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GENOTYPIC REACTION OF CHICKPEA TO *Fusarium oxysporum* AND SEED SANITARY QUALITY

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Abstract

This research investigated the reaction of chickpea genotypes to *Fusarium oxysporum* CML 2878, seed health quality, and seed treatment with Carbendazim + Thiram fungicide. The roots of 15-day-old chickpea seedlings were injured, immersed in fungal suspension (4.5 x 10^7 conidia/mL), and transplanted. Thirty days later, *F. oxysporum* incidence in the genotypes, pathogenicity, and fresh mass and length of the root and shoot were evaluated. The conducted seed health test followed the incubation methodology on a paper substrate. The seed health and sand emergence tests used a factorial arrangement with two seed treatments (treated with fungicide and untreated) and nine genotypes. Total emergence and the number of normal and abnormal plants were evaluated. All genotypes were susceptible to *F. oxysporum* CML 2878, thus yellowing and browning the leaves and reducing root development. The primary fungi associated with the seeds of the studied genotypes were *Aspergillus* sp., *Fusarium* sp., *Rhizopus* sp., and *Penicillium* sp. The fungicide was highly efficient in fungal control but reduced emergence, weight, seedling height, and the number of normal seedlings in the genotypes. This study demonstrated that the evaluated genotypes were susceptible to *F. oxysporum* CML 2878 and *Fusarium* sp., spread by seeds. Although fungicidal control was efficient for *Fusarium*, it may interfere with chickpea germination and vigor.

Keywords: *Cicer arietinum*. Fungicide. Fusariosis. Pathogenicity. Seeds.

1. Introduction

Cicer arietinum is a high-protein legume used in human nutrition that grows well in dry climate regions and is currently spreading worldwide. The Asian continent is the largest chickpea producer and consumer, with India standing out as the top chickpea-producing country, with 11 million tons in 2020 (FAOSTAT 2022).

Although chickpea-planted commercial areas were unknown in Brazil until 2014, and the country imported almost the entirety of grains consumed in the national territory, this crop is expanding into some Brazilian regions. Considering the establishment of cultivation areas in the states of Mato Grosso and Goiás, the chickpea production intercropped with soybean in Brazil in 2018 amounted to approximately

8,000 ha (Nascimento and Silva 2019). However, the prevailing tropical climate in the country challenges production as it favors the development of fungi such as *Fusarium* spp. and pest caterpillars.

Fusarium sp. is the primary fungus that affects chickpeas worldwide (Bekele et al. 2021). The *Fusarium* complex shows various hosts and easily adapts to create a specific pathogen-host relationship. This condition hinders the action of control products, as this fungus survives in the soil and propagates in several ways. *Fusarium* may spread into new areas through contaminated soil, water, host plants, crop remains, and seeds. Once in the soil, *Fusarium* sp. infects the host plant through natural or unnatural root openings (wounds). Inside the plant, the fungus colonizes the conducting vessels, hindering the uptake and transport of water and solutes and causing symptoms such as plant wilt, yellowing, rot, and death (Cota-Barreras et al. 2023).

The Brazilian Agricultural Research Company (Embrapa Vegetables) in Brasilia, Federal District, Brazil, has been studying chickpeas to develop new genotypes with good productivity and adaptability to Brazilian soils and climates. Hence, studies with *Fusarium* sp. isolates known to be pathogenic to chickpeas and found in Brazilian soils (Azevedo et al. 2017) are critical to selecting genotypes resistant to *Fusarium.* However, studies on genotype reaction to *Fusarium* sp. are scarce in Brazil, potentially complicating crop management and diffusion. Furthermore, using high-quality seeds is crucial for increasing crop yield. The sanitary factor is highly relevant among quality attributes because seeds disperse pathogens. From this perspective, seed health tests allow the analysis of whether seeds propagate *Fusarium* and whether conventional seed treatment methods are efficient control strategies. Emergence and seedling development tests also contribute to further clarifying the pathogen-host relationship and the process of genotype selection for future genetic improvement studies.

Therefore, this study evaluated the reaction of chickpea genotypes to the *F. oxysporum* isolate CML 2878 and seed sanitary quality, emergence, and fungal control with Carbendazim + Thiram fungicide.

2. Material and Methods

Reaction of chickpea genotypes to *Fusarium oxysporum*

The Brazilian Agricultural Research Company (EMBRAPA) provided seven chickpea genotypes (BRS Kalifa, BRS Toro, BRS Aleppo, BRS Cristalino, Cicero, Jamu, and Cicero1). The harvested seeds of each genotype were placed in paper bags and stored in a cold chamber at 10 ± 2°C and 50% relative humidity for six months until the experiments. Cicero1 refers to seeds of the Cicero chickpea cultivar cultivated at the Federal University of Minas Gerais (UFMG), Montes Claros, MG, Brazil, and stored for 24 months to determine seed sanitary quality and vigor. The experiment used the *F. oxysporum* isolate CML 2878 stored at the fungal collection of Lavras (CML) of the Federal University of Lavras (UFLA), MG, Brazil (http://www.dfp.ufla.br/cml), which is pathogenic and aggressive to the Cicero chickpea cultivar (Azevedo et al. 2017). This cultivar represented a susceptible control. At the Laboratory of Plant Pathology Research (LPF) of the Federal University of Minas Gerais (UFMG), the *F. oxysporum* isolate CML 2878 was cultured in 9-cm wide Petri dishes containing a malt extract culture medium. The dishes were stored in a BOD incubator at 25°C with a photoperiod of 12 hours of fluorescent white light and 12 hours of darkness for 15 days, aiming at sporulation. After this period, the fungal culture received 20 mL of distilled and sterilized water, and a Drigalski spatula spread the material to obtain the conidial suspension. Then, the suspension was filtered through gauze and had its concentration adjusted to 4.5 x 10^7 conidia/mL using a Neubauer chamber and an optical microscope at a 40x magnification.

