

Article

Use of *Canavalia ensiformis* as Green Manure for Managing the Root Rot and Vine Decline Complex in Melon

Moisés Bento Tavares ¹, Raíssia Andressa Moraes da Costa ¹, Naama Jéssica de Assis Melo ¹ ,
Márcio Thalison de Queiroz Souza ¹, Sabrina Queiroz de Freitas ¹, Carla Sonale Azevêdo Soares Silva ¹,
Allinny Luzia Alves Cavalcante ¹, Andréia Mitsa Paiva Negreiros ¹ , Inês Maria Mendes Sales ²
and Rui Sales Júnior ^{1,*} 

¹ Department of Agronomic and Forest Sciences, Universidade Federal Rural do Semi-Árido, Mossoró 59625-900, RN, Brazil; moises.tavares@alunos.ufersa.edu.br (M.B.T.); raissia.costa@alunos.ufersa.edu.br (R.A.M.d.C.); naama.melo@ufersa.edu.br (N.J.d.A.M.); marcio.souza@alunos.ufersa.edu.br (M.T.d.Q.S.); sabrina.freitas@alunos.ufersa.edu.br (S.Q.d.F.); carla.soares@alunos.ufersa.edu.br (C.S.A.S.S.); allinny.cavalcante@alunos.ufersa.edu.br (A.L.A.C.); mitsa@ufersa.edu.br (A.M.P.N.)

² Department of Applied Social Sciences, Universidade Federal Rural do Semi-Árido, Mossoró 59625-900, RN, Brazil; ines.sales@alunos.ufersa.edu.br

* Correspondence: ruissales@ufersa.edu.br

Abstract

Root rot and vine decline (RRVD) is one of the main root diseases of melon, causing significant production losses due to the isolated or combined action of different phytopathogenic fungi. The absence of registered chemical control methods in Brazil reinforces the need for alternative integrated management strategies. This study aims to evaluate the enzymatic responses of Jack Beans plants inoculated with *M. pseudophaseolina* (Experiment I) and the effectiveness of crop rotation cycles between melon (ME) and Jack Bean (JB) on RRVD and soil-borne pathogens (Experiment II). In Experiment I, the activity of chitinase (CT), β -1,3-glucanase (BG), and phenylalanine ammonia-lyase (PAL) were assessed at five evaluation periods. In Experiment II, nine rotation cycles involving JB and ME, grown in naturally infested soil, were evaluated for disease incidence, severity, and fungal isolation frequency. In Experiment I, inoculated plants showed increased CT, BG, and PAL expression compared to non-inoculated plants. In Experiment II, the control treatment showed no disease incidence or severity, whereas all other rotation cycles exhibited 100% incidence and severity ranging from 4.5 to 4.9. The incorporation of JB demonstrated a suppressive effect on important pathogens associated with branch decline, reducing the frequency of *Macrophomina* spp. isolation (0–4% compared to 16% in the treatment without JB) and delaying the root penetration of *Monosporascus* spp. after two consecutive cycles. However, this management also favored the multiplication of *Fusarium* spp. in all treatments with JB incorporation.



Academic Editor: Filippo De Curtis

Received: 24 May 2026

Revised: 17 June 2026

Accepted: 25 June 2026

Published: 28 June 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

Keywords: Jack Bean; enzymes; *Cucumis melo*; root pathogens

1. Introduction

Melon (*Cucumis melo* L.) exports reached US\$231.45 million in 2025, ranking second in the national fresh fruit export rankings, behind only mangoes (US\$335.12 million) [1], with this crop becoming increasingly established as one of the most important in the national agribusiness sector. The Brazilian Northeast region is the main production hub for this fruit,

accounting for over 98% of total production, with revenues of approximately R\$ 2.87 billion in 2024 [1,2].

The state of Rio Grande do Norte (RN) is the Brazil greatest melon-producing state, with an estimated area of 18.943 ha and a production of 505.212 t in 2024 [2]. However, monoculture predominates in the melon production chain in RN, where the use of high-frequency localized irrigation allows for up to three production cycles annually, which are typically conducted on the same soil [3]. Such conditions predispose agroecosystems to the establishment and persistence of various phytosanitary problems, with soil-borne pathogens being the most concerning in that region for this crop [4].

Root rot and vine decline (RRVD) is considered the primary root disease of this crop, in which the roots and/or the collar of the plants are compromised by the isolated or combined action of multiple phytopathogens. Among these, we can mention the fungi: *Rhizoctonia* [5], *Stagonosporopsis cucurbitacearum* [6], *Fusarium* [7], *Lasiodiplodia brasiliensis* [8], *Macrophomina* [4,9,10], and *Monosporascus* [11,12].

It is worth noting that, to date, there are no registered pesticides for the chemical control of these pathogens in melon crops in Brazil [13]. Therefore, alternative and combined control measures are required within the Integrated Pest Management (IPM) system to maintain crop production.

Currently, species of the genus *Macrophomina* are the root pathogens of greatest concern to farmers, as there is still no established management strategy for this fungus [4,14,15]. This is due, in part, to the fungus's diverse survival mechanisms in the environment, including the production of microsclerotia, saprophytic activity, and a wide range of hosts (more than 800 species) among agricultural crops and weeds [16].

However, crop rotation and green manuring (since they are appropriate for the present pathosystems) can help reduce the survival of various pathogens through the isolated or combined action of different mechanisms, such as the suppression of plant pathogens using non-susceptible plants and the increase in the population of beneficial microorganisms [17,18].

The Jack Bean (*Canavalia ensiformis* [L.] DC.) is a legume considered hardy, capable of adapting to various soil types [19]. This species may be recommended as a crop alternative in areas infested with *Macrophomina*, as it is considered resistant to various species of this genus [20], although its defense mechanisms remain poorly understood, particularly about *M. pseudophaseolina*, which has been reported more recently (compared to *M. phaseolina*) and is prevalent in the production areas of Rio Grande do Norte (4); there is little information available regarding its cultural management, as well as its biochemical interaction with species used for this purpose, such as green manure crops.

Regarding its resistance to specific pathogens, *C. ensiformis* may utilize compounds that inhibit fungal development, such as toxins [21], specific ureases [22], various proteases [23], as well as other pre- or post-formed mechanisms, including the production of other enzymes triggered by the recognition of molecular patterns associated with pathogens [24]. According to Porto et al. (2016), who studied Jack bean (*Canavalia ensiformis*) in the control of melon root rot caused by a pathogen association, they observed that the use of Jack bean wort as a cover crop resulted in a lower incidence of root rot in this crop [25].

Enzymes involved in the degradation of structures vital to pathogens, such as chitinases (CT), which degrade chitin and chitosan, as well as β -1,3-glucanase (BG), which breaks the β -1,3-glycosidic bonds of β -glucans, both components of the cell walls of fungi and bacteria, including plant pathogens [24,26,27], are common mechanisms in many plant species.

Still regarding internal defense, plants can also increase the expression of phenylalanine ammonia-lyase (PAL), an enzyme responsible for catalyzing the deamination

of L-phenylalanine to trans-cinnamic acid, which catalyzes the first step of the phenylpropanoid pathway [28]. From this point on, this metabolic pathway becomes a key step in the synthesis of aromatic compounds, such as flavonoids, isoflavonoids, and lignin-compounds that, among other functions, play a fundamental role in biochemical and structural defense against pathogens [29–31].

Although *C. ensiformis* is resistant to *Macrophomina* spp. and produces substances with an inhibitory effect on certain phytopathogenic fungi [20,21], knowledge about its effects on the entire microbial soil community is still limited. This limitation is relevant because the use of this species in disease management can cause differential responses in the soil microbiota, including adverse effects on non-target organisms. Porto et al. [25], when evaluating the efficiency of green manuring with Jack Bean, observed a higher incidence of RRVD where Jack Bean had been incorporated, with the authors attributing part of this effect to the proliferation of *Fusarium* (previously inoculated into the soil) in the organic matter derived from this plant species, confirming an adverse effect of green manuring on multiple pathosystems present in biologically unbalanced soils with a long history of monoculture.

This study evaluated the enzymatic mechanisms potentially involved in the resistance of *C. ensiformis* to *M. pseudophaseolina* (Experiment I), as well as the effectiveness of Jack Bean as a green manure in controlling RRVD in naturally infested soil and its effects on the population of the main soil-dwelling pathogens associated with RRVD in melon plants (Experiment II).

2. Materials and Methods

2.1. Experiment I: Enzymatic Response of *C. ensiformis* to *M. pseudophaseolina*

2.1.1. Site Description

From June to December 2023, the experiment was conducted in a greenhouse located in the municipality of Mossoró, RN (5°11'17" S, 37°20'39" W), at an altitude of 18 m, with an average annual precipitation of 673.9 mm, and a BSh climate, defined as very hot [32].

Enzyme activity analyses were conducted at the Plant Pathology Laboratory II and the Postharvest Laboratory; both part of the Universidade Federal Rural do Semi-Árido (UFERSA).

2.1.2. Inoculum Preparation

The enzymatic response of *C. ensiformis* was evaluated in plants grown in soil artificially infested with isolate of *M. pseudophaseolina* (A7P15) [4]. The soil infestation method adopted was that of rice grains colonized by the pathogen [33–35], with adaptations.

For the preparation of the inoculum source, unhusked rice seeds were soaked in distilled water for 12 h in Erlenmeyer flasks sealed with cotton. After this period, the grains were autoclaved three times (at 24 h intervals). After the last autoclaving, the Erlenmeyer flasks containing the grains were taken to laminar flow chambers, where they were inoculated with five 8 mm disks cut from Petri dishes in which *M. pseudophaseolina* had been previously grown on potato dextrose agar (PDA) medium (Merck, Darmstadt, Germany). After inoculation, the grains were kept in a BOD-type incubator at 28 °C until complete colonization of the grains (approximately 10 days).

2.1.3. Experimental Design and Conduct

The experiment was conducted in a completely randomized design (CRD), in a 5 × 2 × 2 factorial scheme. The first factor is the five evaluation periods: 7, 14, 21, 28, and 35 days after transplanting (DAT). The second factor corresponds to autoclaved soil and

soil artificially infested with *M. pseudophaseolina*. The third factor would be leaves and roots.

In each evaluation period, six plants from each treatment were assessed to quantify the enzymatic response in Jack Bean inoculated with this pathogen. The experimental unit consisted of one plant per pot in each treatment, totaling 120 plants. The vegetative samples were destructive in each evaluation period.

2.1.4. Experiment Setup

Seedlings of *C. ensiformis* (sown in trays ten days before planting) were transplanted into 2.6 L plastic pots filled with a mixture of soil and commercial substrate (2/1 ratio). The soil–substrate mixture was autoclaved three times, at 24 h intervals, for 60 min each time.

Soil infestation (inoculated treatment) was carried out by depositing 18 colonized grains in each pot, which were mixed into the soil. The control treatment received only uncolonized autoclaved grains. This procedure was performed ten days before transplanting the seedlings so that the pathogen would colonize the soil of the experiment. Plants were irrigated daily to maintain field capacity.

2.1.5. Experimental Evaluation and Variables Analyzed

At each harvest (during each of the five evaluation periods), leaves and roots were collected separately from the plants in the inoculated treatment group as well as from the control group. The roots of each plant were first washed under running water to remove excess soil and substrate. Subsequently, fungal re-isolation was performed using root fragments, confirming Koch's postulates. The leaves and roots were then placed in Falcon tubes and frozen in liquid nitrogen. After freezing, the tubes were stored in an ultra-freezer ($-80\text{ }^{\circ}\text{C}$) until the extraction and quantification of enzymatic activity.

