PLANT SCIENCE TODAY
Vol 12(sp4): 01–08
https://doi.org/10.14719/pst.11687

eISSN 2348-1900

RESEARCH ARTICLE

Organic seed treatment formulations enhance biotic stress tolerance in brinjal cv. CO 2

Elakiya A[1], Jerlin R[1*], Malarkodi K[1], Gnanachitra M[2], Vakeswaran V[1], Renugadevi J[1] & Thirusendura Selvi D[1]

[1]Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[2]Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India

*Correspondence email - rjerlin@tnau.ac.in

Received: 08 September 2025; Accepted: 08 October 2025; Available online: Version 1.0: 12 November 2025

Cite this article: Elakiya A, Jerlin R, Malarkodi K, Gnanachitra M, Vakeswaran V, Renugadevi J, Thirusendura SD. Organic seed treatment formulations enhance biotic stress tolerance in brinjal cv. CO 2. Plant Science Today. 2025;12(sp4):01–08. https://doi.org/10.14719/pst.11687

Abstract

Seed enhancement technologies provide eco-friendly alternatives to synthetic protectants for improving crop establishment under stress. In the present study, organic seed pelleting and coating formulations were developed using volcanic ash (as a silicon-rich mineral source), biochar and a microbial consortium. These formulations were evaluated in brinjal (*Solanum melongena* L.) cv. CO 2 under biotic stresses imposed by *Fusarium oxysporum* and *Pythium aphanidermatum*. Results revealed that both pelleting and coating significantly improved seed germination, seedling vigor, root and shoot growth and biomass accumulation compared to the untreated seeds (control). Among various seed treatments, seed pelleting mixture at 500 g kg^{-1} seed and seed coating formulation at 4 g kg^{-1} seed consistently outperformed others, recording higher emergence, leaf area and chlorophyll content even under pathogen pressure. The superior performance of these treatments is attributed to the synergistic effects of silicon-mediated structural defense, nutrient mobilization by biochar, phytohormone stimulation and pathogen suppression by microbes and biocontrol agents. This study demonstrates the potential of integrating mineral-based carriers with microbial inoculants in organic seed enhancement technologies to strengthen seedling establishment and resilience against biotic stresses. Thus, eco-innovative formulations provide a sustainable strategy to reduce pesticide dependence and enhance crop productivity in solanaceous vegetables.

Keywords: biochar; biotic stress; microbes; seed coating; seed pelleting; volcanic ash

Introduction

Brinjal (*Solanum melongena* L.), also known as eggplant, is one of the most important solanaceous vegetable crops cultivated across India due to its adaptability to diverse agro-climatic conditions and consistent demand. It is valued not only for its wide use in daily diets but also for its nutritional richness, being a good source of dietary fiber, vitamins (such as vitamin C and B-complex), minerals and bioactive compounds, including phenolics, anthocyanins and other antioxidants that contribute to its health-promoting properties (1). This crop occupies about 0.70 million hectares with an annual production of 13.55 million tons, contributing nearly 27 % of the global brinjal output (2). Despite its extensive cultivation, the average productivity of 19.4 t ha^{-1} remains below its potential yield under optimal management conditions (1). A major constraint to realizing higher yields is the prevalence of biotic stresses. Plants suffer from devastating fungal diseases, which adversely affect crop vigor, fruit quality and seed production, including early blight (*Alternaria solani*) and Fusarium wilt (*Fusarium oxysporum* f. sp. melongenae), damping off (*Pythium aphanidermatum*), which impair photosynthesis, vascular transport and reproductive capacity, ultimately reducing productivity (3). The shoot and fruit borer (*Leucinodes orbonalis*) is the most destructive pest of brinjal, capable of inflicting yield losses up to 60-70 % when unmanaged (4).

The widespread use of synthetic pesticides and fungicides to manage these biotic challenges has raised serious concerns regarding environmental safety, soil health and the persistence of pesticide residues in food, water and soil, which pose long-term ecological risks and adverse effects on human wellbeing (5). This highlights the urgent need for eco-friendly and sustainable alternatives that can safeguard crop performance without adverse ecological impacts. In this context, organic seed enhancement technologies, particularly seed pelleting and seed coating, have emerged as promising strategies to mitigate biotic stress while enhancing crop establishment and yield potential (6).