The plants of this experiment came from chickpea seeds sown in polyethylene trays containing a commercial substrate based on coconut fiber (Carolina soil®). After sowing, the trays remained in a plant nursery with daily irrigation. After 15 days, the seedlings were removed, and their roots were washed with sterilized distilled water. Then, the root tips of the mentioned genotypes were cut with scissors to produce wounds and favor infection by *F. oxysporum* CML 2878. Next, the root system of each genotype was immersed in 250 mL of a 4.5 x 10^7 conidia/mL suspension for 20 minutes. The seedlings were then transplanted to 1L pots containing 900 g of soil and sand substrate at a proportion of 1:2 and previously sterilized at 121°C for one hour. The pots remained in the plant nursery over benches for 30 days with daily

irrigation. The experiment had a completely randomized design (CRD) with four replications, considering one treatment per genotype.

After 30 days, the plants were removed from the soil, preserving their root system, and the material was sent to the LPF laboratory to separate the shoot from the root and determine the root system's fresh mass and length. The fresh mass was obtained by weighing the material in an analytical balance accurate to 0.001 g. The root system length was measured from the beginning of the primary roots to the tip of the longest root using a metric tape.

The genotype reaction evaluation considered all plants with fusariosis and root infection symptoms infected and susceptible to the *F. oxysporum* isolate CML 2878. The indirect method of fungal isolation determined the infected plant index. Hence, the root surface was disinfected with 70% alcohol and 0.5% sodium hypochlorite (NaClO) for one minute, followed by a triple wash with sterilized distilled water. Then, the roots were dried, underwent a longitudinal section in the primary root using a sterile scalpel, and deposited on a potato-dextrose-agar (PDA) culture medium in a 9-cm wide Petri dish for *F. oxysporum* CML 2878 re-isolation. Finally, the pathogen was recovered to complete Koch's postulates and confirm pathogenicity.

Seed health test

The seed health test used the previous chickpea genotypes (BRS Kalifa, BRS Toro, BRS Aleppo, BRS Cristalino, Cicero, Jamu, and Cicero1) and two others provided by the Olericulture Laboratory of ICA/UFMG (bic4 and 3bb4). The seeds of this experiment were stored in the dark at ± 7°C. The blotter test proposed in the Seed Health Analysis Manual (Brasil 2009b) was adapted to five replications of 25 seeds.

Fungicide efficiency in seed treatment was evaluated using a commercial product based on Carbendazim (150 g/L) + Thiram (350 g/L) at 200 mL per 100 kg of seeds. The seeds were treated with fungicide in Erlenmeyer flasks, adding volumes to the weight of 100 g of seeds for each genotype according to the recommended dose. The containers were shaken until the fungicide completely covered the seeds and allowed fungicide homogenization on the seeds. Then, the seeds were shade-dried for approximately 20 minutes in Petri dishes. After drying, the seeds were deposited in germination boxes containing three layers of germination paper moistened with sterilized distilled water at 2.5 times the weight of the dry substrate.

The treatments consisted of seeds of the nine genotypes treated with fungicide, and the controls included seeds disinfected with 0.5% sodium hypochlorite for one minute. The experiment used a double factorial arrangement with two seed treatments (treated with Carbendazim + Thiram and disinfected with 0.5% sodium hypochlorite) and nine genotypes. The treatments with four replications of 25 seeds remained in a BOD incubator at 25°C, with a photoperiod of 12 hours of fluorescent white light and 12 hours of darkness in a CRD for 15 days. During incubation, the paper was moistened again to prevent drying.

The fungal incidence evaluations started on the third day of incubation and were performed daily for 15 days. Fungal identification used a stereo microscope, and it was based on morphological features (Barnett and Hunter 1998). Whenever necessary, microscope slides were prepared with the fungal structure in synthetic nutrient agar (SNA) and PDA culture media for identification using a light microscope.

The percentages of incidence and fungal genera in treated seeds were calculated after 15 days of fungal incubation. The contaminated seed rate considered the ratio of the number of seeds with fungi to the number of evaluated seeds multiplied by 100. In turn, the ratio of the number of seeds with fungi of a given genus to the total number of contaminated seeds determined the percentage of fungal genera.

Emergence test

The emergence test in sand included the nine genotypes from the previous experiment and the seed treatment based on Carbendazim + Thiram. Accordingly, the experiment was developed in a double factorial arrangement with nine genotypes and two seed treatments (treated with fungicide and untreated). The seeds were distributed in polyethylene trays containing a washed sand substrate, with four

replications of 50 seeds in each treatment. Then, the trays remained in the plant nursery in a CRD with daily irrigation for 15 days. Substrate irrigation continued until achieving 60% of field capacity.