The analyses were performed using previously prepared protein extracts, according to a methodology adapted from Bezerra Neto and Barreto [36]. For this purpose, 0.5 g of leaves and 0.5 g of roots were weighed for each enzyme analyzed. The leaves or roots were then transferred to a mortar, where 25 mg of polyvinylpyrrolidone (PVP) (BASF SE, Ludwigshafen, Germany) and approximately 5 mL of liquid nitrogen were added; subsequently, the material was macerated using a pestle, and immediately after maceration, 2.5 mL of acetate buffer solution (0.1 M, pH 5.0) and 0.5 mL of EDTA (1 mM) (Sigma-Aldrich, St. Louis, MO, USA) were added. The mixture was then homogenized and transferred to Eppendorf tubes, where it was centrifuged for 10 min at $10,000\times g$ at $4\text{ }^{\circ}\text{C}$. After centrifugation, the supernatant was transferred to another Eppendorf tube and stored in a deep freezer ($-80\text{ }^{\circ}\text{C}$) until the time of enzyme activity assessment. Enzyme activities were normalized by protein concentrations.

Chitinase (CT) quantification was performed according to the method described by Stangarlin et al. [37]. For this purpose, 100 μL of protein extract, 300 μL of extraction buffer (0.1 M acetate buffer, pH 5.0), and 100 μL of Chitin Azure (2.0 mg mL^{-1}) (Sigma-Aldrich, St. Louis, MO, USA) were pipetted into Eppendorf tubes, which were then incubated for 20 min at $40\text{ }^{\circ}\text{C}$. After incubation, the reaction was immediately terminated by adding 100 μL of HCl solution (1 M) and cooling on an ice bath. The tubes were then centrifuged at $10,000\times g$ for 5 min. The supernatant was transferred to quartz cuvettes, and the readings were measured at 550 nm absorbance using a spectrophotometer.

To ensure accurate readings, the values were zeroed using the extraction buffer as a general blank. The “control blank” solution consisted of a mixture of 400 mL of extraction buffer + 100 μL of Chitin. The “control blank” reading was recorded and subtracted from the other readings of the protein solution. Chitin Azure (2.0 mg mL^{-1}) was prepared by diluting 0.01 g of Chitin Azure into 5 mL of acetate buffer. HCl (1 M) was obtained by

diluting 42 mL of concentrated HCl in 500 mL of distilled water. Enzyme activity was expressed in E.U. mg^{-1} protein.

The quantification of phenylalanine ammonia-lyase (PAL) was calculated according to the methodology of Umesha [38], where 300 μL of the protein extract were pipetted into test tubes, to which 1.2 mL of Tris-HCl buffer (25 mM pH 8.8) and 1.5 mL of L-phenylalanine solution (50 mM) were added. The tubes were then kept for 2 h at 40 °C in a water bath and then immediately transferred to an ice bath to stop the reaction. After cooling, the solution was transferred to cuvettes for the determination of enzymatic activity in a spectrophotometer set to 290 nm. A “blank” test was performed by replacing the phenylalanine with distilled water. The preparation of the Tris-HCl buffer was done by diluting 3.0285 g of Tris base in 1000 mL of distilled water. The pH of this solution was adjusted to 8.8 with pure HCl. The L-phenylalanine solution (50 mM) was prepared by diluting 0.8258 g of phenylalanine (Sigma-Aldrich, St. Louis, MO, USA) into 100 mL of Tris-HCl buffer. Enzyme activity was expressed in E.U. min^{-1} mg^{-1} protein.

The quantification of β -1,3-glucanase (BG) was performed according to the methodology of Bezerra Neto and Barreto [36]. For this, 25 μL of the previously prepared enzymatic extract were pipetted into test tubes, where 200 μL of acetate buffer solution (0.1 M, pH 5.0) and 200 μL of laminarin (15 mg mL^{-1}) (Sigma-Aldrich, St. Louis, MO, USA) were added. Then, this solution was incubated for 30 min at 37 °C. After incubation, the glucose content produced from the breakdown of laminarin was quantified. For this, 200 μL of the enzymatic extract (after incubation) were pipetted separately into screw-cap test tubes, to which 1.3 mL of distilled water and 1.0 mL of DNS reagent (3,5-dinitrosalicylic acid) (Sigma-Aldrich, St. Louis, MO, USA) were added. The tubes were then stopped and gently shaken. Next, the tubes were heated for 5 min at 100 °C and then cooled in an ice bath. After cooling, 7.5 mL of distilled water was added, and the tubes were gently shaken. Spectrophotometric readings were taken at 540 nm. Acetate buffer solution (0.1 M, pH 5.0) was prepared in a beaker, where 14.8 mL of acetic acid (0.2 M) were mixed with 35.2 mL of sodium acetate (0.2 M) (Sigma-Aldrich, Darmstadt, Alemanha), and the volume was made up to 100 mL with distilled water. The pH of the mixture was adjusted to 5.0 using one of the two component solutions of the buffer mixture.

The 0.2 M acetic acid solution was prepared by pipetting 2.31 mL of glacial acetic acid into a 200 mL volumetric flask and then making up the remaining volume with distilled water. The 0.2 M sodium acetate solution was prepared by dissolving 1.64 g of sodium acetate (anhydrous) in distilled water, with the volume then made up to 100 mL. The preparation of EDTA (1 mM) involved weighing 0.2922 g of EDTA (MW = 292.2), which was then dissolved in distilled water, with the flask volume made up to 1000 mL. Laminarin (15 mg mL^{-1}) was obtained by dissolving 0.15 g of laminarin in 10 mL of distilled water. A digital analytical balance was used for the respective weighings performed in this experiment. Enzyme activity was expressed in E.U. min^{-1} mL^{-1} .

2.1.6. Statistical Analysis

The results were subjected to analysis of variance (ANOVA), and when a significant difference was detected, the means were compared using the *t*-test of Student at a 5% significance level with the SISVAR statistical software, version 6.6.

2.2. Experiment II: Use of *Canavalia ensiformis* in the Management of Root Rot and Vine Decline (RRVD) in Melon Plants

2.2.1. Site Description

From January 2024 to March 2025, a greenhouse experiment was conducted in the municipality of Mossoró, RN, Brazil (5°11'17" S, 37°20'39" W), at an elevation of 18 m and

with an average annual precipitation of 673.9 mm. The climate is of the BSh type, defined as very hot [32].

2.2.2. Definition of Treatments and Experimental Design

Nine different combinations of crop rotation between three growing cycles of melon (ME) Jack Bean (JB) were cultivated. The evaluation of the effect of each treatment on the RRVD pathosystem was conducted by growing a fourth cycle, with melon plants in all treatments at the same time (Figure 1).

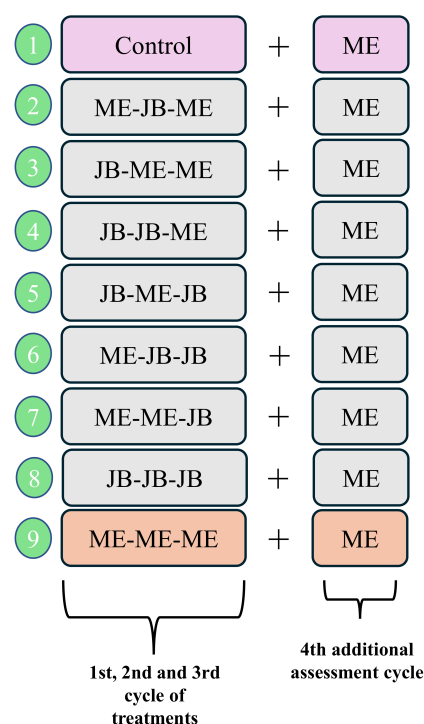


Figure 1. Definition of treatments corresponding to different crop rotation cycles between melon (ME) and Jack Bean (JB).

Each pot had a capacity of 10 L. The experiment was conducted using a CRD with 10 replicates per treatment, where the experimental units consisted of one plant per pot.

2.2.3. Soil Characterization

The soil used in this study was collected from a melon-growing area on the São Romão farm, owned by a melon-producing company in the city of Tibau, RN (4°54′26.62″ S, 37°24′5.22″ W), in an area with a history of recurrent RRVD.

The soil used had sandy texture, with the following physical composition: Coarse sand = 64%; Fine sand = 24%; Total sand = 89%; Silt = 4%; Clay = 7%, and had the following chemical characteristics: pH (H₂O) = 6.6; P = 107.70 mg dm⁻³; K = 78.30 mg dm⁻³; Na = 67.70 mg dm⁻³; Ca = 2.93 cmol dm⁻³; Mg = 0.96 cmol dm⁻³; Al = 0.00 cmol dm⁻³.

The initial diversity of pathogens in the soil used in this experiment was determined through fungal isolation in culture medium, performed on the root systems and collars of 50 melon plants grown in the freshly collected soil after 60 days of cultivation. For isolation, seven fragments of symptomatic tissue from each plant (stem and roots) were disinfected in a 2% sodium hypochlorite (NaClO) solution and washed twice in sterilized distilled water to remove excess NaClO. The fragments were placed on filter paper to remove excess water. The fragments were then placed on Petri dishes containing PDA medium supplemented with 500 ppm streptomycin sulfate. The plates were incubated at 28 ± 1 °C,

and as mycelial growth was observed, the colonies were individually subcultured into Petri dishes containing only PDA for subsequent quantification and fungal identification at the genus level. For the identification of the colonies at the genus level, slides were prepared for microscopy containing fungal vegetative and reproductive structures, stained with lactofenol cotton blue, observed under an optical microscope, and compared to the typical morphological characteristics of the genera *Macrophomina*, *Fusarium*, and *Monosporascus*.

2.2.4. Experimental Procedure

At the end of each of the first three growing cycles (approximately 60 days), the plant residues from each treatment containing *C. ensiformis* were processed in a forage harvester and incorporated into the soil for use in the next cycle. The rest period between cycles was 30 days. The soil was moistened every three days (1.00 L of water) to promote decomposition of the incorporated material.

At the start of each new cycle, the soil in each pot was turned before transplanting the melon or sowing the Jack Bean, to promote root development. At the end of the three rotational cycles for each treatment, a fourth additional cycle was conducted, in which melon plants were grown in all treatments, in order to evaluate the effect of the different rotational cycles on RRVD management, through the observation of symptoms and the relative frequency of pathogens associated with the different treatments, based on isolation frequency.

During the experiment, prior to the final evaluation (the fourth cycle, in which melon was grown in all treatments), the roots of the melon plants (symptoms, signs, and isolation frequency) were also evaluated in each treatment to obtain complementary data on the dynamics of root pathogens present in the soil. The experiment was repeated.

2.2.5. Variables Analyzed

The phytopathogen diversity in the initial soil was assessed based on the isolation frequency of 50 melon plants grown in soil freshly collected after harvest and expressed as a percentage (%).

Disease incidence was evaluated by counting symptomatic melon plants within each treatment, with values expressed as a percentage (%). Disease severity was assessed using the rating scale proposed by Ravf et al. [39], where: 0 = no symptoms; 1 = less than 3% of tissues infected; 2 = 3 to 10% of tissues infected; 3 = 11 to 25% of tissues infected; 4 = 26 to 50% of tissues infected; and 5 = more than 50% of tissues infected. The assessment of disease severity was performed by a single evaluator.

The biometric variables, root length (RL) and shoot length (SL), were measured using a measuring tape graduated in cm. The dry weight of roots (DWR) and the dry weight of shoots (DWS) were determined using a digital analytical balance after drying the plant material in an oven heated to 65 °C for three days, until a constant weight was achieved.