Seed pelleting is a process in which successive layers of inert or bioactive materials are applied to modify the seed shape into a spherical and uniform mass. This improves precision sowing, soil-seed contact and moisture availability during germination (7). Seed coating, on the other hand, involves the application of thin, uniform layers of biofertilizers, biopesticides, micronutrients, or biostimulants directly on the seed surface, ensuring close interaction of active compounds with the emerging radicle (8). Both techniques create a modified microenvironment around the seed that facilitates rapid germination, uniform seedling emergence, enhanced root development and improved tolerance to biotic and abiotic stresses. Beyond seed germination and establishment, organic inputs incorporated through pelleting and coating formulations also enhance rhizosphere microbial diversity, soil structure, nutrient solubilization and hormone regulation, contributing to stronger stress resilience and higher crop productivity (9). In crops like brinjal and tomato, where early seedling vigor directly influences final yield and fruit quality, these seed enhancement approaches hold immense potential for improving stand establishment, reducing yield losses under biotic stress and promoting sustainable production systems (10).

Materials and Methods

The present investigation was undertaken to assess the impact of an organic seed pelleting mixture and seed coating formulation on the performance of brinjal (*S. melongena* L.) cv. CO 2. The fresh seeds of brinjal cv. CO 2 were collected from the Department of Vegetable Science, Horticulture College and Research Institute, Coimbatore. Laboratory experiments were initially carried out to standardize suitable formulations for enhancing seed quality parameters such as germination percentage, seedling length, dry matter production and seedling vigor. The most effective treatments with suitable dosages identified from laboratory evaluations were subsequently tested through pot culture experiments under controlled conditions. These experiments included the imposition of biotic stresses such as diseases through foliar application to evaluate the efficacy of the developed formulations in improving crop growth, vigor and resistance against stress conditions.

The seed pelleting mixture was developed by using volcanic ash, biochar and microbes (*Azosprillum*, Phosphobacteria, *Bacillus subtilis* and pink-pigmented facultative methylotrophs) as the base material. The layers of pellets have been standardized for the brinjal cv. CO 2 under laboratory conditions. The suitable dosages for both seed pelleting and seed coating formulations were standardized under laboratory conditions. The steps involved in seed pelleting for brinjal are given in Fig. 1.

Effect of developed seed pelleting mixture and seed coating formulation

The standardized concentrations of seed pelleting mixture and seed coating formulation were taken from the previously conducted experiments and the effect of seed treatments was tested again for germination parameters under laboratory conditions. The experiment was conducted in a completely randomized design (CRD) with 5 replicates. The treatment details were given as follows:

T_0- Control (untreated seeds)