Next, the total emergence and the percentage of normal and abnormal seedlings were evaluated following the Seed Analysis Rules (Brasil 2009a). Seedlings containing all essential structures that might result in a healthy plant were normal, whereas seedlings incapable of developing a standard plant were abnormal. The abnormal seedlings were classified as damaged and deformed according to structural damage. Therefore, damaged plants did not have all essential structures or had them under inadequate development conditions. Conversely, deformed seedlings showed malformation in primary structures, e.g., disproportionality.

Evaluation of plants from secondary infection in chickpea seeds

This experiment evaluated plants from seeds of the previous lots and genotypes used in item 4.2.2. The seeds were disinfected with NaClO (0.5%) for one minute, then sown on a commercial substrate based on coconut fiber (Carolina soil®) in polyethylene trays. After 15 days, the seedlings were transplanted to 1L pots containing soil and sand substrate (1:2). Each treatment had four replications, comprised a pot with one plant, had a CRD distribution, and remained in benches in the plant nursery. Substrate irrigation occurred daily for 45 days until 60% of field capacity, based on weighing the pots with the plants and directly irrigating the soil.

After 45 days, the plants were removed from the pots, preserving their root system. In the laboratory, the roots were washed and separated from the shoot to determine their fresh weight in a precision balance (0.001 g) and length (cm) with a metric tape. After that, the root system was disinfected by immersion in 70% alcohol and 0.5% NaClO for one minute and three times in sterilized distilled water for one minute. Then, a sterile scalpel cut a longitudinal section in the primary root, which was deposited on a 9-mm wide Petri dish containing a PDA culture medium. Subsequently, the dishes were sent to a BOD incubator, where they remained for seven days at 25°C with a photoperiod of 12 hours of fluorescent light and 12 hours of darkness. The fungi found after this period were streaked on Petri dishes containing the PDA medium and dishes containing the SNA medium and maintained in a BOD incubator for 15 days at 25C°C with 12 hours of fluorescent light and 12 hours of darkness. After incubation, fungal colonies were identified morphologically according to the color of the colonies in the PDA medium and the shape of micro- and macroconidia, chlamydospores, and microconidia aggregated in false heads on short and long monophialides in the SNA medium. The material was observed in microscope slides (Leslie and Summerell 2006).

Statistical analysis

The data underwent analysis of variance, and the Scott-Knott test compared the means at 5% significance using the R Studio statistical software, version 3.2.5.

3. Results

Reaction of chickpea genotypes to *Fusarium oxysporum*

All evaluated genotypes were susceptible to *F. oxysporum* CML 2878 and had shoot yellowing symptoms, leaf wilt, and vascular discoloration. The fungal isolate was confirmed after re-isolating *F. oxysporum* CML 2878 from the root systems of the studied genotypes. Infection by *F. oxysporum* CML 2878 in the genotypes caused differences in plant development, expressed by shoot length and root weight (Table 1). The plants showed, on average, a root system weight of 0.91 g and a shoot weight of 0.17 g.

Seed health test

All studied genotypes showed fungi in chickpea seeds. The primary genera in the seeds were *Aspergillus* sp., *Fusarium* sp., *Rhizopus* sp., and *Penicillium* sp., with 57, 29, 7, and 3% incidence, respectively (Table 2). All studied genotypes showed fungi of the *Fusarium* sp. and *Aspergillus* sp. genera (Table 2). The seeds treated with fungicide did not show fungal growth.

Table 1. Length and fresh mass of chickpea genotypes inoculated with *Fusarium oxysporum* CML 2878 45 days after inoculation.

	Length (cm)		Fresh mass (g)	
Genotype	**Shoot**	**Root**	**Shoot**	**Root**
Jamu	37.33[a]	4.00[a]	2.16[a]	0.08[e]
Cicero	37.35[a]	5.73[a]	1.52[b]	0.04[e]
Cicero1	32.75[a]	5.12[a]	0.44[c]	0.04[e]
BRS Kalifa	30.90[b]	5.45[a]	1.32[b]	0.63[a]
BRS Cristalino	30.50[b]	5.00[a]	1.64[b]	0.22[c]
BRS Aleppo	28.00[b]	6.25[a]	0.61[c]	0.43[b]
BRS Toro	25.08[b]	6.25[a]	0.58[c]	0.15[d]
CV (%)	6.19	10.6	13.97	18.73

Means followed by the same letter in the column did not differ statistically by the Scott-Knott test at 5%.

The fungal mycelium of *Fusarium* sp. in chickpea seeds (cotyledon and radicle) showed an initial white color, changing to pink in some colonies during development (Figures 1C and D). The pure culture of fungal isolates was also pink in the PDA medium (Figure E). Colony cultivation in the SNA medium revealed cylindrical or oval microconidia and oval falciform macroconidia containing three to five septa (Figures 1F and G), like the descriptions of the *Fusarium* genus available in the literature (Barnett and Hunter, 1998).

Table 2. Incidence of fungi associated with seeds of different chickpea genotypes.