The frequency of fungal isolation for each treatment was determined based on the isolation frequency from all 10 melon plants from each treatment, expressed as a percentage (%). The fungal isolation methodology was conducted the same as is described above.

2.2.6. Statistical Analysis

For disease incidence and disease severity, the nonparametric test of Kruskal–Wallis ($p \leq 0.05$) was used with ASSISTAT v. 7.7 software [40]. For the biometric variables (RL, SL, DWR, and DWS), the Tukey test ($p \leq 0.05$) was used to compare means. Data regarding disease severity, incidence, biometric variables, and the frequency of pathogen isolation were subjected to Principal Component Analysis (PCA), performed using the statistical software PAST (version 4.03): Paleontological Statistics software package for education and data analysis.

3. Results

3.1. Experiment I: Enzymatic Response of *C. ensiformis* to *M. pseudophaseolina*

β -1,3-Glucanase (BG), Chitinase (CT), and Phenylalanine-Ammonia Lyase (PAL)

In Factor 1 (leaves vs. roots), a difference was observed only at 21 DAT, when the roots showed higher activity ($0.112 \text{ EU min}^{-1} \text{ mL}^{-1}$) than the leaves ($0.085 \text{ EU min}^{-1} \text{ mL}^{-1}$), according to the *t*-test at the 5% probability level (Table 1). However, there was no significant difference in BG at 28 and 35 DAT.

Table 1. Enzymatic activity of β -1,3-glucanase in *C. ensiformis* grown in soil infested with *M. pseudophaseolina* at 21, 28, and 35 DAT.

Treatments	β -1,3-Glucanase (BG) (E.U. $\text{min}^{-1} \text{ mL}^{-1}$)		
Factor 1	21 DAT	28 DAT	35 DAT
Leaves	0.085 b	0.97 a	0.102 a
Roots	0.112 a	0.096 a	0.102 a
MSD	0.006	0.007	0.005
CV (%)	4.72	5.21	3.82
Factor 2	21 DAT	28 DAT	35 DAT
Non-inoculated	0.085 b	0.084 b	0.089 b
Inoculated	0.112 a	0.110 a	0.115 a
MSD	0.006	0.006	0.005
CV (%)	4.72	5.21	3.82

MSD = Minimum Significant Difference. CV (%) = coefficient of variation; Means followed by the same letter do not differ statistically from each other. A *t*-test was performed at the 5% significance level. DAT = Days After Transplantation. E.U. = Enzyme Units.

In factor 2, there was a significant difference between treatments for BG in all evaluation periods, with the inoculated treatment showing greater activity compared to the non-inoculated treatment at 21 ($0.112 \text{ E.U. min}^{-1} \text{ mL}^{-1}$), 28 ($0.110 \text{ E.U. min}^{-1} \text{ mL}^{-1}$), and 35 DAT ($0.115 \text{ E.U. min}^{-1} \text{ mL}^{-1}$) (Table 1).

There was an interaction between the factors studied for BG activity at 7 and 14 DAT. However, for plants that were not inoculated, there was no difference between leaves and roots (lines) at 7 and 14 DAT. However, there was a difference in the inoculated treatments, where BG expression in the leaves was higher than in the roots (lines) at 7 and 14 DAT, with values of $0.113 \text{ EU min}^{-1} \text{ mL}^{-1}$ (leaves) and $0.097 \text{ EU min}^{-1} \text{ mL}^{-1}$ (roots) at 7 DAT and $0.112 \text{ EU min}^{-1} \text{ mL}^{-1}$ (leaves) and $0.098 \text{ EU min}^{-1} \text{ mL}^{-1}$ (roots) at 14 DAT (Table 2).

It was also observed that BG activity was higher in the leaves of inoculated plants (0.113 and $0.112 \text{ E.U. min}^{-1} \text{ mL}^{-1}$) compared to non-inoculated plants, in both the 7 and 14 DAT, respectively (columns). For the roots, there was no statistical difference between inoculated and non-inoculated plants in both evaluated periods (columns) (Table 2).

Overall, BG activity was consistently higher in inoculated plants, particularly in roots, indicating sustained activation of this defense mechanism over time.

In CT, for factor 1 a significant difference was observed at all DAT, with root activity at 14 ($32.591 \text{ E.U. mg}^{-1} \text{ protein}$), 21 ($28.056 \text{ E.U. mg}^{-1} \text{ protein}$), and 28 DAT ($22.797 \text{ E.U. mg}^{-1} \text{ protein}$) was higher than that observed for leaves (Table 3).

About factor 2, a difference was observed only at 21 DAT, where CT activity in the inoculated treatment ($19.085 \text{ E.U. mg}^{-1} \text{ protein}$) was higher than in the non-inoculated treatment (Table 3).

Table 2. Enzymatic activity of β -1,3-glucanase in *C. ensiformis* grown in soil infested with *M. pseudophaseolina* at 7 and 14 DAT.

β-1,3-Glucanase (BG) (E.U. min ⁻¹ mL ⁻¹)				
7 DAT				
Factor 1 \ Factor 2	Non-inoculated	Inoculated	MSD (Rows)	
Leaves	0.099 aB	0.113 aA	0.009	
Roots	0.103 aA	0.097 bA		
MSD (Columns)	0.009			
CV (%)	4.78			
14 DAT				
Factor 1 \ Factor 2	Non-inoculated	Inoculated	MSD (Rows)	
Leaves	0.081 aB	0.112 aA	0.012	
Roots	0.092 aA	0.098 bA		
MSD (Columns)	0.012			
CV (%)	6.74			

MSD = Minimum Significant Difference. CV (%) = coefficient of variation; Means followed by the same letter do not differ statistically from each other. A *t*-test was performed at the 5% significance level. Lowercase letters = rows. Uppercase letters = columns. DAT = Days After Transplantation. E.U. = Enzyme Units.

Table 3. Enzymatic activity of Chitinase in *C. ensiformis* grown in soil infested with *M. pseudophaseolina* at 14, 21, and 28 DAT.

Treatments	Chitinase (CT) (E.U. mg ⁻¹ Protein)			
Factor 1	14 DAT	21 DAT	28 DAT	
Leaves	6.508 b	6.048 b	8.323 b	
Roots	32.591 a	28.056 a	22.797 a	
MSD	15.278	3.392	7.785	
CV (%)	28.60	14.92	27.51	
Factor 2	14 DAT	21 DAT	28 DAT	
Non-inoculated	15.103 a	15.020 b	15.350 a	
Inoculated	23.995 a	19.085 a	15.771 a	
MSD	15.278	3.392	7.785	
CV (%)	28.60	14.92	27.51	

MSD = Minimum Significant Difference. CV (%) = coefficient of variation; means followed by the same letter do not differ statistically from each other. A *t*-test was performed at the 5% significance level. DAT = Days After Transplantation. E.U. = Enzyme Units.

There was an interaction between the factors for CT expression at 7 and 35 DAT (Table 4), where CT activity at the root level was higher than at the leaf level (lines), both in the non-inoculated treatment (31.124 E.U. mg⁻¹ protein) and in the inoculated treatment (11.755 E.U. mg⁻¹ protein) at 7 DAT, as well as in the non-inoculated treatment (22.683 E.U. mg⁻¹ protein) at 35 DAT. There was no significant difference between leaves and roots in the inoculated treatment at 35 DAT.

There was a difference in CT activity between the non-inoculated treatment and the inoculated treatment (columns) only for the roots, both at 7 (31.124 E.U. mg⁻¹ protein) and at 35 DAT (22.683 E.U. mg⁻¹ protein) (Table 4). Therefore, CT activity was predominantly higher in the roots compared to the leaves throughout the evaluated periods.

Table 4. Enzymatic activity of Chitinase in *C. ensiformis* grown in soil infested with *M. pseudophaseolina* at 7 and 35 DAT.

Chitinase (CT) (E.U. mg ⁻¹ Protein)				
7 DAT				
Factor 1	Factor 2	Non-inoculated	Inoculated	MSD (Rows)
Leaves		3.034 bA	2.806 bA	6.772
Roots		31.124 aA	11.755 aB	
MSD (Columns)		6.772		
CV (%)		28.48		
35 DAT				
Factor 1	Factor 2	Non-inoculated	Inoculated	MSD (Rows)
Leaves		9.758 bA	7.203 aA	5.460
Roots		22.683 aA	7.495 aB	
MSD (Columns)		5.460		
CV (%)		24.56		

MSD = Minimum Significant Difference. CV (%) = coefficient of variation; means followed by the same letter do not differ statistically from each other. A *t*-test was performed at the 5% significance level. Lowercase letters = rows. Uppercase letters = columns. DAT = Days After Transplantation. E.U. = Enzyme Units.

When evaluating factor 1, a difference in PAL activity was observed, with root activity being higher than leaf activity at 14 (0.363 E.U. min⁻¹ mg⁻¹ protein), 28 (1.977 E.U. min⁻¹ mg⁻¹ protein), and 35 DAT (3.801 E.U. min⁻¹ mg⁻¹ protein) (Table 5).

Table 5. Enzymatic activity of Phenylalanine ammonia-lyase (PAL) in *C. ensiformis* grown in soil infested with *M. pseudophaseolina* at 14, 28, and 35 DAT.

Treatments	Phenylalanine Ammonia-Lyase (PAL) (E.U. min ⁻¹ mg ⁻¹ Protein)		
Factor 1	14 DAT	28 DAT	35 DAT
Leaves	0.177 b	0.464 b	0.453 b
Roots	0.363 a	1.977 a	3.801 a
MSD	0.144	0.375	1.517
CV (%)	29.192	23.02	23.47
Factor 2	14 DAT	28 DAT	35 DAT
Non-inoculated	0.219 a	1.344 a	1.743 a
Inoculated	0.321 a	1.097 a	2.511 a
MSD	0.144	0.375	1.517
CV (%)	29.92	23.02	23.47

MSD = Minimum Significant Difference. CV (%) = coefficient of variation; means followed by the same letter do not differ statistically from each other. A *t*-test was performed at the 5% significance level. DAT = Days After Transplantation.

There was no difference for factor 2 non-inoculated versus inoculated at any of the evaluation periods (Table 5).

There was an interaction between the factors for PAL activity at 7 and 21 DAT (Table 6). However, root-level activity was higher than leaf-level activity (rows) only in plants inoculated at 7 (0.502 E.U. min⁻¹ mg⁻¹ protein) and 21 DAT (1.274 E.U. min⁻¹ mg⁻¹ protein).

Table 6. Enzymatic activity of Phenylalanine ammonia-lyase (PAL) in *C. ensiformis* grown in soil infested with *M. pseudophaseolina* at 7 and 21 DAT.

Phenylalanine Ammonia-Lyase (PAL) (E.U. min ⁻¹ mg ⁻¹ Protein)				
7 DAT				
Factor 1	Factor 2	Non-inoculated	Inoculated	MSD (Rows)
Leaves		0.157 aA	0.116 bA	0.191
Roots		0.234 aB	0.502 aA	
MSD (Columns)		0.191		
CV (%)		30.18		
21 DAT				
Factor 1	Factor 2	Non-inoculated	Inoculated	MSD (Rows)
Leaves		0.480 aA	0.512 bA	0.215
Roots		0.375 aB	1.274 aA	
MSD (Columns)		0.215		
CV (%)		17.25		

MSD = Minimum Significant Difference. CV (%) = coefficient of variation; means followed by the same letter do not differ statistically from each other. A *t*-test was performed at the 5% significance level. Lowercase letters = rows. Uppercase letters = columns. DAT = Days After Transplantation.

