra, Rajasthan, Haryana, Assam, Karnataka, Arunachal Pradesh and Mizoram (4).

Maharashtra is one of the leading mandarin-producing states in India, with an area of approximately 1.25 lakh ha and an annual production of 11.27 lakh tonnes, achieving a productivity of 9.01 t ha^{-1}. Nagpur mandarin is predominantly cultivated in the Vidarbha region of Maharashtra, which experiences humid tropical climatic conditions, with summer temperatures rising to 45–46 °C. Vidarbha is the major mandarin-producing region, accounting for nearly 92 % of the total mandarin-growing area in the state (5).

Fusarium spp. is recognised as important contributors to citrus decline. These fungi frequently act in association with other soil-borne pathogens, such as the citrus nematode *Tylenchulus semipenetrans* and *Phytophthora* spp., resulting in increased disease severity than that caused by individual pathogens alone (6). *Fusarium* spp. can induce symptomless infections in citrus roots, however, under stress conditions, they cause severe dry root rot, which can be distinguished from the more common *Phytophthora*-induced foot rot (7, 8). Typical symptoms include twig dieback, chlorosis of the main leaf veins, yellowing and premature leaf drop (6). The severity of the disease depends largely on the rootstock used and prevailing environmental conditions. Several fungi have been isolated from dry root rot-affected citrus trees, including *Coprinus micaceus* and *Diaporthe citri*, although *Fusarium solani* is considered a major causal agent (9). *F. solani* has also been reported from diseased citrus orchards in Florida and is capable of inducing root necrosis in trifoliate orange seedlings and sour orange rootstock in Texas (10). Dry root rot caused by *F. solani* is one of the major soil-borne diseases of acid lime (11) and sweet orange (12) in Andhra Pradesh and Telangana, India. The occurrence of dry root rot of citrus seedlings caused by *F. solani* has also been reported from California (13, 14). The disease incidence of dry root rot has been reported to range from 5 to 50 %, with 10 to 15 % of affected trees dying annually (15).

Citrus species are commercially propagated by budding onto seedling rootstocks. Initially, sour orange dominated as a resistant rootstock because of its resistance to *Phytophthora*; however, it was later replaced by rootstocks such as Rangpur lime, Cleopatra mandarin, Trifoliate orange, Troyer citrange and rough lemon due to its susceptibility to viruses (16). Because of the high rate of losses among susceptible seedlings, interest in the use of tolerant rootstocks has increased. Large quantities of nursery plants are multiplied annually and distributed to orchards without systematic certification for health status, genetic purity, or freedom from pathogens. Such uncertified rootstocks can act as carriers of pathogens, thereby facilitating the dissemination of diseases across nurseries and orchards. The use of resistant rootstocks with desirable horticultural traits is the most effective strategy for managing soil-borne diseases and reducing reliance on fungicide applications (17). Additionally, scion cultivars respond differently in terms of growth, fruit quality and nutrient accumulation when grafted onto different rootstocks; therefore, rootstock selection is crucial for orchard management (18). Disease resistance is one of the most significant functions of a rootstock. Differences in biochemical composition among rootstocks contribute to variations in disease resistance, highlighting the need to screen alternative rootstocks against *Fusarium* to minimise losses in the citrus industry.

Materials and Methods

Collection of citrus rootstocks for pot study

Eight citrus rootstocks, namely NRCC-1, NRCC-2, NRCC-3, NRCC-4, CRH-12, Alemow (*Citrus macrophylla*), Rangpur lime (*Citrus limonia*) and Jambheri or Rough lemon (*Citrus jambhiri*), were obtained from the All India Coordinated Research Project (AICRP) on Fruits scheme and grown in pots for further evaluation. Eleven to thirteen-month-old seedlings were used in the current investigation of the individual rootstocks.

Isolation, production of spore suspension of pathogen and pathogenicity

The pathogen was isolated from the root-zone soil of infected plants on Potato Dextrose Agar (PDA) medium using the tissue isolation method, as described in previous studies (19). The culture was purified using the hyphal tip method. Preliminary identification of the pathogen was carried out based on cultural and microscopic characteristics such as colony colour, mycelial type and the formation of microconidia and macroconidia. Morphological identification was further confirmed with reference to standard descriptions (20). The pure culture of the fungus was sent to the Agharkar Research Institute, Pune, for molecular identification. Based on internal transcribed spacer (ITS4/ITS5) region analysis using species-specific primers, the fungal pathogen isolated from dry root samples was identified as *F. solani*. The pathogen was multiplied on the Potato Dextrose Broth (PDB) for mass culture production and spore suspension was prepared as described in previous studies (10). Soil inoculation was carried out using a suspension prepared from a seven-day-old culture and the spore suspension was adjusted to a concentration of 10^5 spores mL^{-1}. The pathogen suspension (20 mL) was inoculated onto thirteen-month-old Jambheri rootstocks by removing the upper 1-inch soil layer from the pots and mixing the inoculum with the soil. Jambheri seedlings used as controls were treated similarly but received only sterile water. The seedlings were maintained in a greenhouse and disease progression was observed. Seedling mortality was recorded six weeks after inoculation.