T_1- Seed pelleting mixture at 300 g Kg^{-1}

T_2- Seed pelleting mixture at 500 g Kg^{-1}

T_3- Seed coating formulation at 2 g Kg^{-1}

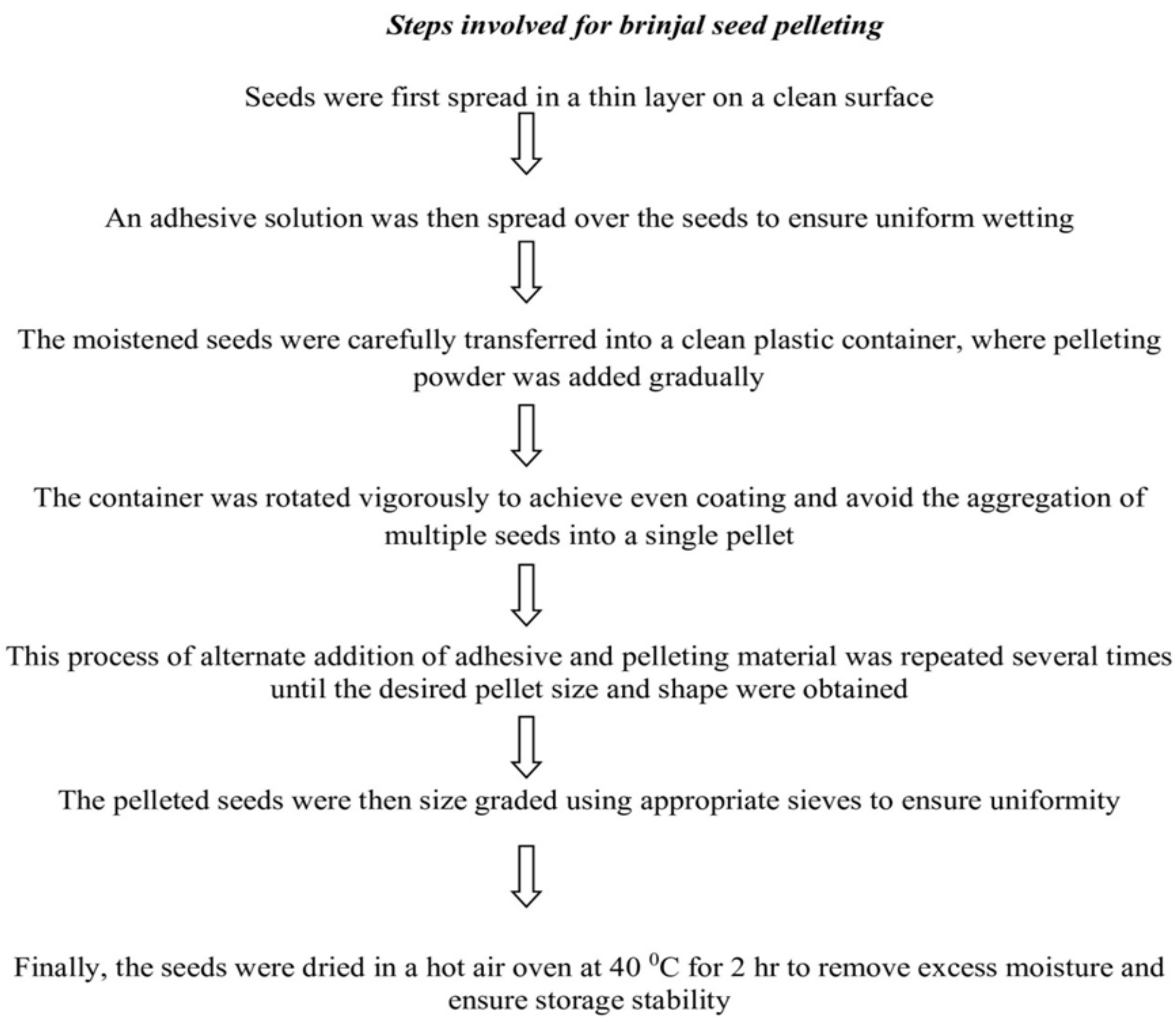

Fig. 1. Steps involved in seed pelleting for brinjal.

T_4- Seed coating formulation at 4 g Kg^{-1}

A germination test was carried out in a controlled environment (germination chamber) at an even temperature of 25 ± 2 °C, a relative humidity of 95 ± 3 % and illumination of 1500 lux to enhance seedling growth. Germination percentage was determined according to International Seed Testing Association (ISTA) (11) guidelines. The seedling vigor index (12) and final dry matter production (DMP) (13) were subsequently calculated.

Preparation of pathogen inoculum

Pure cultures of *F. oxysporum* and *P. aphanidermatum* were obtained from the Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore and maintained on potato dextrose agar (PDA) medium. Fungal cultures of *F. oxysporum* and *P. aphanidermatum* were separately grown on PDA plates and incubated at 25 + 2 °C for 7-10 days. After full growth, conidial suspensions were prepared by flooding the plates with sterile distilled water and gently scraping the surface to release spores. The resulting suspensions were filtered through sterile muslin cloth to remove mycelial debris. Spore concentrations were adjusted to 1 % (v/v) by dilution with sterile water and the final concentration was standardized to approximately 1 x 10^6 spores ml^{-1} using a haemocytometer.

T_0- Control (Untreated seeds)	Pathogen spray
T_1- Seed pelleting mixture at 300 g Kg^{-1}	P_0-Without biotic stress
T_2- Seed pelleting mixture at 500 g Kg^{-1}	P_1-1 % *F. oxysporum* suspension
T_3- Seed coating formulation at 2 g Kg^{-1}	P_2-1 % *P. aphanidermatum* suspension
T_4- Seed coating formulation at 4 g Kg^{-1}	

Evaluating the effect of seed treatments on the brinjal cv. CO 2 growth under biotic (disease) stress conditions

Brinjal seeds cv. CO 2 was weighed and the seeds were coated and pelleted with the seed coating formulation and seed pelleting mixture, respectively. The spores of *F. oxysporum* and *P. aphanidermatum* were applied as a foliar spray for brinjal plants. The seeds were treated as per the schedule given below:

Treatment details

The experiment was conducted in factorial completely randomized design (FCRD) with 4 replications. The growth parameters were assessed to study the impact of seed treatments on biotic stress of brinjal cv. CO 2 with the following observations:

Emergence %

At 15 days after sowing seedling (DAS) emergence was noted for each treatment. A percentage (%) representing the average emergence rate across treatments was determined.