PAL activity in the inoculated treatment was higher than in the non-inoculated treatment (columns) only in the roots, both at 7 (0.502 E.U. min⁻¹ mg⁻¹ protein) and at 21 DAT (1.274 E.U. min⁻¹ mg⁻¹ protein) (Table 6). Overall, PAL was predominantly higher in roots compared to leaves throughout the evaluated stages, with a more evident increase in plants inoculated in the initial phases, while the effect of inoculation was restricted to roots at certain periods.

When evaluating enzymatic activities throughout the growth cycle, it can be observed that for β -1,3-glucanase, there was higher enzymatic activity in the inoculated treatment compared to the non-inoculated. However, for the roots, this activity increases gradually over the DAT, where in the leaves this rate remains constant over the DAT (approximately E.U. min⁻¹ mL⁻¹) (Figure 2a,b).

For chitinase in leaves, both treatments showed similar activity at 7 DAT; however, the inoculated treatment showed a rapid increase compared to the control, peaking at 14 DAT, followed by a decline in subsequent DAT. The control remained stable until approximately 21 DAT, then showed a higher rate until 35 DAT (Figure 2c).

In roots, CT activity was initially higher in the non-inoculated treatment at 7 DAT, but increased in inoculated plants up to 14 DAT, surpassing the control around 11 DAT before declining to 35 DAT. In contrast, the control showed a continuous decrease in activity overtime, reaching its lowest level at 35 DAT (Figure 2d).

For PAL at the leaf level, activity increased in both treatments over the course of the DAT, up to 21 DAT. The inoculated treatment surpassed the non-inoculated starting at 10 DAT, maintaining higher PAL expression in subsequent DAT, although both treatments showed a reduction in PAL expression after 21 DAT (Figure 2e). At the root level, there was an increase in the activity of this enzyme between 14 and 21 DAT, which intensified up to 35 DAT, where the inoculated treatment showed higher mean expression than the non-inoculated, although the non-inoculated treatment also showed an increase over the DAT (Figure 2f).

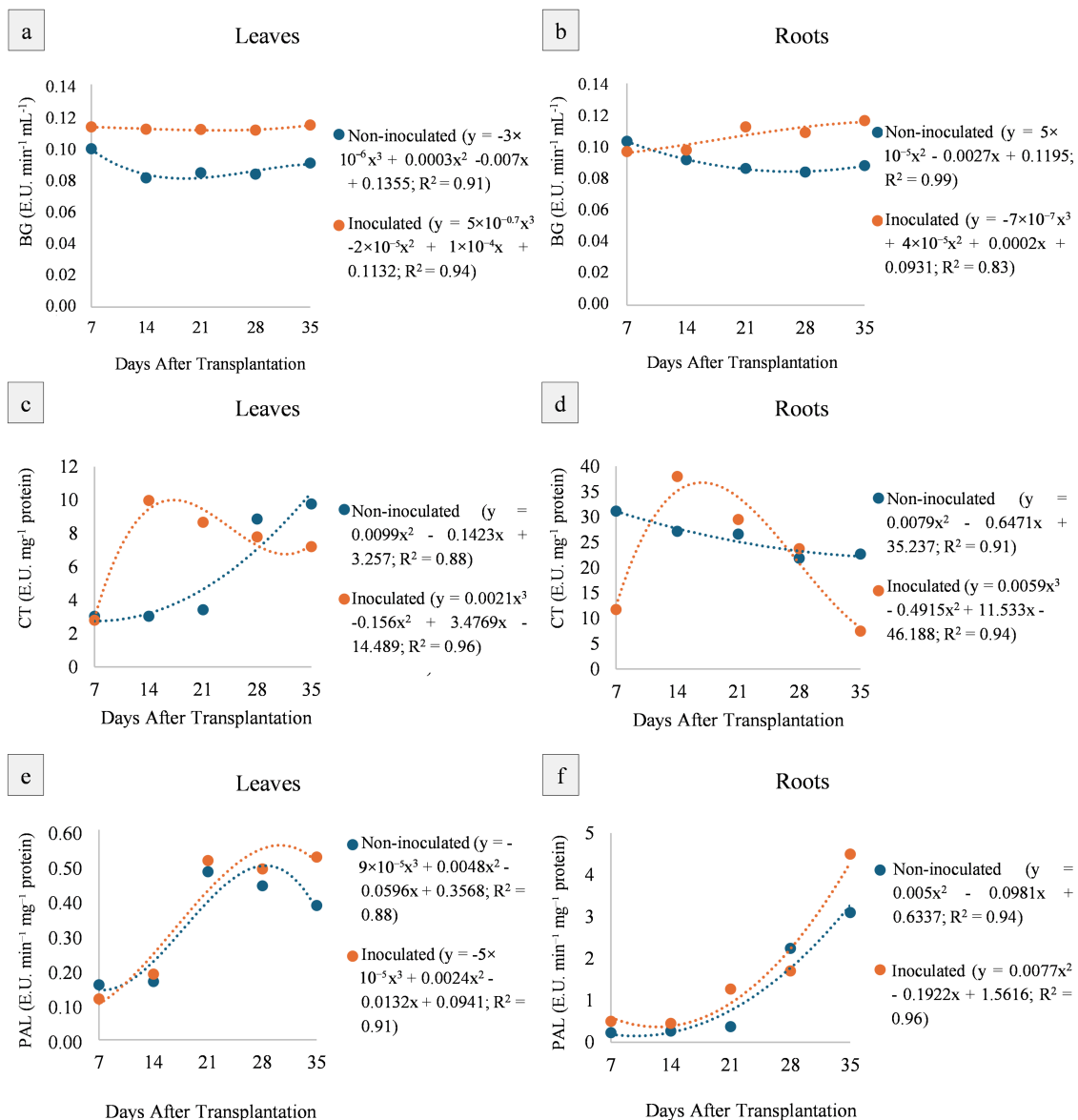


Figure 2. Enzyme activity of β -1,3-glucanase (BG) (a,b), chitinase (CT) (c,d), and phenylalanine-ammonia lyase (PAL) (e,f) in leaves and roots of *C. ensiformis* inoculated with *M. pseudophaseolina* compared to the control at 7, 14, 21, 28, and 35 days after transplantation.

3.2. Experiment II: Use of *Canavalia ensiformis* in the Management of Root Rot and Vine Decline (RRVD) in Melon Plants

3.2.1. Initial Isolation Frequency of Pathogenic Fungi Associated with RRVD, Disease Incidence, and Disease Severity

Regardless of rotation strategy, RRVD incidence remained at 100%, indicating limited effectiveness of green manure alone under high inoculum pressure. The frequency of isolates of phytopathogenic fungi associated with RRVD from the fifty plants was as follows at the genus level (Figure 3).

Analysis of the experiment indicated that there was no significant difference in disease incidence ($\chi^2 = 15.51$; $p \leq 0.05$) or disease severity ($\chi^2 = 15.51$; $p \leq 0.05$), according to the test of Kruskal–Wallis, in which all treatments evaluated were inferior to the control treatment (Table 7).

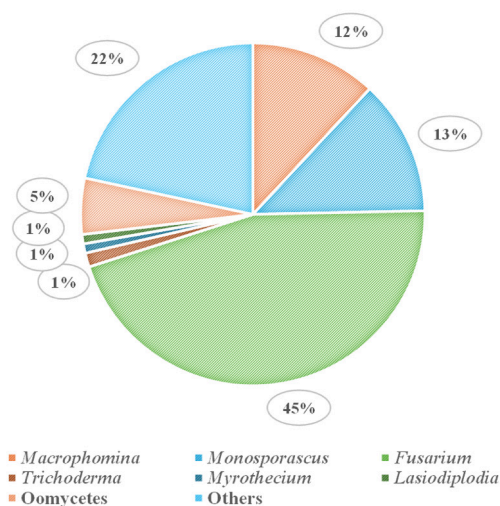


Figure 3. Percentage frequency of isolation of plant pathogenic fungi associated with RRVD in the initial soil.

Table 7. Disease incidence and disease severity of root rot and vine decline (RRVD) in melon plants following different rotation cycles between Jack Bean (JB) and melon (ME).

Treatments	Disease Incidence (%)		Disease Severity	
	Rank	Mean	Rank	Mean
Control	5.5 a	0	5.5 a	0
JB-JB-JB	50.5 b	100	46.3 b	4.7
JB-JB-ME	50.5 b	100	46.3 b	4.9
JB-ME-ME	50.5 b	100	46.3 b	4.6
JB-ME-JB	50.5 b	100	46.3 b	4.9
ME-ME-ME	50.5 b	100	46.3 b	4.9
ME-JB-JB	50.5 b	100	46.3 b	4.5
ME-ME-JB	50.5 b	100	46.3 b	4.9
ME-JB-ME	50.5 b	100	46.3 b	4.9
χ^2 tab	15.51	-	15.51	-
χ^2	46.88	-	89.00	-

Control: ME-ME-ME (autoclaved soil); χ^2 tab = tabulated significant chi-square values. χ^2 = significant chi-square values; values followed by the same letter in the columns show no statistically significant difference from each other according to the nonparametric test of Kruskal–Wallis ($p \leq 0.05$).

The different rotation cycles did not affect the development of the disease, which had a 100% incidence rate across all treatments; high average severity scores were also observed for all treatments, ranging from 4.5 to 4.9.

3.2.2. Biometric Variables

According to the Tukey test ($p \leq 0.05$), a significant effect of treatment was observed for all biometric variables analyzed (Table 8). Treatments including at least one cycle of Jack Bean generally showed improved vegetative performance compared to continuous melon cultivation.

For the RL variable, the JB-JB-JB treatment (25.24 cm) did not differ from the control treatment (24.80 cm), and both were greater than the JB-ME-JB treatment (17.30 cm) and the ME-JB-ME treatment (16.16 cm). The other treatments did not differ from one another or from the control treatment.

For SL, the control treatment (143.50 cm) was superior to the JB-JB-ME (72.80 cm), ME-ME-ME (75.45 cm), and ME-JB-ME (69.30 cm) treatments. The other treatments did not differ from one another or from the control (Table 8).

Table 8. Root length (RL), shoot length (SL), dry weight of roots (DWR), and dry weight of shoots (DWS) of melon plants on different cycles of crop rotation between Jack Bean (JB) and melon (ME).

Treatments	RL (cm)	SL (cm)	DWR (g)	DWS (g)
Control	24.80 a	143.50 a	0.45 a	20.22 a
JB-JB-JB	25.24 a	97.00 ab	0.33 b	10.00 b
JB-JB-ME	18.40 abc	72.80 b	0.18 de	6.45 c
JB-ME-ME	18.50 abc	106.50 ab	0.28 bcd	10.03 b
JB-ME-JB	17.30 bc	91.80 ab	0.18 de	7.38 bc
ME-ME-ME	21.14 abc	75.45 b	0.21 cde	6.45 c
ME-JB-JB	21.00 abc	103.20 ab	0.30 bc	8.74 bc
ME-ME-JB	20.00 abc	100.80 ab	0.28 bcd	9.11 bc
ME-JB-ME	16.16 c	69.30 b	0.17 e	6.01 c
MSD	7.54	55.61	0.09	3.54
CV (%)	26.7	20.8	24.49	26.46

Control: ME-ME-ME (autoclaved soil); MSD = Minimum Significant Difference. CV (%) = coefficient of variation; values followed by the same letter in the columns show no statistically significant difference from each other according to the test of Tukey ($p < 0.05$).