Screening of rootstocks using different screening methods

Rootstock susceptibility and tolerance were assessed using different screening methods, as follows:

a) Seedling inoculation method

The seedling inoculation method was performed as described previously (21). Citrus rootstock seedlings aged 11–13 months were inoculated with 20 mL of *F. solani* spore suspension (10^5 spores mL^{-1}) by making 5-6 cm deep and 2 cm diameter holes in the potting mixture around the root zone of respective rootstock seedlings. Pots were watered on a regular basis to maintain adequate moisture for pathogen development. Control pots of each rootstock were maintained without inoculum. Data were recorded 6 weeks after pathogen inoculation. The experiment was laid out in a completely randomized design (CRD) with three replications, each consisting of ten plants. Disease severity index (%) was recorded using the scale described in previous studies (22). The grades of disease severity (%) response are shown in Table 1.

Table 1. Grades of disease severity (%) response

Grade	Disease severity index (%)	Reaction
0	0	Immune (I)
1	1-20	Resistant (R)
3	21-40	Moderately Resistant (MR)
5	41-60	Moderately susceptible (MS)
7	61-80	Susceptible (S)
9	> 81	Highly susceptible (HS)

b) Screening of citrus rootstocks with injury to leaf

Full-expanded leaves were collected from the respective rootstocks. Mycelial bits (4.0 mm) were excised from the periphery of fresh *F. solani* cultures and placed upside down on the top of each leaf previously perforated with a sterilised needle. The experiment was conducted in a CRD with 3 replications, each replication consisting of three leaves. The uninoculated leaves were maintained as control. The inoculated leaves were placed on a moist filter paper fixed in a Petri dish. The Petri dishes were covered with lids and black carbon paper and incubated at 25 ± 2 °C for three days. After incubation, data on length (mm), breadth (mm) and area / size (mm^2) of necrotic lesions were recorded.

c) Screening of citrus rootstocks without injury to leaf

Susceptibility of the respective rootstocks was assessed using the non-injury leaf method following the procedure described in previous studies (23). A total of 20 mL of *F. solani* spore suspension was mixed with 80 mL sterile water in a beaker. Healthy leaves from six-months-old seedlings of respective rootstocks were surface disinfected with 95 % alcohol and used as baits. No injury was inflicted on the leaf surface prior to exposure to the spore suspension. Lesion development was monitored at 48 hr intervals up to 120 hr and lesion size was measured in millimetres. The experiment was conducted in a CRD with three replications.

d) Application of culture filtrate on wounded leaf segments

The phytotoxic effects of *F. solani* culture filtrate on respective rootstocks were evaluated following the method described in earlier studies (24). Leaf segments (20 mm) from eleven-months-old seedlings were excised, placed on moist filter paper in Petri dishes,

punctured with a fine needle and 5 µL of culture filtrate was applied to the wound area. All leaf segments were incubated on moist filter paper in Petri dishes under continuous fluorescent light at 22 ± 2 °C. Sterile water was used as control. The assay consisted of 5 leaf segments per rootstock, with a total of 20 leaf segments. The reactions of leaves were recorded 35 hr after incubation and necrotic spot area was calculated using the following equation:

$A = (\pi/4) \times a \times b$

Where:

A = Necrosis spot area (mm^2), a = radius longer (mm), b = radius shorter (mm) and $\pi = 3.1415$.

Results and Discussion

Sample collection and symptomatology

Seedlings infected with *F. solani* exhibited above-ground symptoms such as sudden wilting of leaves, often occurring without prior yellowing. This was followed by gradual yellowing, occasional leaf drop (defoliation), twig dieback and overall stunted growth. The root system of infected seedlings showed brownish discoloration and numerous decaying roots that broke easily upon handling, along with dark brown to black discoloration of the feeder roots. The root cortex often shed off, exposing the central vascular cylinder (Fig. 1).