Plant height (cm), leaf length (cm) and leaf breadth (cm)

For each treatment and for every replication, the height of the plant was measured and leaf length and breadth at 30, 45 and 60 DAS were recorded. The average mean values were expressed in cm.

Leaf area (cm²)

The leaf area was calculated by using the formula (14).

$$\text{Leaf area } (cm^2) = L \times B \times 0.747 \times n$$

Where, L = length of leaf, B = breadth of leaf, 0.747 = correction factor and n = number of leaves per plant

Chlorophyll content [soil plant analysis development (SPAD) value]

The chlorophyll content of the leaves was observed by the use of SPAD meter at 30, 45 and 60 DAS.

Percent disease incidence (%)

The percent disease incidence (PDI) was calculated by using the following formula:

$$\text{PDI } (\%) = \frac{\text{Number of seedlings}}{\text{Total number of seedlings examined}} \times 100$$

Results and Discussion

Effect of different treatments on seed quality parameters

Significant differences were observed among treatments for speed of germination, germination percentage, root length, shoot length, dry matter production, vigor index I and vigor index II.

Speed of germination varied from 5.75 (T_1) to 6.54 (T_3). The highest value recorded in T_3, indicating accelerated and uniform emergence, whereas the lowest in pelleting treatment (T_1) suggested that multiple pelleting layers delayed germination speed (Table 1). This might be attributed to cellular activation, which enhances mitochondrial activity, thereby facilitating the synthesis of additional high-energy compounds and essential biomolecules during the initial phase of germination (15). Germination percentage increased from 74 % in T_0 to a maximum of 90 % in T_2, highlighting the effectiveness of the treatment in enhancing germination efficiency. Treatments T_3 (88 %) and T_4 (84 %) also performed better than the control (Table 1). The higher germination percentage in coated and pelleted seeds may be linked to reduced microbial infection on the seed surface, protection from unfavorable micro environmental conditions and the presence of organic nutrients enhancing viability (16). Volcanic ash, being a multi-nutrient mineral fertilizer, supports seedling establishment and embryo development (17). Hence, the control seeds marked a decreased germination percentage than the treated seeds. The enhanced performance of pelleted brinjal seeds correlated with increased efficacy of the pelleting material and reduced permeability of the cell membrane (18).

Root length was significantly higher in T_2 (8.18 cm), followed by T_3 (7.87 cm), while the lowest was noted in T_0 (6.86 cm). Similarly, shoot length ranged from 3.68 cm (T_0) to 4.90 cm in T_2 (Table 1). The improved root and shoot growth in treated seeds indicates better vigor and seedling growth potential. This improvement may be attributed to continuous nutrient supply and increased cytokinin production, which promotes cell division and growth regulation (19). Beneficial microbes might have enhanced auxin production, facilitating earlier radicle protrusion (20).

Dry matter production registered notable improvement with treatments, with T_2 recording the highest value (0.023 g) compared to 0.015 g in T_0 (Table 1). The improved dry matter accumulation is a strong indicator of enhanced metabolic activity and photosynthetic efficiency in seedlings. The enhanced dry weight may be attributed to greater lipid utilization and enzyme activity, stimulated by bioactive compounds such as auxin (21).

Among the vigor indices, vigor index I was markedly superior in T_2 (1177), followed by T_1 (1005), whereas the lowest

Table 1. Effect of developed organic seed pelleting technology and seed coating formulation on germination parameters of brinjal cv. CO 2 under laboratory conditions

Treatments	Speed of germination	Radicle length (mm)	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production	Vigor index I	Vigor index II
T_0	6.32	1.32	74	6.86	3.68	0.015	780	1.11
T_1	5.75	1.54	82	7.53	4.73	0.019	1005	1.56
T_2	6.27	2.10	90	8.18	4.90	0.023	1177	2.07
T_3	6.54	1.98	88	7.87	4.86	0.020	1120	1.76
T_4	6.21	1.43	84	7.59	4.67	0.018	1029	1.51
Mean	6.22	1.67	84	7.61	4.57	0.019	1022	1.60
SE	0.28	0.076	3.721	0.339	0.20	0.0009	45.520	0.07
CD	0.56	0.159	7.761	0.707	0.42	0.0018	94.953	0.15