Regarding DWR, the control treatment (0.45 g) was higher than all others. The second-highest mean was observed in the JB-JB-JB treatment (0.33 g), which was higher than the ME-ME-ME (0.21 g), JB-JB-ME (0.18 g), JB-ME-JB (0.18 g), and ME-FE-ME (0.17 g) treatments.

For DWS, all treatments were lower than the control treatment. The JB-JB-ME (6.45 g), ME-ME-ME (6.45 g), and ME-JB-ME (6.01 g) treatments had the lowest DWS means and were lower than the JB-JB-JB (10.0 g) and JB-ME-ME (10.03 g) treatments. The remaining treatments were lower only in relation to the control and did not differ from each other or from the other treatments (Table 8).

3.2.3. Isolation Frequency Among Treatments

A difference was observed in the isolation frequency of *Macrophomina* spp., where the ME-ME-ME treatment maintained a high isolation frequency of this pathogen (16%), whereas the other treatments, which included at least one growing cycle of Jack Bean, showed frequencies ranging from 0% to 4%. The ME-ME-ME treatment maintained a high isolation frequency of *Monosporascus* spp. (20%) compared to the others (0% to 7%), and it is also the only treatment where fungi of the genus *Trichoderma* was isolated. It is also observed that the isolation frequency of *Monosporascus* spp. tends to increase in treatments with two or more consecutive melon cycles following Jack Bean cultivation, such as JB-ME-ME (7%), in contrast to the ME-ME-JB treatment (0%). All treatments with at least one cycle of Jack Bean cultivation showed an increase in the frequency of *Fusarium* spp. isolation (80% to 92%) compared to the ME-ME-ME treatment (54%) (Figure 4).

In this study, it was also found that the symptoms present in the roots of diseased plants, regardless of whether they exhibited collar rot symptoms, were consistent with the damage caused by *Monosporascus*, including root browning, a fragile root system, and loss of root hairs. The production of reproductive structures (perithecia) of *Monosporascus* were also observed, visible to the naked eye and under an optical microscope, even in the JB-JB-JB treatment, which received the highest deposition of *C. ensiformis* plant debris (Figure 5).

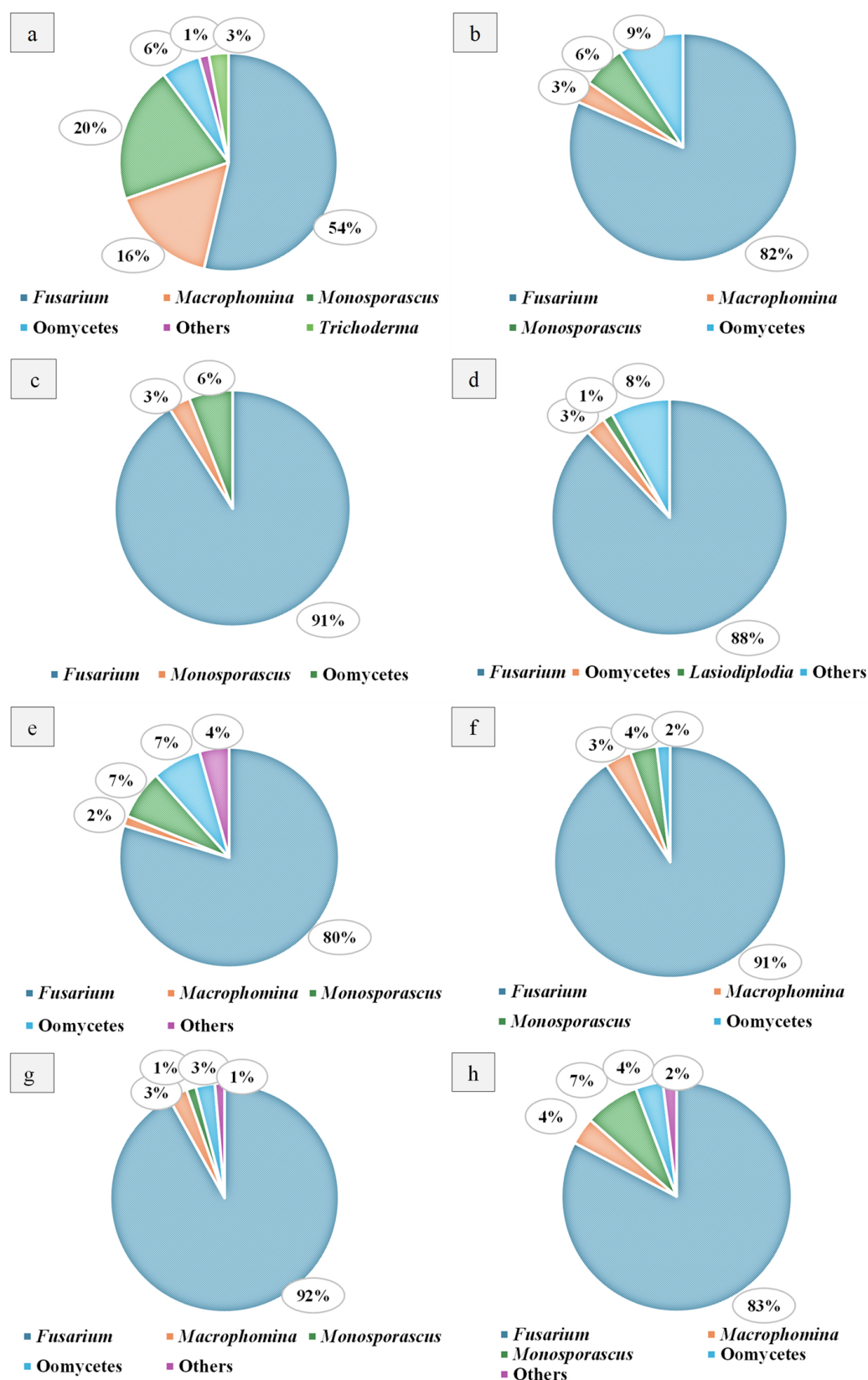


Figure 4. Percentage distribution of the frequency of fungal genus isolates associated with RRVD in different treatments using crop rotations of melon (ME) and Jack Bean (JB) grown in naturally infested soil. (a) ME-ME-ME; (b) JB-JB-JB; (c) ME-JB-JB; (d) ME-ME-JB; (e) JB-ME-ME; (f) JB-JB-ME; (g) JB-ME-JB; and (h) ME-JB-ME.

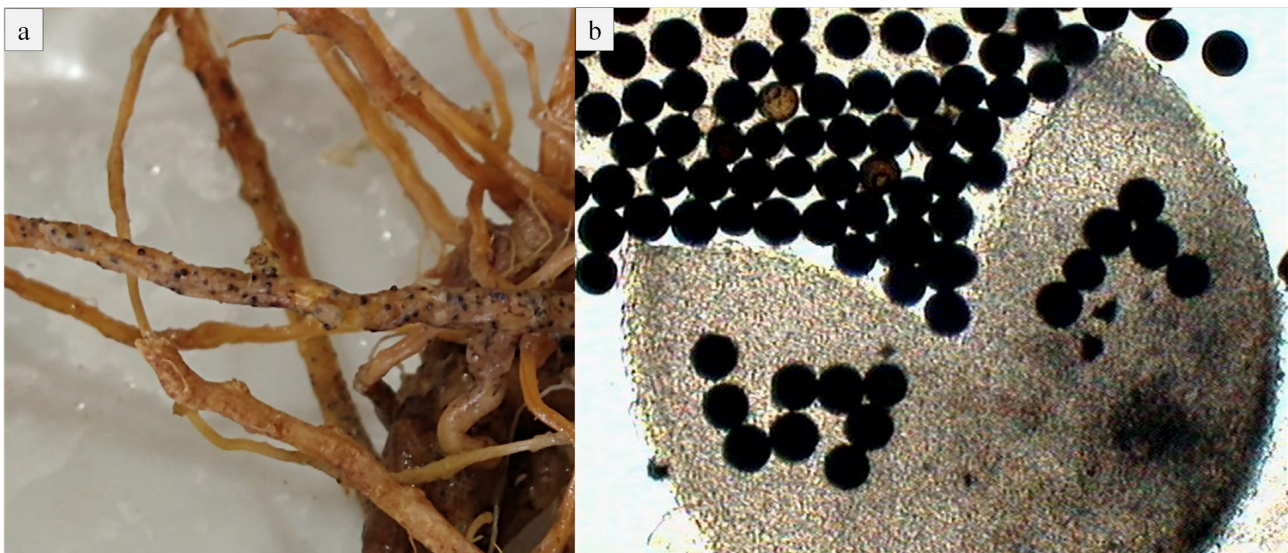


Figure 5. (a) Perithecia of *Monosporascus* embedded in a melon roots, viewed under a stereomicroscope; (b) Perithecium and ascospores of *Monosporascus* sp., viewed under an optical microscope (40× magnification).

3.2.4. Principal Component Analysis (PCA)

The PCA conducted to examine the relationship between the different treatments and the variables disease incidence, disease severity, root length (RL), shoot length (SL), dry weight of roots (DWR), and dry weight of shoots (DWS) yielded two components. Component 1 (horizontal) accounts for 84.52% of the total experimental variation, and Component 2 (vertical) accounts for 10.63% of the experimental variation, totaling 95.15% of the total observed experimental variation (Figure 6).

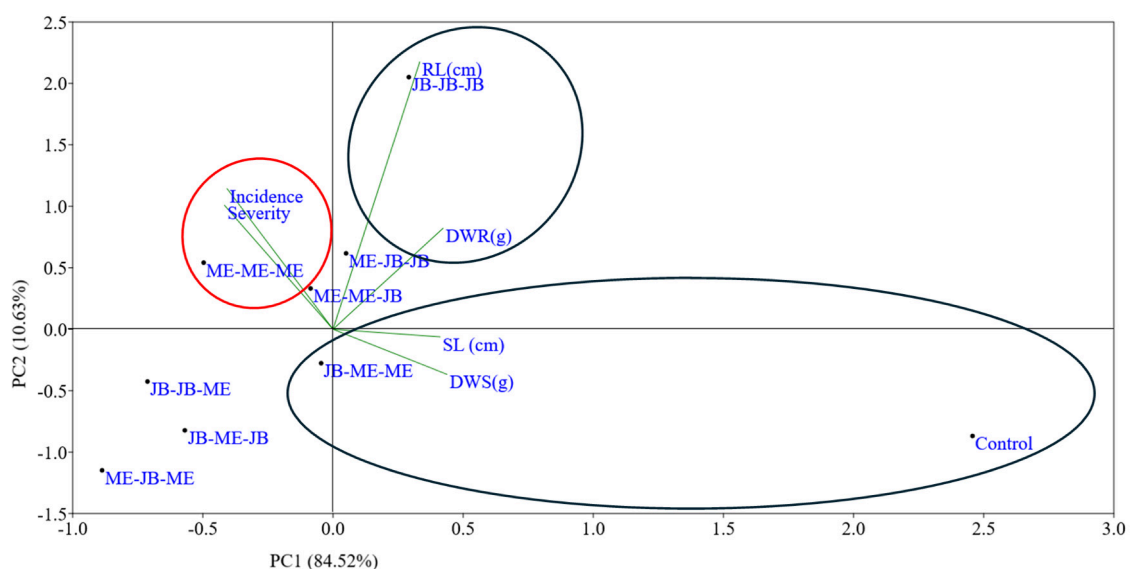


Figure 6. Principal component analysis (PCA) of the correlation between different rotation cycles of Jack Bean (JB) and melon (ME) with respect to the variables disease incidence, disease severity, root length (RL), shoot length (SL), dry weight of roots (DWR), and dry weight of shoots (DWS). PC1 = Principal Component 1 (horizontal); PC2 = Principal Component 2 (vertical).