Genera	**Genotypes**									**Incidence (%)**
	Cicero	**BRS Toro**	**BRS Aleppo**	**3bb4**	**Jamu**	**Cicero1**	**bic4**	**BRS Cristalino**	**BRS Kalifa**	
Aspergillus sp.	12.87	10.05	6.7	7.58	3.53	3.7	8.47	2.47	1.76	57.14
Penicillium sp.	0	0.35	0.18	1.59	0.35	0.53	0	0	0.88	3.88
Rhizopus sp.	0.88	0.88	1.76	2.29	0.35	0.53	0.18	0	0.18	7.05
Fusarium sp.	0.35	4.41	3.53	1.06	4.94	5.11	0.88	3.7	2.65	29.63
Fungi genotype[1]	17.11	15.87	12.52	12.7	10.23	9.88	9.52	6.53	5.82	
Contaminated seeds	97[a]	90[a]	85[a]	72[a]	58[b]	56[b]	54[b]	36[b]	33[b]	
CV = 31%										

Means followed by the same letter in the row did not differ statistically by the Scott-Knott test at 5%. [1]Fungi incidence in chickpea genotypes.

Seedling emergence test

The tests showed that seed treatment reduced seedling emergence by 57% and increased the number of abnormal plants by 68.06% in the tested genotypes. Seed treatment with Carbendazim + Thiram, although efficient in seed protection against fungi, drastically reduced the emergence and growth of chickpea seedlings in the evaluated genotypes (Table 3). Furthermore, the fungicide reduced the length and fresh mass of the shoot and root of seedlings of all genotypes except for the fresh root mass of BRS Aleppo (Table 4).

The absence of seed treatment caused a higher emergence, especially in genotypes BRS Cristalino and BRS Kalifa, which showed the highest emergence rates, with 93 and 92%, respectively. The worst test performances occurred in genotypes BRS Toro and Cicero1. These genotypes showed the lowest emergence (43 and 40%, respectively) without seed treatment and the lowest values for treated seeds,

with BRS Toro showing no emergence at all. Seed treatment did not allow the emergence of normal seedlings in genotypes Cicero and BRS Toro, highlighting their higher susceptibility to the phytotoxic effect.

Genotypes 3bb4, BRS Cristalino, and bic4 showed the highest seedling germination percentages with treated seeds, and genotype bic4 stood out with the lowest abnormal seedling rate. However, the performance was well below that of untreated seeds. Genotypes 3bb4 and bic4 might have performed otherwise because they belong to the 'Desi' group, unlike the others from the 'Kabuli' group. The seeds of the Desi group are harder, and their angular shape might have reduced the contact surface with the product. Also, these seeds have a thicker cover, which might hinder product translocation.

Untreated seeds generated more damaged seedlings than deformed ones, as their development was compromised by the absence of one of the essential plant structures, e.g., the roots (Figure 1H). Treated seeds generated a similar proportion of seedlings without some essential structure (51%) and seedlings with structural deformities (38%) (Figure 1 I).

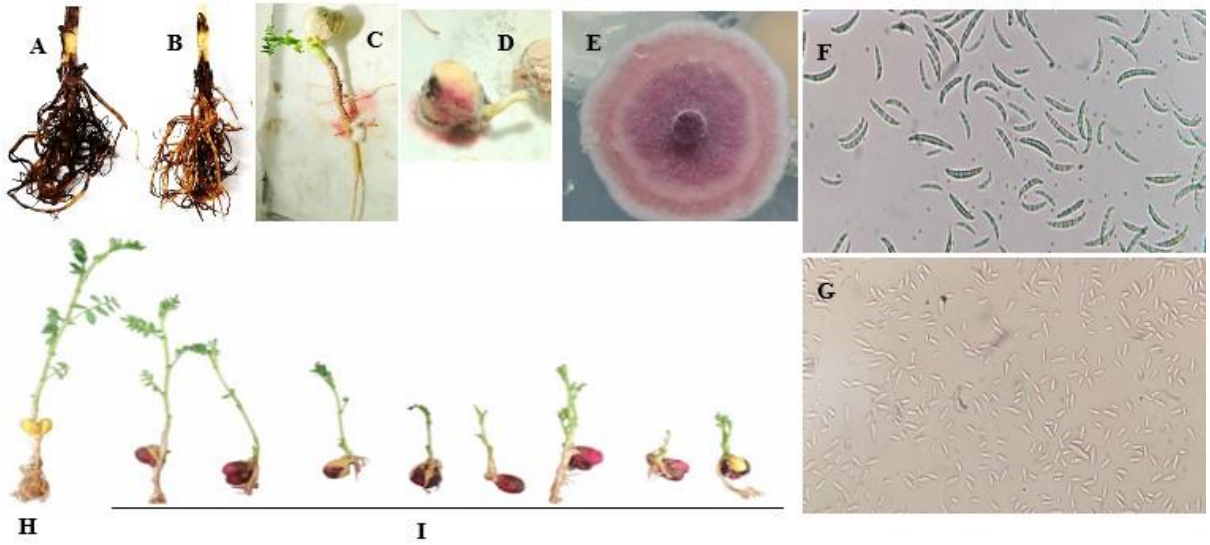

Figure 1 - Chickpea genotypes BRS Cristalino (A) and BRS Kalifa (B) with root necrosis caused by *F. oxysporum* CML 2878 30 days after inoculation. *Fusarium* sp. in the radicle (C) and cotyledon (D) of chickpea seeds after germination. Pure culture of *Fusarium* sp. with a pink color (E), macroconidia (F), and microconidia (G) of *Fusarium* sp. Healthy chickpea seedlings (H) and seedlings with abnormal development (I) after Carbendazim + Thiram fungicide treatment.