T_0- Control (Untreated seeds), **T_1**- Seed pelleting mixture at 300 g kg^{-1}, **T_2**- Seed pelleting mixture at 500 g kg^{-1}, **T_3**- Seed coating formulation at 2 g kg^{-1}, **T_4**- Seed coating formulation at 4 g kg^{-1}.

was observed in T_0 (780). A similar trend was noted in vigor index II, where T_3 registered the highest value (2.07) compared to 1.11 in T_0 (Table 1). This might be due to the beneficial bacterial strains, which have promoted growth of regulators like indole acetic acid (IAA) for increasing the germination rate, which leads to enhanced seedling vigor (22). The higher the germination percentage results in higher the seedling vigor in increased dosage of seed pelleting mixture at 500 g Kg^{-1} and seed coating formulation at 4 g Kg^{-1}.

Effect of seed treatments on the growth of brinjal cv. CO 2 under biotic stress conditions

Under biotic stress, T_3 exhibited greater tolerance across all observed parameters. Specifically, the developed product has effectively controlled the *F. oxysporum* pathogens than the *P. aphanidermatum*. Control plants showed reduced growth parameters and high disease incidence. Significant differences were observed among treatments and pathogen conditions.

In the absence of pathogen stress (P_0), maximum emergence (100 %) was recorded in T_2 (seed pelleting mixture at 500 g Kg^{-1}) and T_4 (seed coating formulation at 4 g Kg^{-1}). The control (T_0) recorded the lowest emergence percentage of 75 %. Under *F. oxysporum* spray (P_1), T_2 maintained the highest emergence (100 %), followed by T_4(94 %). As shown in Fig. 1., the better performance of treated seeds under *F. oxysporum* stress may be attributed to the protective effect of pelleting and coating materials, which likely created a physical barrier and enhanced resistance mechanisms against pathogen invasion (23). Microbes are known to colonize the phyllosphere and seed surface, producing cytokinins and improving stress tolerance, which could account for better emergence under pathogen stress (24). When subjected to *P. aphanidermatum* spray (P_2), emergence percentage was substantially reduced across all treatments, with values ranging from 61 % in T_0 to 85 % in T_2(Fig. 2). Among the treatments, T_2 consistently exhibited the least reduction in emergence (15 % higher emergence percentage compared to P_0), In contrast, T_0 showed a drastic reduction of 14 %, highlighting its high susceptibility to *P. aphanidermatum* infection. The increased emergence percentage in seedlings is attributed to the organic amendments along with biocontrol agents such as *Bacillus* sp., which would have effectively controlled the *P. aphanidermatum* pathogen during stress conditions (25).

The plant height recorded significant variation among all the treatments. The tallest plants were observed in T_2 (26.04 cm), followed by T_4 (25.32 cm), while T_0 (control) recorded the minimum plant height of 17.94 cm in the absence of pathogen stress. With respect to *F. oxysporum*, seed pelleting with the pelleting mixture at 500 g Kg^{-1} (T_2) recorded the maximum plant height of 24.50 cm. The untreated seeds (T_0) registered reduced height of the plant (16.83 cm) (Fig. 3). This might be due to the presence of microbes, which enhance metabolic activity and are involved in the production of phytohormones such as IAA and auxin, which lead to cell elongation and cell division for increased growth of the plant (26).

Leaf length varied significantly across treatments and was also affected by pathogen stress. On average, the maximum leaf length was recorded in T_2 (9.8 cm), followed by T_4 (9.4 cm), while the minimum was observed in T_0 (5.8 cm). A similar trend was observed for leaf breadth, where T_2 (6.80 cm) and T_4 (7.00 cm) recorded superior values, while the lowest was noted in T_0 (6.18 cm) (Fig. 3). Pathogen stress (both *F. oxysporum* and *P. aphanidermatum*) consistently reduced leaf length and breadth across all treatments, with the reduction being most severe under *P. aphanidermatum*. However, the magnitude of reduction was markedly less in T_2 and T_4