The symptomatology recorded in the present study aligns

Fig. 1. Seedling specimens showing dry root rot symptoms.

with earlier studies (8, 10). Affected roots were found to be blackened and decayed, with brown vascular discoloration in the rootstock stem. The disease has been documented on citrus seedlings under both nursery and field conditions (13). Infection by *F. solani* commonly results in rot of the crown and scaffold roots. Rotting of fibrous roots is often associated with reduced canopy size, wilting, defoliation, dieback, sloughing of the root cortex and water-soaked root tissues (25). The symptoms observed in the present study agree with earlier findings, which reported that dry root rot causes detachment of the root cortex and decay of the stele, thereby impairing water and nutrient uptake from the soil. This ultimately results in leaf yellowing and wilting (26, 27).

Isolation and identification of the pathogen

Following purification, the cultural and morphological characters of the pathogen were examined for identification. The pathogen initially exhibited slow growth and then completely colonised the PDA medium within 7–8 days (Fig. 2). The mass multiplication of the pathogen in PDB is shown in (Fig. 3). Colonies on PDA developed abundant white cream-colored aerial mycelium, forming downy colonies with pink centres. The morphological characteristics observed included hyaline, septate mycelium and microconidia that were ovoid, ellipsoid or reniform, oval to kidney-shaped with 1–2 cells but predominantly single-celled (Fig. 4). The macroconidia were hyaline, 3–5 septate, blunt and slightly rounded with beaked

Fig. 2. Pure culture of *Fusarium solani*.

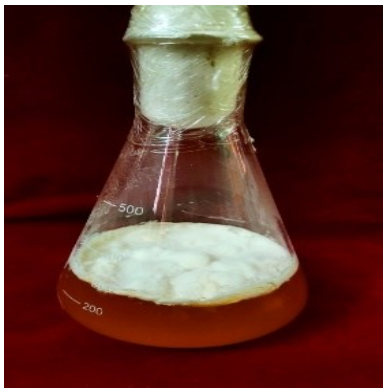

Fig. 3. Broth of pure culture of *Fusarium solani*.

apical cells (Fig. 5). Based on these observed characteristics, the fungal isolate obtained from dry root rot-affected seedlings was identified as *F. solani*. The culture was maintained in a biological oxygen demand incubator at 25 ± 2 °C for further studies. The findings of the present study agree with earlier reports (10, 28, 29). The purified pathogen culture was sent to the Agharkar Research Institute, Pune for molecular identification. Based on internal transcribed spacer (ITS4/ITS5) region analysis using species -specific primers, the pathogen isolated from dry root samples was identified as *F. solani*. The sequence was deposited in the NCBI GenBank under accession number PX286120. The BLAST analysis of the obtained ITS sequences against the NCBI GenBank database

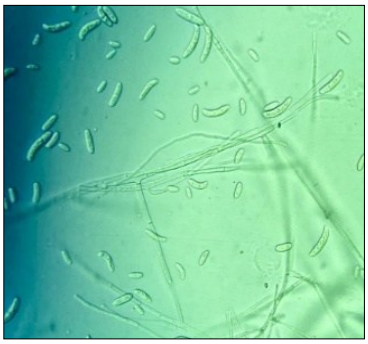

Fig. 4. Microconidia and septate hyaline mycelium of *Fusarium solani*.

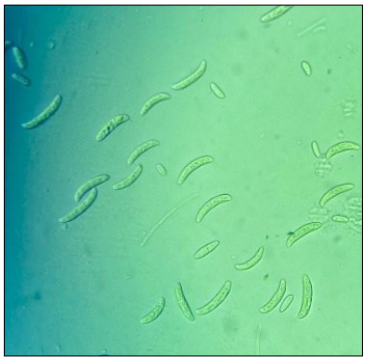

Fig. 5. Macroconidia of *Fusarium solani*.

revealed 100 % similarity with *F. solani* (GenBank accession number MK075013.1). The sequences were subsequently subjected to multiple sequence alignment using the ClustalW algorithm implemented in MEGA version 12 with default parameters for further analysis.

Numerous soil-borne infections, including *Fusarium*, *Rhizoctonia* and *Diplodia* species, infect citrus and cause root rot disease in both nurseries and main fields, resulting in reduced plant life and productivity (30). Among these, *F. solani* is a major causal agent of dry root rot in citrus seedlings as well as in mature plants.

Pathogenicity test

The results of the pathogenicity test (Table 2) confirmed the ability of *F. solani* to induce dry root rot. In Jambheri rootstock pots inoculated with *F. solani*, seedling mortality rate of 73.33 % was recorded six weeks after inoculation, whereas no mortality was observed in the control treatment. Symptom development in inoculated Jambheri seedlings began shortly after inoculation. Affected seedlings exhibited yellowing of leaves and signs of general decline. The most prominent symptoms were observed in the root system, including brown discoloration and extensive root decay. Additional symptoms included leaf drying and wilting. In contrast, control seedlings remained healthy with no visible root or foliar symptoms.