Table 3. Percentage of seedlings emerged in the last count, abnormal seedlings, deformed seedlings, and damaged seedlings emerged from chickpea seeds untreated and treated with fungicide.

	Emerged seedlings		Abnormal		Deformed		Damaged	
Genotypes	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
BRS Kalifa	14.0^{bB}	92.0^{aA}	97.0^{aA}	6.0^{aB}	52.0^{aA}	54.0^{cA}	48.0^{bA}	46.0^{bA}
3bb4	34.0^{aB}	86.0^{bA}	97.0^{aA}	1.0^{aB}	50.0^{aA}	50.0^{dB}	50.0^{bA}	50.0^{bA}
BRS Cristalino	26.0^{aB}	93.0^{aA}	90.0^{aA}	10.0^{aB}	57.0^{aA}	8.0^{dB}	43.0^{bB}	92.0^{aA}
Cicero1	3.0^{cB}	40.0^{dA}	63.0^{bA}	15.0^{aB}	25.0^{bB}	100.0^{aA}	75.0^{aA}	0.0^{cB}
BRS Toro	-	43.0^{dA}	-	11.0^{aB}	-	20.0^{dA}	-	79.0^{aA}
Jamu	10.0^{bB}	79.0^{bA}	88.0^{aA}	0.0^{aB}	0.0^{bA}	0.0^{dB}	100.0^{aA}	0.0^{cB}
bic4	31.0^{aB}	83.0^{bA}	49.0^{cA}	11.0^{aB}	43.0^{aA}	0.0^{dB}	57.0^{bB}	100.0^{aA}
Cicero	12.0^{bB}	63.0^{cA}	95.0^{aA}	3.0^{aB}	56.0^{aA}	75.0^{bA}	43.0^{bA}	25.0^{cB}
BRS Aleppo	17.0^{bB}	85.0^{bA}	97.0^{aA}	4.0^{aB}	57.0^{aA}	0.00^{dB}	43.0^{bB}	100.0^{aA}
CV (%)	12.5		34.67		43.4		37.72	

Means followed by the same lowercase letter in the column and uppercase letters in the row, when comparing treated and untreated seeds in each category, did not differ statistically by the Scott-Knott test at 5%.

Table 4. Mean values of shoot length (PA), root length (CR), shoot fresh mass (MFPA), and root fresh mass (MFR) of seedlings emerged from chickpea seeds untreated and treated with fungicide.

Genotypes	PA (cm)		CR (cm)		MFPA (g)		MFR (g)	
	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated
Jamu	2.90^{dB}	17.07^{aA}	1.55^{bB}	5.15^{aA}	0.36^{cB}	0.99^{aA}	0.40^{bB}	0.92^{aA}
Cicero	4.58^{cB}	16.26^{aA}	1.91^{bB}	5.26^{aA}	0.54^{bB}	0.86^{bA}	0.48^{bB}	0.85^{aA}
Cicero1	-*	12.37^{cA}	-	5.16^{aA}	-	0.79^{dA}	-	0.86^{aA}
BRS Kalifa	4.85^{cB}	12.70^{cA}	2.40^{bB}	4.70^{aA}	0.57^{aB}	0.87^{bA}	0.54^{aB}	0.71^{cA}
BRS Cristalino	6.92^{bB}	14.72^{bA}	3.43^{aB}	4.80^{aA}	0.61^{aB}	0.80^{cA}	0.57^{aB}	0.75^{bA}
BRS Aleppo	7.30^{bB}	11.27^{cA}	3.13^{aB}	4.68^{aA}	0.62^{aB}	0.73^{dA}	0.53^{aA}	0.55^{dA}
BRS Toro	-	11.71^{cA}	-	4.60^{aA}	-	0.80^{cA}	-	0.79^{bA}
3bb4	5.96^{bB}	9.79^{dA}	1.96^{bB}	4.59^{aA}	0.51^{bB}	0.76^{dA}	0.45^{bB}	0.67^{cA}
bic4	10.67^{aB}	14.43^{bA}	3.45^{aB}	4.33^{aA}	0.48^{bB}	0.79^{cA}	0.46^{bB}	0.56^{dA}
CV (%)	11.93		15.3		6.02		10.87	

* There were no normal seedlings for evaluation. Means followed by the same lowercase letter in the column and uppercase letters in the row, when comparing treated and untreated seeds in each category, did not differ statistically by the Scott-Knott test at 5%.

Evaluation of plants from secondary infection in chickpea seedlings

All evaluated plants showed fungi of the *Fusarium* spp. genus within the roots. The indirect method of fungal re-isolation and morphological evaluation of micro- and macroconidia confirmed these fungi.

The length and weight of the shoot and root were not associated. Therefore, the genotypes with the highest shoot length values (Cicero and bic4) were not the same with the highest root length values (BRS Toro, 3bb4, and BRS Cristalino). That also occurred for weight, with the shoot weight of the BRS Toro genotype differing negatively from the others and the BRS Kalifa genotype showing the best mean root weight value (Table 5).

Table 5. Length and weight of chickpea plants from seeds infected by *Fusarium* sp. 45 days after planting.