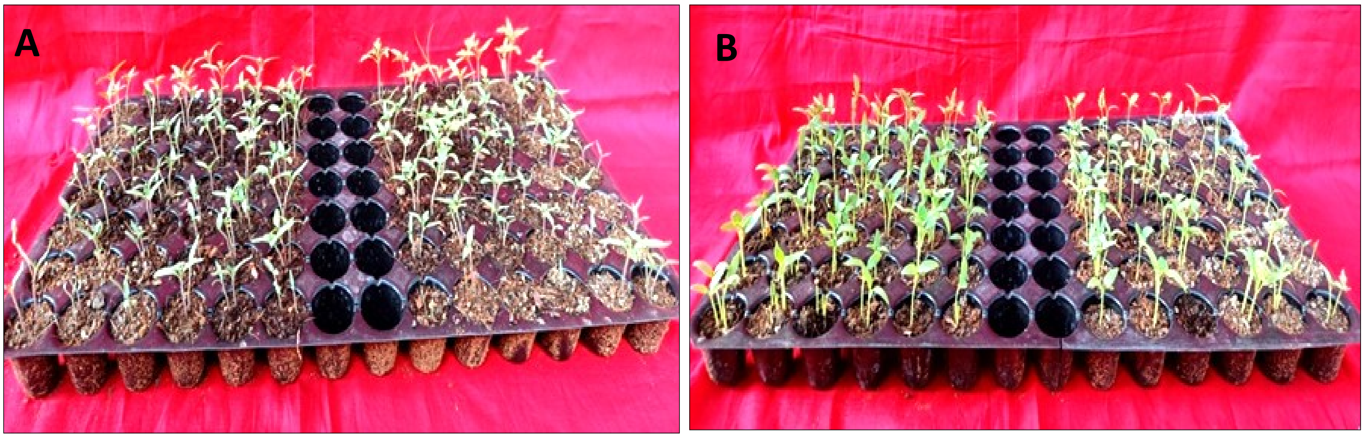

Fig. 2. Influence of volcanic ash, biochar and microbes on emergence (%) of brinjal cv. CO 2 under biotic stress condition (**A**) Control along with *F. oxysporum*; (**B**) Seeds treated with seed pelleting mixture at 500 g Kg^{-1} along with *F. oxysporum* spray.

Fig. 3. Influence of volcanic ash, biochar and microbes on crop growth of brinjal cv. CO 2 under biotic stress conditions (**A**) Control along with *F. oxysporum* (**B**) Seeds treated with the seed pelleting mixture at 500 g Kg^{-1} along with *F. oxysporum* spray.

compared to the untreated control. Microorganisms, such as Phosphobacteria, solubilize phosphorus, which is vital for energy metabolism and leaf tissue development (27). Additionally, *B. subtilis,* a biocontrol agent, suppressed the deleterious effects of *F. oxysporum* and *P. aphanidermatum* by producing antifungal metabolites and inducing systemic resistance, thereby sustaining leaf growth under pathogen stress (28).

Across pathogen regimes, P_0 (no pathogen spray), P_1 (*F. oxysporum*) and P_2 (*P. aphanidermatum*) leaf area increased markedly with seed enhancement and declined most in the untreated control (T_0). T_2 recorded 85.86, 72.75 and 59.40 cm^2 of leaf area under P_0, P_1 and P_2 respectively whereas, T_0 performed lower than all other treatments with 33.39, 25.20 and 18.36 cm^2 under P_0, P_1 and P_2, respectively. Silicon (Si) fortifies epidermal and mesophyll cell walls, lowers cuticular microcracks and improves light interception and water status, which together support larger leaves and higher chlorophyll stability especially under biotic stress (29). By preventing pathogen entry and restricting colonization, Si helps maintain chloroplast integrity and chlorophyll content, thereby sustaining photosynthesis under infection (30). Biochar's high porosity enhances water retention and cation exchange capacity, smoothing diurnal water stress and sustaining photosynthetic pigments (Fig. 4). Its surfaces adsorb pathogen exudates and phenolics and favor beneficial biofilms (31).

Percent disease incidence is a key indicator for assessing plant tolerance to biotic stress. Regarding percent disease incidence, the highest disease incidence was found in the untreated seeds sprayed with *P. aphanidermatum,* a causal organism of damping off disease, which is one of the most prevalent nursery diseases in vegetable crops. However, the