These results demonstrate that *F. solani* is pathogenic to Jambheri rootstock, inducing dry root rot in inoculated pots. Similar findings were reported in earlier studies, where the pathogenicity of *F. solani, F. proliferatum* and *F. sambucinum* has been tested on potted trees and found that they can cause disease, particularly under conditions of water stress, excessive fertilisation, or root injury (31). Typical symptoms such as interveinal chlorosis, wilting, defoliation, poor vigour, brownish discoloration and extensive root decay have also been reported in seedlings inoculated with *F. solani* (25). These symptoms may be caused by the naphthazarin toxins produced during *F. solani* infection. The pathogenicity of *F. solani* has been further confirmed in sour orange rootstock by inoculating seedlings with conidial suspensions using the standard root-dip method (10). Infected plants exhibited stunted growth and characteristic dry rot symptoms and the pathogen was successfully re-isolated from symptomatic root and stem tissues.

Table 2. Pathogenicity test

Sl. No.	Particulars	No. of inoculated pots	Seedling mortality	Mortality (%)
1	*Fusarium solani*	30	22	73.33%
2	Control (Un-inoculated)	30	00	---

Screening of rootstocks using different screening methods

a) Seedling inoculation method

The effect of seedling inoculation and disease reaction of different rootstocks was studied under greenhouse conditions and percent mortality due to pathogen infection was recorded. Significant differences in seedling mortality were observed among the rootstocks (Table 3, Fig. 6). Rangpur lime recorded the lowest mortality (20.00 %), followed by NRCC-2 and Alemow (23.33 % each). In contrast, rough lemon (Jambheri) exhibited the highest mortality (60.67 %), indicating high susceptibility to root rot.

All tested rootstocks developed infection under artificial

Table 3. Screening by seedling inoculation method

Sl. No.	Rootstocks	% Mortality of seedlings	Reaction
T_1	NRCC-1	26.67 (31.00)*	Moderately Resistant
T_2	NRCC-2	23.33 (28.78)	Moderately Resistant
T_3	NRCC-3	26.67 (31.00)	Moderately Resistant
T_4	NRCC-4	33.33 (35.22)	Moderately Resistant
T_5	CRH-12	33.33 (35.22)	Moderately Resistant
T_6	Rangpur lime	20.00 (26.07)	Resistant
T_7	Alemow	23.33 (28.78)	Moderately Resistant
T_8	Jambheri	60.67 (50.85)	Susceptible
	SE (m) ±	2.68	
	CD (P=0.05)	8.04	

*Figure in parentheses are arc sine values

Fig. 6. Inoculated pots showing dry root rot symptoms following seedling inoculation method: (a) NRCC-1; (b) NRCC-2; (c) NRCC-3, (d) NRCC-4; (e) CRH-12; (f) Alemow; (g) R. lime and (h) Jambheri. Left: inoculated pots; Right: control pots.

inoculation conditions. Rangpur lime exhibited a resistant response, while the remaining rootstocks were moderately resistant, except rough lemon (Jambheri), which was categorised as susceptible. These results are consistent with earlier reports indicating that rough lemon (Jambheri) is highly susceptible to dry root rot (13, 25). Similar susceptible rootstocks have also been documented for Changsha mandarin, Cleopatra mandarin and rough lemon (20). Rangpur lime has also been reported to be more resistant than rough lemon strains (15).

In the present study, the evaluated rootstocks exhibited varying levels of response to *Fusarium solani* infection. This differential resistance may be attributed to the absence of specific targets for pathogenic effectors, thereby hindering hyphal colonisation (32). However, further studies are required to elucidate the underlying mechanisms responsible for variation in resistance among citrus rootstocks to *Fusarium*-induced root rot. Utilising resistant rootstocks serves as an effective strategy to minimise losses caused by dry root rot. Resistance to fungal pathogens ensures that the feeder and crown roots are not extensively colonised by the pathogen (33).

b) Screening of citrus rootstocks with injury to leaf

After 72 hr of incubation, lesion size varied significantly among rootstocks (Table 4, Fig. 7 & 8). Rangpur lime exhibited the smallest lesion size (14.45 mm), followed by CRH 12 (16.06 mm), whereas Jambheri showed the largest lesion size (24.43 mm), followed by Alemow (21.29 mm). Intermediate lesion sizes were observed in other rootstocks, namely NRCC 1 (17.22 mm), NRCC 3 (18.23 mm) and NRCC 2 (19.89 mm).