The control treatment and the variables DWS and SL were closely associated with PC1, where the sterile soil provided better vegetative development of the melon plant. However, the JB-JB-JB treatment and the RL variable were more closely associated with PC2.

The ME-ME-ME treatment (without JB cultivation) correlated closely with high disease incidence and disease severity and negatively with the SL and DWS variables, while the JB-JB-ME and JB-ME-JB treatments correlated negatively with the RL and DWR variables and showed low correlation with the SL and DWS variables, whereas the ME-JB-JB, ME-ME-JB, and JB-ME-ME treatments showed moderate performance and low correlation with all analyzed variables (Figure 6).

The PCA performed to examine the relationship between the frequency of isolates and the different treatments that yielded two components, with PC 1 (horizontal) accounting for 98.1% of the experimental variation, and PC2 (vertical) accounts for an additional 1.70% of the experimental variation, totaling 99.71% of the total observed experimental variation (Figure 7).

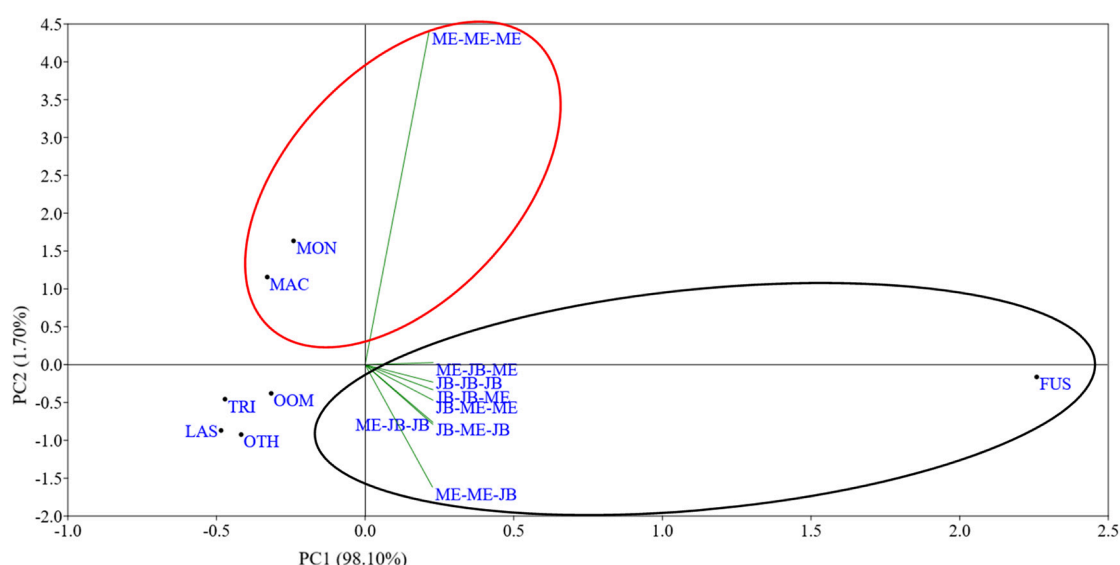


Figure 7. Principal component analysis (PCA) of the correlation between different crop rotations of Jack Bean (JB) and melon (ME) in relation to plant pathogenic species associated with RRVD. MAC = *Macrophomina*; MON = *Monosporascus*; FUS = *Fusarium*; OOM = *Oomycetes*; TRI = *Trichoderma*; LAS = *Lasiodiplodia*; OTH = others. PC1 = Principal Component 1 (horizontal); PC2 = Principal Component 2 (vertical).

According to PCA, the main variable associated with PC1 was the genus *Fusarium* (FUS), which showed a quantitative predominance of isolates in all treatments with at least one cycle of Jack Bean incorporation (Figure 7).

Regarding PC2, it was strongly associated with the ME-ME-ME treatment (without any Jack Bean cultivation), which is most closely related to the isolation rates of *Macrophomina* (MAC) and *Monosporascus* (MON) (Figure 7).

4. Discussion

In general, inoculation with *M. pseudophaseolina* in a greenhouse induced changes in the activity of defense-related enzymes in *C. ensiformis*, although the intensity and pattern of response varied among enzymes, plant organs, and evaluation periods. Consistently, higher enzymatic activity was observed in root tissues for CT and PAL, BT showed responses more dependent on treatment and time.

For the mechanisms of *C. ensiformis* resistance to *M. pseudophaseolina*, our studies found strong evidence of the contribution of key enzymatic mechanisms, as there was an increase in activity of the enzymes Chitinase (CT) and β -1,3-Glucanase (BG) in plants inoculated with this pathogen, especially in the roots. This increase in response to inoculation may

be related to Jack Bean promoting the degradation of the cell wall of *M. pseudophaseolina* (through the action of CT and BG) [24,26].

Furthermore, the observation that the expression level of PAL is higher in inoculated plants compared to the control suggests that the production of phenolic compounds generated through this metabolic pathway such as phytoalexins and lignins [28–31] is intensified in response to pathogen detection in the root tissues of *C. ensiformis* (post-formation mechanisms), and further studies are needed to elucidate this. The increase in PAL expression over the course of the DAT, even in the control treatment, is because this metabolic pathway is also fundamental for plant growth and phenological development [28].

Regarding CT, at the root level, the higher expression rate in the control treatment (7 DAT) compared to the inoculated treatment, may involve additional defense mechanisms not evaluated in the present study. This fact may be related to the release of oligomers resulting from the degradation of the present chitin in the cell wall of *M. pseudophaseolina*. This finding may be related to the release of oligomers resulting from the degradation of chitin present in the cell wall of *M. pseudophaseolina*. These oligomers, once recognized by the defense system of *C. ensiformis*, can trigger the expression of specific genes that are more effective in neutralizing this pathogen and halting the progression of infection [41–43], thereby acting as elicitors.

These findings regarding the role of enzymatic mechanisms in response to the inoculation of Jack Bean with *M. pseudophaseolina* complement studies such as that by Lima et al. [20], which demonstrated the resistance of this species to *Macrophomina* spp., and point to its viability for cultivation in areas infested with these pathogens.

About the use of *C. ensiformis* as green manure, although there was no significant difference among the treatments in terms of disease incidence and severity, the fact that most treatments involving at least one cycle of cultivation and incorporation of Jack Bean showed higher means for the biometric variables indicates a beneficial effect of this technique on the management of the root pathosystems of the melon plant and that the different pathogens present in the soil respond differently to the use of this technique.

In this regard, the reduced frequency of *Macrophomina* and *Monosporascus* isolates in treatments involving the cultivation and incorporation of cowpeas compared to the ME-ME-ME treatment (naturally infested soil) clearly reinforces the importance of crop rotation and green manuring within cultural management and integrated disease management, since continuous cultivation of the same crop maintains high levels of inoculum of the main pathogens associated with the primary crop [44,45].

These findings also point to a greater efficiency of *C. ensiformis* inhibiting *Macrophomina*, which maintained a lower isolation frequency among the treatments in which this legume was used, although this species also favored the development of *Fusarium* in the different rotational cycles, where this pathogen increased its predominance. These findings express the complexity existing within the cultural management of multiple pathosystems, where different techniques (such as crop rotation and green manure) act differentially on the various soil inhabitants. In this sense, the work of Jain et al. [21] corroborates these results, where the authors observed that a 2% concentration of canatoxin (a toxic protein present in seeds) inhibited the mycelial growth of *M. phaseolina*, but without a significant effect on the development of *Fusarium oxysporum*. In our studies, *C. ensiformis* was cultivated until the milky grain stage, close to maturity, possibly contributing to the deposition of this substance in the soil. The action of other mechanisms, such as the BC and CT enzymes and other metabolites produced from phenolic compounds (obtained through increased expression of PAL), as verified in experiment I, may also have acted in an inhibitory manner on the development of *Macrophomina* throughout the development of Jack Bean plants and jointly contributed to the suppression of this pathogen in the cultivation soil.

In greenhouse conditions, although the frequency of *Macrophomina* isolation was reduced in the treatments with Jack Bean, the incidence of the disease remained high throughout the experiment. This result suggests that other variables may be involved in disease development, such as the involvement of other soil-borne pathogens. Thus, the results obtained indicate that Jack Bean should be considered as a component of integrated disease management. The reduction in the frequency of *Macrophomina* isolation demonstrates its potential to reduce inoculum pressure in the soil and improve soil health.

The increased percentage of *Fusarium* isolation observed in all treatments with at least one cycle of Jack Bean indicates a high multiplication rate of this pathogen during crop development, as well as on the plant residues of the incorporated fertilizer. *Fusarium* can infect roots of Jack Bean [46], and is not affected by *Canavalia*, one of the most fungitoxic proteins produced by this fabaceous plant [21]. Furthermore, tris fungal can survive in the environment by producing chlamydospores and by colonizing the organic matter in the soil [47]. These facts generate a high inoculum pressure of this pathogen in the melon crop cultivated subsequently, and may be related to the high levels of incidence and severity of the disease observed in this study. Porto et al. [25], when evaluating the efficiency of green manure with Jack Bean in controlling RRVD in melon, observed that the incidence of the disease was higher (100%) where Jack Bean was incorporated. The authors attributed part of this effect to the multiplication of *Fusarium* (previously inoculated into the soil) in the organic matter from this plant species.

The frequency of isolation of the genus *Monosporascus*, although it showed a reduction after treatments consisting of previous cycles of Jack Bean cultivation, tends to increase in subsequent cycles of melon cultivation (JB-ME-ME), for example, the presence of green manure in the soil only delays the penetration of *Monosporascus* spp. into melon roots.

Another fact that corroborates this hypothesis was the visualization of *Monosporascus* perithecia in melon roots, even in treatments with the incorporation of *C. ensiformis* in previous cycles. This may be related to the saprophytic survival of this fungus in the crop residues of green manure (same for *Fusarium*), in which the metabolites produced by *C. ensiformis*, as well as those from the decomposition of its tissues, exert little or no inhibitory effect on these fungi [21,46], and that *Monosporascus* is competitive and has the ability to survive with other soil inhabitants in organic matter.

Trials conducted by Sales Junior et al. [48], in which the authors worked with different green manures, including *C. ensiformis*, support this hypothesis, where they did not obtain success in controlling the disease caused by *Monosporascus* with the use of Jack Bean, obtaining disease levels similar to that of naturally infested soil without any treatment (89.06% and 100% respectively) with the use of this species, where only the number of incorporation cycles were different. The same study also detected an increase in the inoculum density of *M. cannonballus* in soil where Jack Bean had been cultivated in alternating cycles with melon (1.10 ascospores g⁻¹ of soil) compared to soil without incorporation (0.89 ascospores g⁻¹ of soil); whereas, in soil where two consecutive cycles of incorporation of Jack Bean had occurred, the inoculum density was lower, indicating that the pathogen survived in the incorporated organic matter, and that the return of *Monosporascus* to parasitic activity depended mainly on the availability of organic matter in the soil, which in fact served as food for this pathogen. Together, these findings demonstrate that green manuring with *C. ensiformis* has pathogen-specific and sometimes antagonistic effects within the RRVD complex.