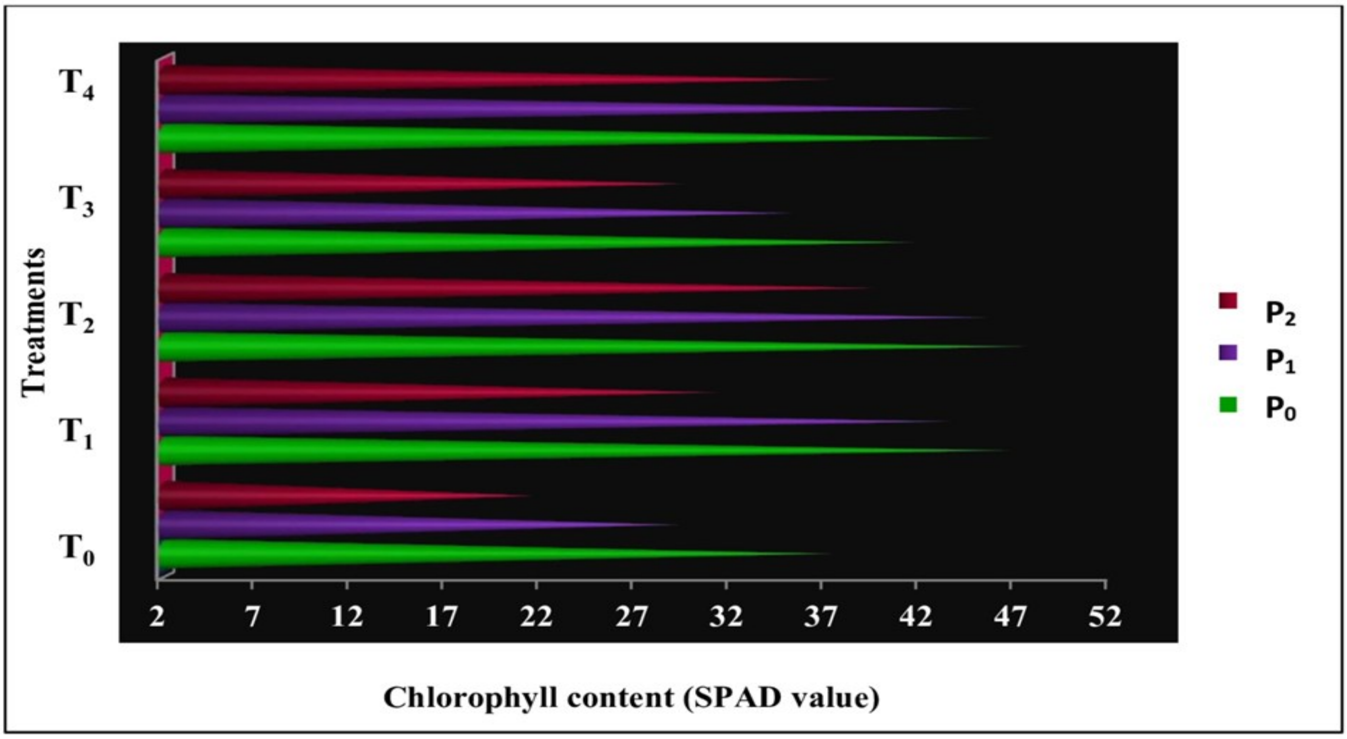

Fig. 4. Impact of seed treatments on chlorophyll content of brinjal cv. CO 2 under biotic stress. **T_0**- Control, **T_1**- Seed pelleting mixture at 300 g Kg^{-1}, **T_2**- Seed pelleting mixture at 500 g Kg^{-1}, **T_3**- Seed coating formulation at 2 g Kg^{-1}, **T_4**- Seed coating formulation at 4 g Kg^{-1}. **P_0**- Without pathogen stress, **P_1**- *F. oxysporum* spray and **P_2**- *P. aphanidermatum* spray.