Genotypes	Length (cm)		Weight (g)	
	Shoot	Root	Shoot	Root
BRS Kalifa	31.20^{c}	15.80^{b}	7.86^{a}	11.80^{a}
BRS Toro	36.00^{c}	17.40^{a}	3.52^{b}	5.49^{c}
Jamu	46.00^{b}	13.20^{b}	8.19^{a}	4.92^{d}
Cicero	50.60^{a}	14.60^{b}	6.71^{a}	4.07^{d}
BRS Cristalino	36.40^{c}	19.80^{a}	7.53^{a}	9.28^{b}
BRS Aleppo	44.40^{b}	14.40^{b}	6.74^{a}	7.80^{b}
Cicero1	43.80^{b}	15.60^{b}	6.22^{a}	4.07^{d}
bic4	54.80^{a}	12.60^{b}	9.07^{a}	6.48^{c}
3bb4	43.20^{b}	16.60^{a}	7.53^{a}	8.29^{b}
CV %	5.06	8.27	9.46	8.54

Means followed by the same letter in the column did not differ statistically by the Scott-Knott test at 5%.

The plants of all tested genotypes showed few secondary roots, vascular discoloration, and an outer black or brown color, typical root symptoms of the presence of *Fusarium* spp. (Rocha et al., 2020). The visual evaluation of the root system of sick plants highly contributes to disease management. The faster the pathogen identification, the sooner control measures can be taken, reducing damage and losses.

4. Discussion

Reaction of chickpea genotypes to *Fusarium oxysporum*

The low root and shoot weight values are the indirect effects of colonization by *F. oxysporum* on the root system, causing plant wilt. Roots perform water and nutrient uptake, and phloem and xylem transport them; therefore, the fungi inside the conducting vessels of the root system might have limited plant development, reflecting on the weight and length of this structure. Genotypes Jamu, Cicero, and Cicero1 showed statistically higher shoot length values and lower root weight, although they did not differ statistically from the other evaluated genotypes (Table 1). Without the stress caused by the pathogen, the Cicero genotype cultivated in a 1L pot reaches a shoot length of 34.2 cm and 4.31 g and a root length of 27.2 cm and 6.9 g (Azevedo et al. 2020).

Zaim et al. (2018) also observed weight and length reductions in plants exposed to *F. oxysporum* f. sp. *ciceris* (FOC). The authors observed mean values of 41.33 cm and 3.53 g and 16.10 cm and 1.96 g for the length and weight of the shoot and root, respectively. They used the FOC suspension at 1 x 10^6 conidia/mL, finding 37.40 cm and 14.76 g and 2.92 cm and 1.27 g for the length and weight of the shoot and root, respectively. A high inoculum concentration (4.5 x 10^7 conidia/mL) and direct inoculation into the roots might explain the lower length and weight values. Azevedo et al. (2020) used the *F. oxysporum* isolate CML 2878 in plants of the Cicero cultivar with lower concentration and inoculation into the soil, verifying no symptoms of pathogen attack.

Besides the low weight and length, the root system manifested external symptoms of *F. oxysporum* CML 2878, with colors ranging from brown to black (Figures 1 A and B). The symptoms from *F. oxysporum* CML 2878 may help identify strains by differentiating between pathotypes. Therefore, the higher the genotype's susceptibility, the sooner it usually presents symptoms from the pathogen. Commonly, symptoms are more visible at the beginning of flowering (six to eight weeks after planting), called late wilt (Bhar et al. 2021). Late wilt manifests by the fall of petioles and leaflets, yellowing, and leaf necrosis. Plants presenting symptoms within 25 days after sowing undergo early wilting and are highly susceptible (Rocha et al. 2020). Other early wilt symptoms are an opaque green color and drooping leaves. The present study demonstrated that symptoms occurred approximately 15 days after inoculation, with crucial temperature and soil moisture for disease progression.

The transverse root section exposed the darkening of conducting vessels caused by discoloration. Rocha et al. (2020) reported the impossibility of differentiating *Fusarium* species based on the reflex symptoms. However, in Brazil, *F. oxysporum* darkens the stem tissue (root rot), while *F. solani* colonization is restricted to root tissues and causes plant wilt. From this perspective, observing morphological features may help confirm the present pathogen (Rodrigues and Menezes 2005).

The indirect method of fungal isolation did not provide fungal cultures for morphological evaluation based on their coloration in the PDA medium and the shape of micro- and macroconidia in the SNA medium. All studied plots showed fungal colony growth. Coloration in PDA is a relevant morphological feature, and *F. oxysporum* CML 2878 colonies expressed white and violet colors and an abundant cotton-like aerial mycelium (Summerell et al. 2003). In the PDA medium, the colonies showed a white cotton-line aerial mycelium and a violet backside, the same color features reported by Azevedo et al. (2017) when identifying the *F. oxysporum* isolate CML 2878.

The suspension of colonies cultivated in the SNA medium revealed abundant macro- and microconidia. The macroconidia showed a falciform shape with two to five septa, and the microconidia had an oval non-septate shape, agreeing with Qureshi et al. (2021) on the morphology of *F. oxysporum* f. sp. *ciceris* isolates.