The tested rootstocks exhibited variable lesion sizes following pathogen inoculation using the leaf injury method. Similar observations have been previously reported, wherein *Phytophthora* tolerance was assessed in 22 *Citrus* and 8 *Poncirus* accessions by measuring brown rot lesion development on leaves after inoculation (34). The study reported that lesion size varied depending on the genotype and that resistance levels inferred from leaf lesion assays corresponded well with resistance responses observed under artificial stem inoculation. Furthermore, variation in lesion length ranging from 5.9 to 45.0 mm and 2.8 to 46.9 mm was reported in infected leaves of parents and hybrids, respectively, caused by *Phytophthora* (35).

Table 4. Screening of citrus rootstocks with injury to leaf

Sl. No.	Rootstocks	Leaf lesion mean (mm)
T_1	NRCC-1	17.22
T_2	NRCC-2	19.89
T_3	NRCC-3	18.23
T_4	NRCC-4	22.94
T_5	CRH-12	16.06
T_6	Rangpur lime	14.45
T_7	Alemow	21.29
T_8	Jambheri	24.43
	SE (m) ±	0.75
	CD (P=0.01)	3.12

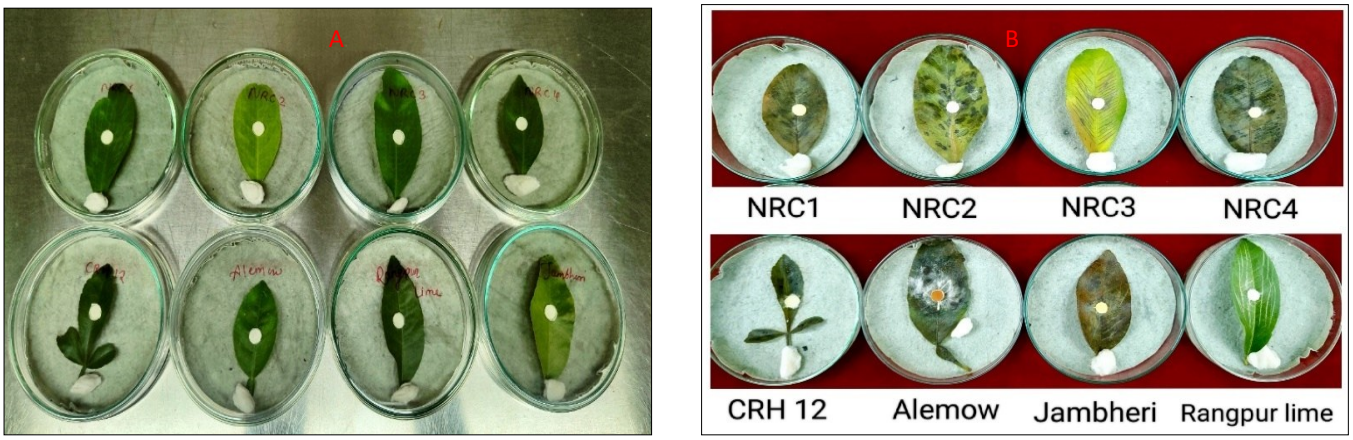

Fig. 7. Screening of citrus rootstocks with injury to leaf. (A) Leaves incubated at 25 ± 2 °C for three days; (B) Necrotic lesions observed on leaves (right): (a) NRCC-1; (b) NRCC-2; (c) NRCC-3; (d) NRCC-4; (e) CRH-12; (f) Alemow; (g) Jambheri and (h) Rangpur lime.