In our studies, it was also observed that in melon plants grown in succession to a single cycle of Jack Bean, as in the alternating treatments JB-ME-JB and ME-JB-ME, the symptoms of the disease in the melon appeared earlier at the base of the plant, probably due to the phytopathogenic synergy of *Fusarium* with the other pathogens [25] remaining in the soil

after the incorporation of *C. ensiformis*, where the root injuries caused by these causal agents favored the rapid penetration and colonization of other tissues by the *Fusarium* species that prevailed in the soil.

One of these remnants, *Monosporascus cannonballus*, produces a wide range of metabolites potentially responsible for weakening the host even before its penetration. Studies conducted by Al-Rawahi et al. [49], using a filtrate containing metabolites (non-fungal structures) of *M. cannonballus*, which was applied to cucumber leaves, was able to induce necrosis symptoms 5 to 7 days after application, and this same filtrate reduced the vigor of cucumber seedlings. Additionally, Hosseini et al. [50] found that *M. cannonballus* produces a wide range of heat-tolerant proteins that are resistant to the action of proteolytic enzymes, as well as low molecular weight compounds that remained phytoactive, i.e., capable of causing symptoms in sensitive plants even after boiling and autoclaving.

These same metabolites are also produced by *Monosporascus* in the soil, as they are part of the survival and pathogenicity mechanisms of this species [50] and, due to their biochemical stability, may have accumulated in the treatments where Jack Bean were incorporated and, consequently, acted on the melon roots since transplanting, favoring the early penetration mainly of *Fusarium* (which multiplied in the crop residues of cowpea) and other remaining pathogens.

It should also be noted that in the pathogenesis process, injuries caused to the host, whether by the action of physical or biological agents, can serve as an entry point for the penetration and establishment of other secondary infections. This type of synergy that can occur between phytopathogens contributes to the worsening of the host's physiological state, culminating in a faster rate of disease development and severity of lesions [31,51–53], similar to the results observed in this study. This study has limitations, including the use of greenhouse conditions, a single soil source with high inoculum pressure, and the absence of direct functional assays to confirm pathogen inhibition mechanisms. These factors should be considered when extrapolating the results to field conditions.

However, although the increase in chitinase, β -1,3-glucanase, and PAL activities in Jack Beans plants inoculated with *M. pseudophaseolina* indicates the activation of defense mechanisms associated with resistance, these results should be interpreted with caution regarding their potential for disease control in subsequent crops. The responses observed in Experiment I reflect the Jack Bean's ability to recognize the presence of the pathogen and activate biochemical defense mechanisms in its own tissues. However, such responses do not necessarily imply a reduction in inoculum density in the soil or effective suppression of the causal agents of root rot and stem decline in melon. This interpretation is corroborated by the results of Experiment II, in which the incidence of the disease reached 100% in all treatments with naturally infested soil, and the severity remained high.

As seen in this study, the cultural management of multiple root pathosystems is a challenge, since different pathogens respond differently to each technique, and can be suppressed or favored in a particular way according to their evolutionary and adaptive characteristics.

Our findings reinforce the need to integrate knowledge on the dynamics and complexity of multiple pathosystems involved in root rot and vine decline (RRVD), highlighting the challenges these interactions pose to the effective implementation of integrated disease management strategies.

5. Conclusions

Jack Bean showed higher activity of the enzymes chitinase (CT), β -1,3-glucanase (BG) and phenylalanine ammonia-lyase (PAL), mainly in roots grown in soils with the presence of *M. pseudophaseolina*.

The incorporation of Jack Bean into the soil did not reduce the incidence and severity of the disease in soils naturally infested with multiple pathogens, due to the differential sensitivity of these pathogens to this plant species. Although greater activity of CT, BG, and PAL was observed in Experiment I, this biochemical response did not translate into a reduction in the incidence or severity of the disease under conditions of natural infestation with multiple pathogens.

In greenhouse, the use of *C. ensiformis* as green manure reduces the frequency of *Macrophomina* spp. isolation after one or more cultivation cycles, although it only delays the penetration of *Monosporascus* spp. into melon roots and promotes the multiplication of *Fusarium* spp.

Author Contributions: Conceptualization, A.M.P.N. and R.S.J.; methodology, M.B.T., R.A.M.d.C., N.J.d.A.M., M.T.d.Q.S., S.Q.d.F., C.S.A.S.S. and A.L.A.C.; formal analysis, M.B.T., R.A.M.d.C., N.J.d.A.M., M.T.d.Q.S., S.Q.d.F., C.S.A.S.S., A.L.A.C., A.M.P.N. and I.M.M.S.; investigation, M.B.T., A.M.P.N. and R.S.J.; resources, R.S.J.; data curation, A.M.P.N.; writing—original draft preparation, M.B.T., A.M.P.N. and R.S.J.; writing—review and editing, M.B.T., A.L.A.C., A.M.P.N., I.M.M.S. and R.S.J.; supervision, A.M.P.N. and R.S.J.; project administration, R.S.J. All authors have read and agreed to the published version of the manuscript.

Funding: This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior–Brasil (CAPES)–Finance Code 001 and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ANOVA	Analysis of variance
BG	β -1,3-glucanase
BOD	Biochemical oxygen demand
CRD	Completely randomized design
CT	Chitinase
CV	Coefficient of variation
DAT	Days after transplanting
DNS	3,5-dinitrosalicylic acid
DWR	Dry weight of roots
DWS	Dry weight of shoots
EDTA	Ethylenediamine tetra acetic acid
EU	Enzyme units
FUS	<i>Fusarium</i>
HCl	Hydrochloric acid
IPM	Integrated pest management
JB	Jack Bean
LAS	<i>Lasiodiplodia</i>
MAC	<i>Macrophomina</i>
ME	Melon
MON	<i>Monosporascus</i>
MSD	Minimum significant difference
NaClO	Sodium hypochlorite
OOM	Oomycetes

OTH	Others
PAL	Phenylalanine ammonia-lyase
PCA	Principal component analysis
PDA	Potato dextrose agar
pH	Potential of hydrogen
PVP	Polyvinylpyrrolidone
RL	Root length
RN	Rio Grande do Norte
RRVD	Root rot and vine decline
SL	Shoot length
TRI	<i>Trichoderma</i>

References

1. ABRAFRUTAS—Associação Brasileira dos Produtores Exportadores de Frutas e Derivados. Painéis de Produção. 2026. Available online: <https://abrafrutas.org/paineis-de-producao/> (accessed on 18 February 2026).
2. IBGE—Instituto Brasileiro de Geografia e Estatística. SIDRA—Sistema IBGE de Recuperação Automática. 2026. Available online: <https://sidra.ibge.gov.br/home/pnadct/> (accessed on 28 February 2026).
3. Figueirêdo, M.C.B.; Gondim, R.S.; Aragão, F.A.S. *Produção de Melão e Mudanças Climáticas: Sistemas Conservacionistas de Cultivo para Redução das Pegadas de Carbono e Hídrica*; Embrapa: Brasília, Brazil, 2017; 302p.
4. Alves, C.P.S.S.; Negreiros, A.M.P.; Cavalcante, A.L.A.; De Barros, A.P.O.; Correia, K.C.; Viana, D.M.; Tavares, M.B.; Sales Junior, R. Pathogenicity of *Macrophomina* spp. Associated with Watermelon Plants. *Trop. Plant Pathol.* **2025**, *50*, 8. [\[CrossRef\]](#)
5. Medeiros, A.C.; Melo, D.R.M.; Ambrósio, M.M.Q.; Nunes, G.H.S.; Costa, J.M. Methods of Inoculation of *Rhizoctonia solani* and *Macrophomina phaseolina* in Melon (*Cucumis melo*). *Summa Phytopathol.* **2015**, *41*, 281–286. [\[CrossRef\]](#)
6. Nuangmek, W.; Aiduang, W.; Swannarach, N.; Kumla, J.; Lumyong, S. First Report of Gummy Stem Blight Caused by *Stagonosporopsis cucurbitacearum* on Cantaloupe in Thailand. *Can. J. Plant Pathol.* **2018**, *40*, 306–311. [\[CrossRef\]](#)
7. Medeiros Araujo, M.B.; Moreira, G.M.; Nascimento, L.V.; Nogueira, G.A.; Nascimento, S.R.C.; Pfenning, L.H.; Ambrósio, M.M.Q. Fusarium Rot of Melon Is Caused by Several *Fusarium* Species. *Plant Pathol.* **2021**, *70*, 712–721. [\[CrossRef\]](#)
8. Da Silva, R.M.; Ambrósio, M.M.Q.; Silva Neto, J.A.; Silva, J.L.S.; Costa, T.E.; Figueiredo, F.R.A.; Barroso, K.A.; Da Silva, W.; Holanda, I.S.A. First Report of *Lasiodiplodia brasiliensis* Causing Root Rot in Melon Plants in Northeastern Brazil. *Plant Dis.* **2024**, *108*, 219. [\[CrossRef\]](#)
9. Porto, M.A.F.; Ambrósio, M.M.Q.; Nascimento, S.R.C.; Cruz, B.L.S.; Torres, R.M. Interaction of *Fusarium solani*, *Macrophomina phaseolina* and *Rhizoctonia solani* as root rot pathogens of *Cucumis melo*. *Summa Phytopathol.* **2019**, *45*, 355–360. [\[CrossRef\]](#)
10. Moreira, G.V.S.; Tavares, M.B.; Cavalcante, A.L.A.; Alves, C.P.S.S.; Sales Junior, R.; Negreiros, A.M.P.; Souza, M.T.Q.; Da Silva, W.L. Reaction of Two Melon Hybrids to *Macrophomina phaseolina* and *M. pseudophaseolina*. *Rev. Caatinga* **2025**, *38*, 12875. [\[CrossRef\]](#)
11. Castro, G.; Perpiñá, G.; Esteras, C.; Armengol, J.; Picó, B.; Pérez-de-Castro, A. Resistance in melon to *Monosporascus cannonballus* and *M. eutypoides*: Fungal pathogens associated with *Monosporascus* root rot and vine decline. *Ann. Appl. Biol.* **2020**, *177*, 101–111. [\[CrossRef\]](#)
12. Cavalcante, A.L.A.; Negreiros, A.M.P.; Tavares, M.B.; Barreto, E.S.; Armengol, J.; Sales Junior, R. Characterization of Five New *Monosporascus* Species: Adaptation to Environmental Factors, Pathogenicity to Cucurbits and Sensitivity to Fungicides. *J. Fungi* **2020**, *6*, 169. [\[CrossRef\]](#) [\[PubMed\]](#)
13. AGROFIT—Sistema de Agrotóxicos Fitossanitários, do Ministério da Agricultura, Pecuária e Abastecimento. Consulta Aberta. 2025. Available online: https://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons (accessed on 11 March 2026).
14. Alves, C.P.S.S.; Negreiros, A.M.P.; Cavalcante, A.L.A.; Viana, D.M.; Oliveira, S.H.F.; Sales Junior, R. Efficacy of Fungicides for Controlling *Macrophomina* spp. in Melon Plant. *Trop. Plant Pathol.* **2024**, *49*, 218–231. [\[CrossRef\]](#)
15. Marquez, N.; Giachero, M.; Declerck, S.; Ducasse, D.A. *Macrophomina phaseolina*: General characteristics of pathogenicity and methods of control. *Front. Plant Sci.* **2021**, *12*, 634397. [\[CrossRef\]](#) [\[PubMed\]](#)
16. USDA—United States Department of Agriculture. Fungal Databases. 2025. Available online: <https://fungi.ars.usda.gov/> (accessed on 2 January 2026).
17. Dou, Y.; Yu, S.; Liu, S.; Cui, T.; Huang, R.; Wang, Y.; Wang, J.; Tan, K.; Li, X. Crop rotations reduce pathogenic fungi compared to continuous cropping. *Rhizosphere* **2025**, *34*, 101074. [\[CrossRef\]](#)
18. Flower, K.C.; Hüberli, D.; Collins, S.J.; Thomas, G.; Ward, P.R.; Cordingley, N. Progression of Plant-Parasitic Nematodes and Foliar and Root Diseases under No-Tillage with Different Crop Rotations. *Soil Tillage Res.* **2019**, *191*, 18–28. [\[CrossRef\]](#)
19. Formentini, E.A. Cartilha sobre Adubação Verde e Compostagem. 2019. Available online: <https://biblioteca.incaper.es.gov.br/digital/bitstream/123456789/3718/1/cartilha-adubacao-verde-compostagem.pdf> (accessed on 11 March 2026).