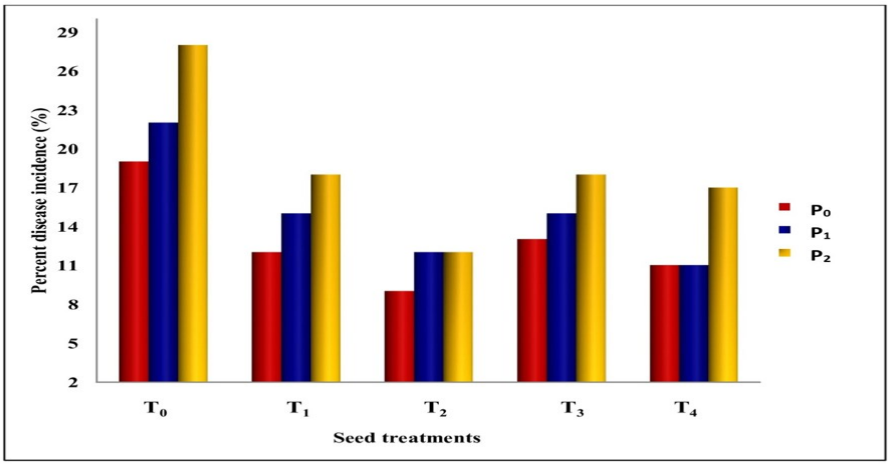

Fig. 5. Impact of seed treatments on percent disease incidence (%) of brinjal cv. CO 2 under biotic stress conditions. **T_0**- Control, **T_1**- Seed pelleting mixture at 300 g Kg^{-1}, **T_2**- Seed pelleting mixture at 500 g Kg^{-1}, **T_3**- Seed coating formulation at 2 g Kg^{-1}, **T_4**- Seed coating formulation at 4 g Kg^{-1}. **P_0**- Without pathogen stress, **P_1**- *F. oxysporum* spray and **P_2**- *P. aphanidermatum* spray.

disease severity was reduced in the seeds pelleted with the pelleting mixture at 500 g Kg^{-1} and seed coated with the seed coating formulation of 4 g Kg^{-1} (Fig. 5). This shows that the nutrients present in the mixture have helped to create a defense mechanism in the plant against biotic stresses. The major contribution to this tolerance might be due to the presence of Si in volcanic ash. Once absorbed as monosilicic acid [$Si(OH)_4$], Si is polymerized and deposited beneath the cuticle as silica-cuticle double layers. This reinforcement reduces leaf epidermal permeability and creates a rigid barrier that prevents penetration by fungal appressoria and restricts entry of bacterial pathogens (32). As a result, Si-treated plants often exhibit delayed or reduced lesion formation. Silicon accumulation at infection sites strengthens cell walls, reducing pathogen entry through stomata or wounds. In solanaceous crops, Si has been shown to reduce penetration by *F. oxysporum*, *P. aphanidermatum* and *Alternaria*, lowering systemic spread (33).

Conclusion

The present study demonstrated the effectiveness of organic seed pelleting and coating formulations in enhancing seedling vigor and mitigating biotic stresses in brinjal cv. CO 2. Across all growth parameters, including germination percentage, speed of germination, root and shoot length, vigor indices, emergence under pathogen challenge, plant height, leaf morphology (length and breadth), leaf area and chlorophyll content, the treated seeds consistently outperformed the untreated control. Disease severity was also reduced in treated plants compared to the control. Among the treatments, seed pelleting mixture at 500 g Kg^{-1} of seed (T_2) and seed coating formulation at 4 g Kg^{-1} (T_4) proved as the most effective treatment in sustaining growth and physiological performance under *F. oxysporum* and *P. aphanidermatum* stresses. These treatments maintained higher emergence percentages, improved root and shoot growth and recorded superior leaf area and chlorophyll content compared to the control.

The enhanced performance of T_2 and T_4 can be attributed to the synergistic role of volcanic ash (as a source of Si and micronutrients), biochar (improving microbial habitat) and microbial inoculants, which promoted nutrient uptake and hormonal regulation. In addition, the biocontrol agent effectively suppressed pathogen incidence through antibiosis and systemic resistance induction. The presence of silicon further contributed to resistance by reinforcing leaf epidermis, preventing pathogen penetration and sustaining chlorophyll content. Overall, the study establishes that bio-based seed pelleting and coating formulations are promising eco-friendly technologies for improving germination, vigor and resilience of solanaceous crops under biotic stress conditions. Adoption of such organic seed enhancement techniques offers a sustainable alternative to synthetic chemical protectants while ensuring better crop establishment and productivity.

Exploring novel organic carriers such as nanostructured biochar or Si-based composites may improve nutrient release and microbial colonization. Expanding this technology to other solanaceous crops and pulses could contribute to broader adoption and reduce dependence on synthetic inputs. Ultimately, scaling up through seed companies and farmer-producer organizations will accelerate the transition towards eco-friendly seed enhancement practices, thereby improving productivity, soil health and long-term sustainability of vegetable production systems.

Acknowledgements

The authors are thankful to the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore, for providing the facilities to conduct the work.

Authors' contributions

EA performed the study and wrote the manuscript. JR planned the layout and participated in drafting and editing of the manuscript. MK, GM, VV, RJ and TSD helped in reviewing and further drafting of the manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

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