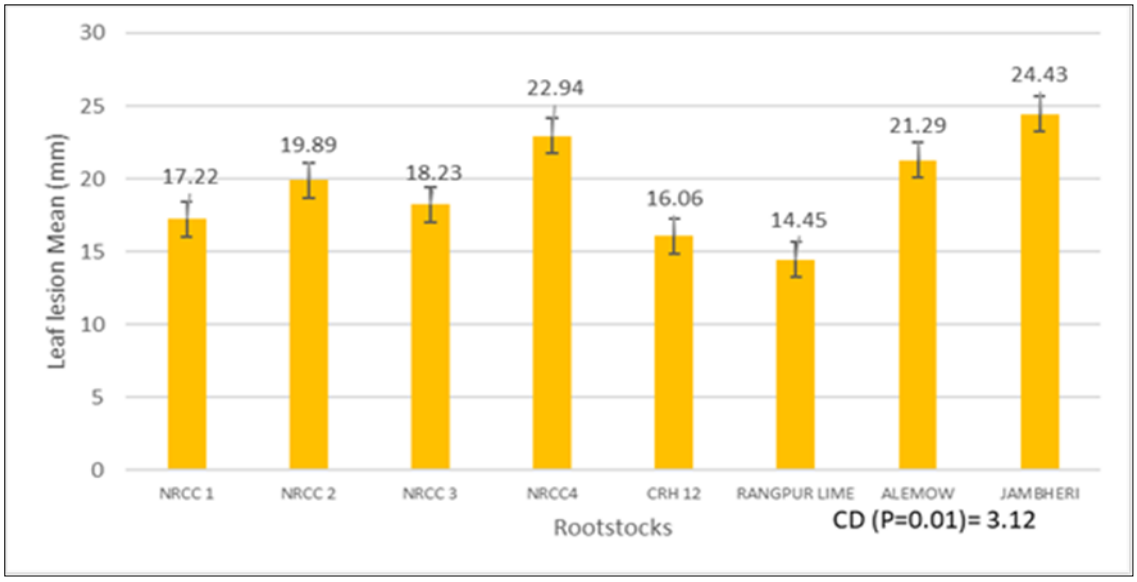

Fig. 8. Mean leaf lesion size of citrus rootstocks with injury to leaf method.

The present findings are also in agreement with earlier screening of eleven citrus rootstocks using the leaf injury method, which revealed significant variation in lesion size among rootstocks (23). Sour orange exhibited the smallest lesion size (1.5 cm), whereas rough lemon (2.5 cm) and Cleopatra (2.7 cm) developed the largest lesions after 48 hr of incubation. Other rootstocks such as Pectinifera (1.6 cm) and Troyer (1.8 cm) showed smaller lesions, while Rangpur lime (2.1 cm) and Volkameriana (2.2 cm) exhibited moderate lesion sizes.

The present investigation revealed that the leaf injury method produced rapid and comparable results to the young seedling inoculation method and can therefore be used as an alternative approach for screening citrus rootstocks. However, more in-depth research is required to prove tolerance or resistance reactions.

c) Screening of citrus rootstocks without injury to leaf

The effect of the no-injury leaf method on the tested rootstocks was evaluated and the results are presented (Table 5, Fig. 9 & 10). Variation in lesion size was observed among the tested rootstocks. The data revealed that the minimum lesion size was recorded in Rangpur lime (11.95 mm), followed by NRCC-3 (13.35 mm), NRCC-1 (14.56 mm) and CRH-12 (15.91 mm). In contrast, the maximum lesion size was observed in NRCC-4 (20.43 mm), followed by NRCC-2 (19.36 mm), Jambheri (19.33 mm) and Alemow (19.13 mm) after 72 hr of incubation.

In screening of citrus rootstocks without injury to leaf method, Rangpur lime exhibited the smallest lesion size, followed by NRCC-3, NRCC-1 and CRH-12. In contrast, NRCC-4 showed the largest lesion size. A similar pattern of responses was observed when rootstocks were screened using the leaf injury method, indicating that the pathogen can infect the respective rootstocks both through mechanical injury and natural infection (non-injury) routes. This suggests a lack of effective defense response in the rootstocks against the pathogen. Therefore, *Fusarium*-induced disease remains a significant concern for nursery growers.

Table 5. Screening of citrus rootstocks without injury to leaf

Sl. No.	Rootstocks	Leaf lesion mean (mm)
T_1	NRCC-1	14.56
T_2	NRCC-2	19.36
T_3	NRCC-3	13.35
T_4	NRCC-4	20.43
T_5	CRH-12	15.91
T_6	Rangpur lime	11.95
T_7	Alemow	19.13
T_8	Jambheri	19.33
	SE (m) ±	0.91
	CD (P=0.01)	3.79