Seed health test

Saido et al. (2020) studied the isolation and identification of fungi associated with different species of stored grains, finding a relative frequency of *Aspergillus niger* (34%), *Penicillium* spp. (42,5%), *Rhizoctonia* sp. (18.9%), *Alternaria* sp. (1.9%), and *Curvularia* sp. (0.9%) in chickpea seeds. Getaneh et al. (2020) found 17 fungi species in chickpea seeds, such as *Fusarium* spp., *Aspergillus* sp., *A. niger*, *A. flavus*, *A. nidulans*, *A. candidus*, *A. fumigatus*, *Penicillium* sp., *Rhizopus* sp., *Verticillium* sp., *Rhizoctonia* sp., *Pythium* sp., *Alternaria* sp., *Helminthosporium* sp., *Phyllosticta* sp., *Cladosporium* sp., and *Nigrospora* sp. The same authors observed that *A. flavus* was the most dominant with recovery (25.59% infection frequency) from all seed lots. Our study showed the highest incidence for *Aspergillus* sp. (57.14%) and *Fusarium* (29.63%) in the seed coat and cotyledons, followed by the radicle and plumule in chickpea seeds. Among the fungi genera found in seeds, *F. solani* and *F. oxysporum* were the primary pathogens that caused disease in chickpea crops in Brazil (Rocha et al. 2020). Hazzat et al. (2023) reported that *F. equiseti* is a main *Fusarium* species in Morocco, causing seed rot, wilt, root rot, root and crown necrosis, and leaf yellowing. Moparthi et al. (2021) obtained several species of *Fusarium* (*F. oxysporum*, *F. culmorum*, *F. poae*, *F. redolens*, and *F. solani*) from chickpea seeds and roots in the growing regions of Montana, United States, verifying that *F. solani* was the most aggressive species in chickpeas. The same authors also identified several species of *Fusarium* in seeds and roots of peas, lentils, and barley (*F. avenaceum, F. solani, F. poae, F. equiseti, F. oxysporum, F. culmorum, F. redolens, F. sporotrichioides, F. solani, F. graminearum, F. torulosum*, and *F. tricinctum*), concluding that *Fusarium* species from seeds and roots may cause root rot in both pulse and cereal crops.

The different seed production, origin, and storage conditions interfere with the observed microflora, justifying the variations in the number and diversity of fungi. Nevertheless, the fungi found in the present study are the most common in seed health tests in chickpeas (Getaneh et al. 2020; Moparthi et al. 2021; Hazzat et al. 2023). This test allows the growth of fungi in different seed parts. *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., and *Fusarium* sp. are storage fungi commonly found in seeds. Seed contamination by these genera is mainly related to harvest and storage, with seeds contaminated by these fungi showing germination defects and vigor loss. These storage fungi usually prevail over other fungi throughout the storage of chickpea seeds (Khatun et al. 2018). However, seed surface disinfestation reduces the number of fungi depending on the disinfectant product and concentration (Gilbert et al. 2023).

The most frequent genus in our study was *Aspergillus* sp. (57.14%), a saprophyte fungus that contaminates seeds pre- and postharvest. *Aspergillus flavus* is among the most common species in hot climates, and it is found in the soil, seeds, and plant tissues. This fungus may cause diseases in relevant crops, such as cotton, maize, peanut, and soybean (Rocha et al. 2014; Nahar et al. 2019; Ali et al. 2021; Erasto et al. 2023). Although considered saprophytes, fungi of the genus *Aspergillus* spp. produce substances that affect the defense system and kill the cells of host plants. Rocha et al. (2014) studied the physiological quality of soybean seeds naturally infected with different contamination levels by *A. ochraceus*, finding that contamination levels of up to 50% of inoculum reduced the emergence speed index, seedling emergence, seedling length, shoot and root fresh weight, shoot dry weight, and root dry mass. The same authors found that soybean seeds with incidences above 50% of *A. ochraceus* showed total vigor loss and tissue decay.

Azevedo et al. (2017) also reported the fungal mycelium of *Fusarium* sp. in chickpea seeds (cotyledon and radicle) found in this study, with velvety white colonies of rapid growth and the production of a red pigment in seeds infected by *F. oxysporum*. The authors also reported that the fungus was found in the seed integument and lower amounts in the cotyledons of chickpea seeds.

The fragments of fungal mycelium from different genotypes and seeds originated colonies with salmon, violet, cream, and brown colors when grown in PDA, suggesting more than one *Fusarium* species. Azevedo et al. (2017) also identified *F. oxysporum* colonies with violet and cream colors and *F. solani* with brown color. However, more studies are required to confirm different fungal species in chickpea seeds. The evaluation of morphological features exclusively does not allow a safe differentiation between *Fusarium* species, and some species may express different colors even when sharing the same growth conditions (Leslie and Summerell 2006).

Chickpea seeds with *Fusarium* may introduce the fungus into the soil, and the pathogen population tends to increase at every crop cycle. From this perspective, crop rotation with susceptible crops may also

benefit this population increase. Moreover, the high fungus capacity to colonize organic matter may favor population continuity. These factors highlight the relevance of preliminary tests to attest to seed quality and using healthy seeds and fungicides for seed treatment as a preventive measure.