20. Lima, W.N.; Alves, C.P.S.S.; Negreiros, A.M.P.; Ambrósio, M.M.Q.; Medeiros, E.V.; Sales Junior, R. *Macrophomina pseudophaseolina* Isolated from Weeds Is Pathogenic against Cowpea, Mung Bean, Corn, and Sorghum. *Eur. J. Plant Pathol.* **2024**, *168*, 225–230. [\[CrossRef\]](#)
21. Jain, M.; Amera, G.M.; Muthukumaran, J.; Singh, A.K. Insights into biological role of plant defense proteins: A review. *Biocatal. Agric. Biotechnol.* **2022**, *40*, 102293. [\[CrossRef\]](#)
22. Kumar, M.; Bhardwaj, M.; Yadav, P.; Vashishth, D.; Chahal, S.; Dalal, S.; Kataria, S.K. A review on distribution, properties, genetic organization, immobilisation and applications of urease. *J. Appl. Nat. Sci.* **2022**, *14*, 1413–1429. [\[CrossRef\]](#)
23. Gonçalves, R.N.; Gozzini Barbosa, S.D.; Silva-López, R.E. Proteases from *Canavalia ensiformis*: Active and Thermostable Enzymes with Potential of Application in Biotechnology. *Biotechnol. Res. Int.* **2016**, *2016*, 3427098. [\[CrossRef\]](#) [\[PubMed\]](#)
24. Dos Santos, C.; Franco, O.L. Pathogenesis-Related Proteins (PRs) with Enzyme Activity Activating Plant Defense Responses. *Plants* **2023**, *12*, 2226. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Porto, M.F.; Ambrósio, M.M.Q.; De Freitas, F.C.L.; Nascimento, S.R.C.; Da Cruz, B.L.S.; Guimarães, L.M.S. Feijão-de-Porco (*Canavalia ensiformis*) no Controle da Podridão Radicular do Meloeiro Causada por Associação de Patógenos. *Summa Phytopathol.* **2016**, *42*, 327–332. [\[CrossRef\]](#)
26. Zribi, I.; Ghorbel, M.; Brini, F. Pathogenesis-Related Proteins (PRs): From Cellular Mechanisms to Plant Defense. *Curr. Protein Pept. Sci.* **2021**, *22*, 396–412. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Boccardo, N.A.; Segretin, M.E.; Hernandez, I.; Mirkin, F.G.; Chacón, O.; Lopez, Y.; Borrás-Hidalgo, O.; Bravo-Almonacid, F.F. Expression of Pathogenesis-Related Proteins in Transplastomic Tobacco Plants Confers Resistance to Filamentous Pathogens under Field Trials. *Sci. Rep.* **2019**, *9*, 2791. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Singh, M.; Pandita, S.; Upadhyay, S.K.; Verma, P.C. Genomic and Functional Characterization of the Phenylalanine Ammonia Lyase (PAL) Gene Family in Wheat (*Triticum aestivum*) Reveals Roles in Salt Stress Adaptation. *Biotechnol. Appl. Biochem.* **2026**, *73*, 1200–1216. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Whetten, R.; Sederoff, R. Lignin Biosynthesis. *Plant Cell* **1995**, *7*, 1001. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Weisshaar, B.; Jenkins, G.I. Phenylpropanoid Biosynthesis and Its Regulation. *Curr. Opin. Plant Biol.* **1998**, *1*, 251–257. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Mo, F.; Li, L.; Zhang, C.; Yang, C.; Chen, G.; Niu, Y.; Si, J.; Liu, T.; Sun, X.; Wang, S.; et al. Genome-Wide Analysis and Expression Profiling of the Phenylalanine Ammonia-Lyase Gene Family in *Solanum tuberosum*. *Int. J. Mol. Sci.* **2022**, *23*, 6833. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Alvares, C.A.; Stape, J.L.; Sentelhas, P.C.; Gonçalves, J.L.M.; Sparovek, G. Köppen's Climate Classification Map for Brazil. *Meteorol. Z.* **2014**, *22*, 711–728. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Songa, W.; Hillocks, R.J.; Mwangi, A.W.; Buruchara, R.; Ronno, W.K. Screening Common Bean Accessions for Resistance to Charcoal Rot (*Macrophomina phaseolina*) in Eastern Kenya. *Exp. Agric.* **1997**, *33*, 459–468. [\[CrossRef\]](#)
34. Souza, E.M.S.; Pinto, F.A.C.; Capucho, A.S.; Da Silva, L.A.B.; Borel, J.C.; Ishikawa, F.H. Inoculation Methods and Aggressiveness of *Macrophomina phaseolina* Isolates in Cowpea. *Cienc. Rural* **2021**, *52*, e20210072. [\[CrossRef\]](#)
35. De Freitas, S.Q.; Negreiros, A.M.P.; Cavalcante, A.L.A.; Viana, D.M.; Santos, F.J.Q.; Nunes, G.H.S.; Pinto, P.S.L.; Silva, C.S.A.S.; Tertulino, E.J.A.; Sales Junior, R. Comparison of Soil Infestation and Toothpick Methods for *Macrophomina* Species in Melon Plants. *J. Plant Pathol.* **2026**, *108*, 1311–1317. [\[CrossRef\]](#)
36. Bezerra Neto, E.; Barreto, L.P. *Análises Químicas e Bioquímicas em Plantas*; UFRPE: Recife, Brazil, 2011.
37. Stangarlin, J.R.; Pascholati, S.F.; Labate, C.A. Efeito de *Phaeoisariopsis griseola* na Atividade de Ribulose-1,5-Bifosfato Carboxilase-Oxygenase, Clorofilase, β -1,3-Glucanase e Quitinase em Cultivares de *Phaseolus vulgaris*. *Fitopatol. Bras.* **2000**, *25*, 59–66.
38. Umesha, S. Phenylalanine Ammonia Lyase Activity in Tomato Seedlings and Its Relationship to Bacterial Canker Disease Resistance. *Phytoparasitica* **2006**, *34*, 68–71. [\[CrossRef\]](#)
39. Ravf, B.A.; Ahmad, I. Studies on Correlation of Seed Infection to Field Incidence of *Alternaria alternata* and *Macrophomina phaseolina* in Sunflower. In Proceedings of the 13th Iranian Plant Protection Congress, Karaj, Iran, 23–27 August 1998.
40. Silva, F.A.Z.; Azevedo, C.A.V. The Assistat Software Version 7.7 and its use in the analysis of experimental data. *Afr. J. Agric. Res.* **2016**, *11*, 3733–3740. [\[CrossRef\]](#)
41. Finkina, E.I.; Melnikova, D.N.; Bogdanov, I.V.; Ovchinnikova, T.V. Plant Pathogenesis-Related Proteins PR-10 and PR-14 as Components of Innate Immunity System and Ubiquitous Allergens. *Curr. Med. Chem.* **2017**, *17*, 1772–1787. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Héloir, M.C.; Adrian, M.; Brulé, D.; Claverie, J.; Cordelier, S.; Daire, X.; Dorey, S.; Gauthier, A.; Lemaître-Guillies, C.; Negrel, J.; et al. Recognition of Elicitors in Grapevine: From MAMP and DAMP Perception to Induced Resistance. *Front. Plant Sci.* **2019**, *10*, 1117. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Ali, Z.; Serag, M.F.; Demirer, G.S.; Torre, B.; Di Fabrizio, E.; Landry, M.P.; Habuchi, S.; Mahfouz, M. DNA–Carbon Nanotube Binding Mode Determines the Efficiency of Carbon Nanotube-Mediated DNA Delivery to Intact Plants. *ACS Appl. Nano Mater.* **2022**, *5*, 4663–4676. [\[CrossRef\]](#)

44. Liu, Q.; Wang, S.; Li, K.; Qiao, J.; Guo, Y.; Liu, Z.; Guo, X. Responses of Soil Bacterial and Fungal Communities to the Long-Term Monoculture of Grapevine. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 7035–7050. [[CrossRef](#)] [[PubMed](#)]
45. Wu, H.; Yan, W.; Wu, H.; Zhang, J.; Zhang, Z.; Zhang, Z.; Rensing, C.; Lin, W. Consecutive Monoculture Regimes Differently Affected the Diversity of the Rhizosphere Soil Viral Community and Accumulated Soil-Borne Plant Viruses. *Agric. Ecosyst. Environ.* **2022**, *337*, 108076. [[CrossRef](#)]
46. Dhingra, O.D.; Coelho Neto, R.A. Reservoir and Non-reservoir Hosts of Bean-Wilt Pathogen, *Fusarium oxysporum* f. sp. *phaseoli*. *J. Phytopathol.* **2001**, *149*, 463–467. [[CrossRef](#)]
47. Gordon, T.R. *Fusarium oxysporum* and the Fusarium Wilt Syndrome. *Annu. Rev. Phytopathol.* **2017**, *55*, 23–39. [[CrossRef](#)] [[PubMed](#)]
48. Sales Junior, R.; Senhor, R.F.; Michereff, S.J.; Medeiros, E.V. Influência da Adubação Verde no Declínio de *Monosporascus* em Solo Naturalmente Infestado. *Hortic. Bras.* **2017**, *35*, 135–140. [[CrossRef](#)]
49. Al-Rawahi, A.Y.; Al-Mahmooli, I.H.; Al-Sadi, A.M.; Al-Sabahi, J.N.; Velazhahan, R. Toxin Production by Melon Root Rot Fungus, *Monosporascus cannonballus*. *Australas. Plant Pathol.* **2018**, *47*, 543–546. [[CrossRef](#)]
50. Hosseini, B.; Sarpele, A.; Farrokhi, N. Functional Secretomics of Phytotoxic Compounds of *Monosporascus cannonballus*. *J. Agric. Sci. Technol.* **2018**, *20*, 621–632.
51. Luiz, D. Interação entre *Pratylenchus brachyurus* e *Macrophomina phaseolina* na Cultura da Soja. Master's Dissertation, Instituto Federal Goiano, Goiânia, Goiás, 2022.
52. Hu, Y.J.; Yang, H.M.; Jin, J.; Yan, H.H.; Wang, J.P.; Zhang, R.Q. Synergistic Activity of Antagonistic *Trichoderma* spp. and *Rhizoctonia solani* Increases Disease Severity on Strawberry Petioles. *Eur. J. Plant Pathol.* **2022**, *164*, 375–389. [[CrossRef](#)]
53. Da Costa, R.V.; De Almeida, R.E.M.; Cota, L.V.; Da Silva, D.D.; Lima, L.S.; De Sousa, C.W.A.; De Souza, M.R. Corn Stunt Disease Complex Increases Charcoal Rot (*Macrophomina phaseolina*) under Field Conditions. *Trop. Plant Pathol.* **2023**, *48*, 283–292. [[CrossRef](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.