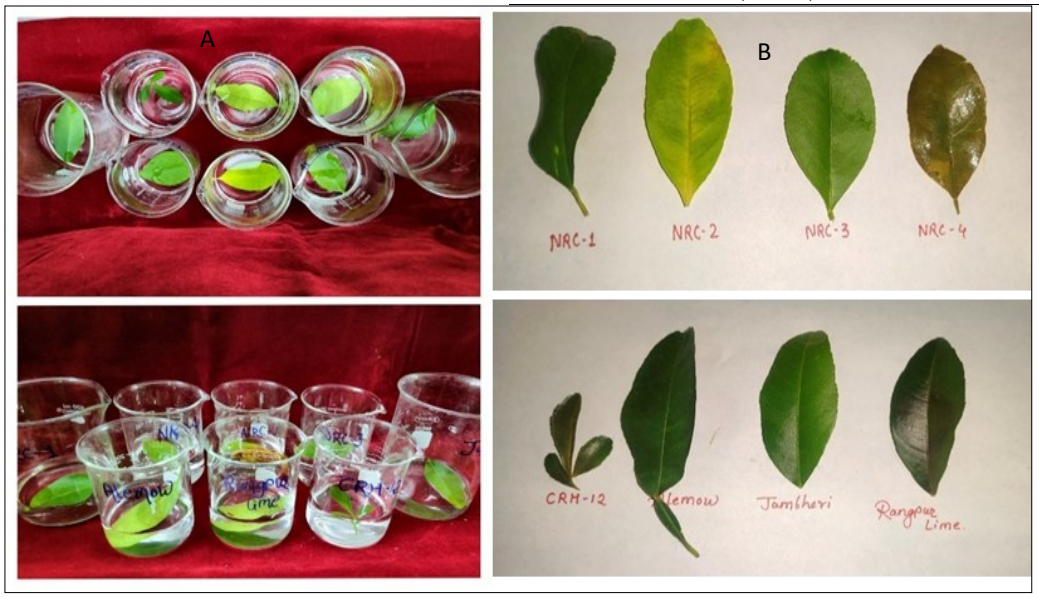

Fig. 9. Screening of citrus rootstocks without leaf injury (leaf baiting method). (A) Leaf baiting with spore suspension; (B) lesion size observed on leaves.

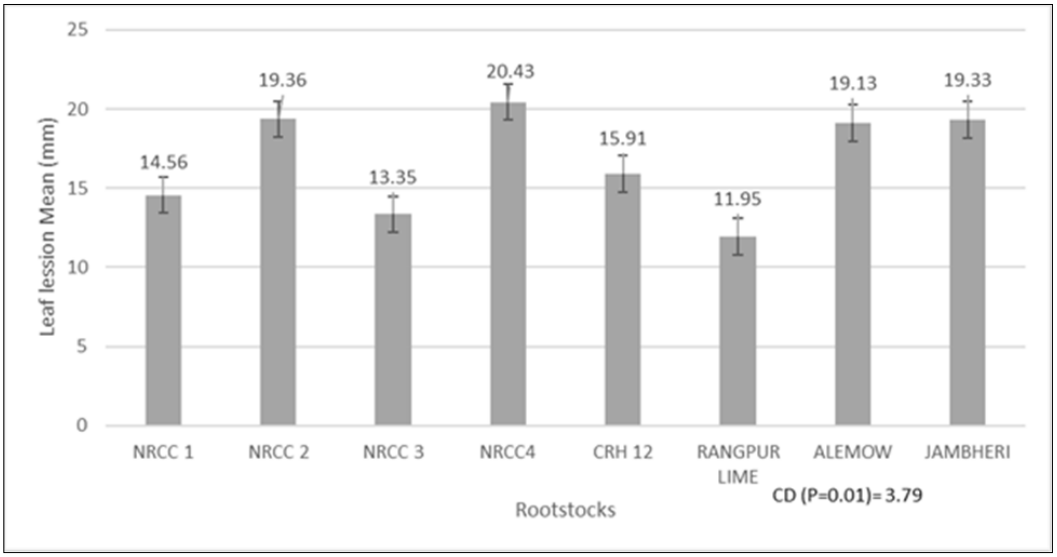

Fig. 10. Mean leaf lesion size of citrus rootstocks without injury to leaf method

Among the two methods evaluated, leaf baiting with injury proved to be more effective than the non-injury method, as indicated by the larger lesion sizes observed. The present findings are consistent with earlier reports wherein various screening methods were employed to evaluate citrus rootstocks for tolerance or resistance to *Phytophthora parasitica* (23). They reported that leaf baiting with injury was more effective than the non-injury method, as lesion size comparisons were more precise in this approach. They recommended the leaf baiting technique as a reliable screening method for assessing rootstock reactions to pathogens. Similarly, variable levels of susceptibility were reported among citrus rootstocks screened for resistance to root rot and gummosis caused by *Phytophthora parasitica* and *F. semitectum* (33).

d) Applying the culture filtrate on wounded leaf segments

The effect of *F. solani* culture filtrate on wounded leaf segments on the growth of the different rootstocks was investigated *in vitro* and the resulting data was processed and presented (Table 6, Fig. 11 & 12). The culture filtrate induced necrotic spots on citrus leaf segments of all tested rootstocks and the size of necrotic spots was measured after 72 hr. The size of the necrotic spot varied significantly among rootstocks. Rangpur lime exhibited a lower degree of necrotic spot area (1.01 cm^2) following treatment with *F. solani* culture filtrate. Comparatively lower necrotic spot areas were also recorded in Alemow (1.22 cm^2), CRH-12 (1.24 cm^2) and NRCC-4 (1.27 cm^2). In contrast, NRCC-1, Jambheri, NRCC-2 and NRCC-3 were the most sensitive citrus rootstocks, developing larger necrotic areas of 1.69, 1.49, 1.43 and 1.35 cm^2, respectively.