The Carbendazim and Thiram fungicides, used individually or jointly to treat seeds, control, and inhibit the mycelial growth of *F. oxysporum* f. sp. *ciceris,* while using only Carbendazim promotes higher germination, inhibiting *Fusarium* wilt in chickpea (Yadav et al. 2018; Golakiya et al. 2018). The treatment in this study used a fungicide with a commercial product based on Carbendazim and Thiram, which was efficient for fungal control as there was no microorganism growth in the seeds. In another study using Carbendazim for seed treatment, Yadav et al. (2018) improved germination by approximately 100%, reducing the incidence by 85.7% and 52.4% of *Fusarium* wilt in chickpeas using seed treatments with Carbendazim (0.2%) and Thiram (0.2%), respectively. The same authors also verified that Carbendazim and Thiram inhibited *Fusarium* wilt by 100% *in vitro*, but Carbendazim performed best for seed treatment at *in vivo* conditions. Golakiya et al. (2018) evaluated different fungicides in seed treatment to control *Fusarium* wilt, reporting that Tebuconazole (25.9%-EC) effectively inhibited mycelial growth in the systemic group of fungicides. In the non-systemic group, copper oxychloride (50%-WP) and Carbendazim (12%) + Mancozeb (63%-WP) combinations, in the case of fungicides, significantly inhibited fungal growth. The *in vivo* experiment showed that the combination of Carbendazim (12%) + Mancozeb (63%-WP) provided a minimum disease incidence percentage, followed by Carbendazim (50%-WP) (Golakiya et al. 2018).

Seedling emergence test

The negative results regarding the use of Carbendazim + Thiram found in this study might be partly due to the type of seeds of the genotypes associated with physical damage due to fungal contamination, which increased fungicide concentration in the seeds. Rehman et al. (2013) evaluated the effect of seed treatment with fungicide application using 2.5 g/Kg (product/seed) in the management of *Fusarium* wilt, finding 8% incidence, 78% germination, a plant weight of 22.3 g, and roots with 24.8 cm. These results did not differ significantly from untreated seeds, which showed 12% incidence, 74% germination, a plant weight of 20.7 g, and roots with 22.33 cm. The increase in fungicide levels might harm or benefit crop management, depending on the application form and the increase in applied levels. Golakiya et al. (2018) observed that the higher fungal control was proportional to the higher Carbendazim concentration from 100 to 500 ppm in the *in vitro* experiment. When drenching the soil with the aqueous fungicide suspension, the same authors found disease reduction rates of 76% for Carbendazim (50%-WP) and 83.3% for the combination of Carbendazim (12%) + Mancozeb (63%-wp) in the *in vivo* experiment. Therefore, studies on the dosage and application forms may improve the use of such substances.

Root length and seedling vigor may show product phytotoxicity in seed treatment (Carvalho et al. 2020). The present study demonstrated that, except for the root fresh mass of the BRS Aleppo genotype, seed treatment with the fungicide combination of Carbendazim + Thiram at the dosage used per kg of seeds reduced the length and fresh mass values of the shoot and root of seedlings of all genotypes (Table 4), indicating a phytotoxic effect on chickpea seeds.

Even the genotypes with higher values in the evaluated parameters differed regarding the presence and absence of seed treatment. The Jamu genotype stood out among untreated seeds, showing the best means. Conversely, genotypes BRS Cristalino and BRS Aleppo obtained the highest values of root length, root fresh mass, and shoot fresh mass among treated seeds. Regarding shoot length, the bic4 genotype showed the highest mean in the group. In soybean plans, Carbendazim + Thiram may reduce seedling length while causing no abnormalities, resulting in lower seedling uniformity (Carvalho et al. 2020).

The fungicide dose of the present study reduced *Fusarium* incidence but affected chickpea seedling development. Carbendazim below recommended levels may promote higher dry mass values, whereas levels above the recommended may reduce plant weight and leaf pigments (Garcia et al. 2002). Therefore, even with the positive action on disease and pathogen reduction, Carbendazim may be phytotoxic to seedling development depending on the physical damage and level and the fungal contamination level of the seeds, as discussed.

Evaluation of plants from secondary infection in chickpea seedlings

The plants of all tested genotypes showed few secondary roots, vascular discoloration, and an outer black or brown color, typical root symptoms of the presence of *Fusarium* spp. The visual evaluation of the root system of sick plants highly contributes to disease management. The faster the pathogen identification, the sooner control measures can be taken, reducing damage and losses.

The genus *Fusarium* sp. in seeds may cause diseases in chickpea plants, affecting plant germination, mortality, and size. The fungal isolates identified as *Fusarium* in the roots expressed the same morphological features as in the fungi from the seed health tests, with salmon, violet, and cream colors. Pande et al. (2007) confirmed the transmissibility of *F. oxysporum* from chickpea seeds to seedlings or new areas, establishing the soil disease to economic threshold levels within three seasons. In the seeds, the authors also found necessary chlamydospore structures to germinate and infect the plants, justifying the delayed symptom appearance in the first crop cycle and underestimating the problem, which usually aggravates at each cycle with the increase in the soil's fungal population. Moreover, these findings may explain why plants performed better even when containing *Fusarium* compared to the plants of the resistance test against *F. oxysporum* in the same study.

5. Conclusions

Genotypes BRS Kalifa, BRS Toro, BRS Aleppo, BRS Cristalino, Cicero, Jamu, Cicero1, bic4, and 3bb4 were susceptible to the *F. oxysporum* isolate CML 2878. Chickpea seeds were relevant to disseminating *Fusarium* into cultivation areas. Fungicides based on Carbendazim + Thiram in the treatment of chickpea seeds are efficient in fungal control but reduce seedling emergence and vigor of contaminated seeds.

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