The observed variation in the severity of necrotic spots induced by the culture filtrate of *F. solani* on the tested rootstocks suggests that the culture filtrate contains specific phytotoxic metabolites or toxins capable of inducing necrosis on wounded leaf segments. Rangpur lime exhibited a low degree of necrotic spotting, while NRCC-1 and Jambheri showed a higher intensity of necrosis, indicating differential responses of the rootstocks to the pathogen.

These findings are consistent with earlier findings, wherein the culture filtrate of *F. subglutinans*, the causal agent of pineapple leaf spot, induced necrotic lesions on pineapple leaves after 35 hr of treatment, with susceptible cultivars developing larger necrotic areas than resistant ones (36). Similarly, phytotoxic culture filtrate of *F. solani* caused more severe symptoms in susceptible cultivars than in resistant ones when applied to wounded leaves, highlighting the differential sensitivity among cultivars (37). Overall, the present assays demonstrate the effectiveness of culture filtrate-based bioassays for *in vitro* screening of citrus rootstocks and highlight their potential application in assessing resistance or tolerance to *F. solani*.

Table 6. Applying the culture filtrate on wounded leaf segments

Sl. No.	Rootstocks	Leaf lesion (cm^2) mean
T_1	NRCC-1	1.69
T_2	NRCC-2	1.43
T_3	NRCC-3	1.35
T_4	NRCC-4	1.27
T_5	CRH-12	1.24
T_6	Rangpur lime	1.01
T_7	Alemow	1.22
T_8	Jambheri	1.49
	SE (m) ±	0.04
	CD (P=0.01)	0.18

Fig. 11. Application of pathogen culture filtrate on wounded leaf segments. (A) Culture filtrate (5 µL) applied to the wounded area; (B) comparison between control and inoculated leaf segments.

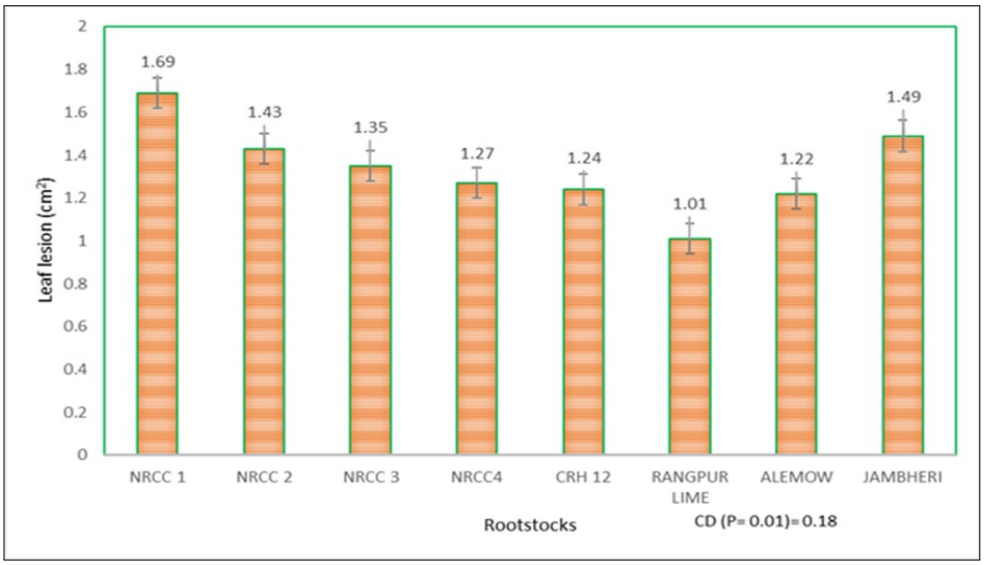

Fig. 12. Mean leaf lesion area of citrus rootstocks following application of culture filtrate on wounded leaf segments.

Conclusion

Among the citrus rootstocks evaluated, Rangpur lime was identified as the most tolerant to the dry root rot pathogen *Fusarium solani* when compared with the other tested rootstocks. The results also indicate that the leaf injury method can be effectively used for *in vitro* screening of citrus rootstocks against *F. solani*, due to its rapid and reliable assessment of disease response.

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Authors' contributions

LYP carried out the experimental work and performed data analysis. YVI designed and supervised the work related to rootstock screening. RSC contributed to data interpretation and resource management. VPS and AMB contributed to review and editing of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that there is no conflict of interest.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used ChatGPT to make the research paper easier to understand and more accurate. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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