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Title: **Efficacy of Organic Farming Against Charcoal Rot of Chickpea (*CICER ARIETINUM L.*)**

Author (s): Sundus Akhtar, Asma Maryyum, Ayesha Shafqat, Sajida Sharif, and Fahad Riaz

Affiliation (s): Minhaj University Lahore, Lahore, Pakistan

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Efficacy of Organic Farming Against Charcoal Rot of Chickpea (*CICER arietinum* L.)

Sundus Akhtar*, Asma Maryyum, Ayesha Shafqat, Sajida Sharif and Fahad Riaz

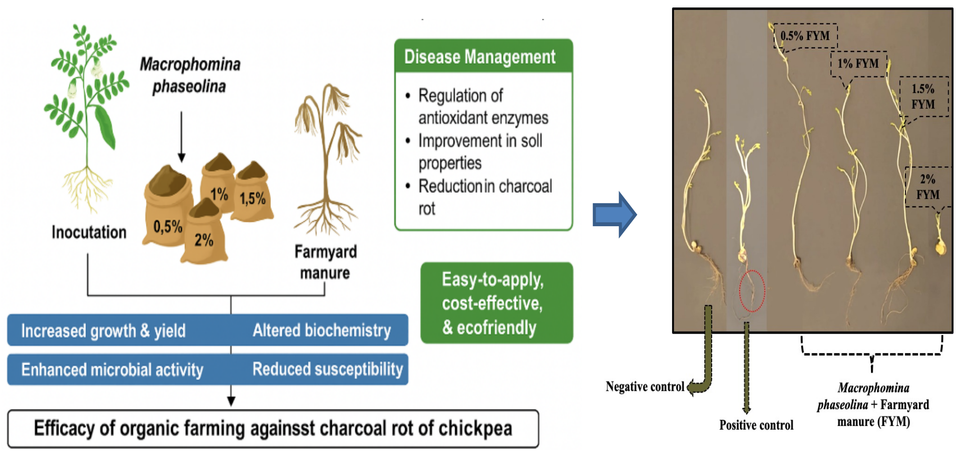
School of Biology, Minhaj University Lahore, Pakistan

ABSTRACT

Charcoal rot disease, caused by *Macrophomina phaseolina* Goid., significantly impacts chickpea (*Cicer arietinum* L.) production in Pakistan. This study evaluated the efficacy of farmyard manure (FYM) as an ecofriendly management strategy. FYM treatments (0.5%, 1%, 1.5%, and 2%) were applied to *M. phaseolina*-inoculated soil (positive control), and their effects on disease severity, plant growth, and biochemical parameters were assessed. Results showed that FYM significantly ($p \leq 0.05$) reduced disease infestation in positive control up-to 70%. Moreover, the incorporation of FYM in the soil with *M. phaseolina* enhanced the physiological traits (total chlorophyll content and soluble sugar (SS)) by 40 to 64% in comparison with positive control. Furthermore, the antioxidant enzyme activities significantly ($p \leq 0.05$) enhanced in *C. arietinum,* that is, 3 to 4 folds as compared to positive control. Likewise, physiological parameters, for instance, the root and shoot length (SL) and fresh and dry biomass of tested plant significantly ($p \leq 0.05$) increased (up to 70%) in comparison with positive control. The findings suggested that FYM is a cost-effective, sustainable, and environmentally-friendly option for managing charcoal rot disease in chickpea, offering a promising alternative to chemical fungicides.

Keywords: biological disease management, charcoal rot, chickpea, *Macrophomina phaseolina* organic farming

GRAPHICAL ABSTRACT



*Corresponding Author: dr.sundas@mul.edu.pk

Highlights

- Eco-friendly disease management approach enhancing plant resilience
- Improved physiological and biochemical traits for better growth performance
- Sustainable yield enhancement through environmentally responsible practices

1. INTRODUCTION

The word ‘chickpea’ (*Cicer arietinum* L.) has been derived from a Latin word “*Cicer”,* which refers to family Fabaceae. It is also commonly called as gram or Bengal gram, garbanzo bean, and Egyptian pea. Chickpeas are mainly classified into two main types based on size, colour, and coat thickness. These include Kabuli (large, beige, smooth coat) and desi (small, dark, rough coat). Moreover, chickpeas are highly versatile and possess a natural nutty, sweet, and buttery flavour with a creamy texture. The younger chickpeas with sweet flavour are green in colour. The Kabuli-type chickpeas are commonly found in United States, whereas desi-type are found worldwide [1].

Chickpeas are the major source of proteins (20-25%), carbohydrates (51-62%), fibre (raffinose), vitamin B, thiamine, folate, polyunsaturated and monounsaturated fatty acids including linoleic and oleic acid, iron, phosphorous, zinc, sodium, potassium, magnesium, and calcium [2]. Chickpeas are used both for human consumption as well as for animal feed. These are beneficial to reduce blood sugar and increase insulin sensitivity. Chickpeas are also useful to reduce blood cholesterol level and improve bowel function. Additionally, these are also beneficial in reducing the number of bad bacteria from intestines [3].

According to the report of United Nation of Food and Agriculture (FAO) in the world, Pakistan comes at the 4th place for the production of chickpea [4]. Major areas of Pakistan for the production of chickpea include Thal desert and Cholistan of Punjab and Khyber Pakhtunkhwa (KPK) in rainfed conditions. In Baluchistan and Sindh, chickpea crop is cultivated after the rice crop. The production of chickpea in Pakistan is affected by various biotic and abiotic factors [5]. The various biotic factors affecting chickpea production include fungal diseases (fusarium wilt, dry root rot and stunt), insects (homoptera, diptera, coleoptera, lepidoptera), pests (pod borer, semi-looper, aphids, black cutworm, leaf miner, pulse beetle), and plant parasitic nematodes (reniform nematodes, root-knot nematodes, root-lesion nematodes and cyst forming nematodes), causing drastic loss of chickpea yield in Pakistan [6]. On the other hand, drought, terminal heat, low temperature, salt, and water-logging are the major abiotic factors affecting chickpea production in Pakistan [7].

Charcoal rot in chickpea is caused by the fungus *Macrophomina phaseolina*. Asia, Africa, and Australia are the warm, dry parts of the world where charcoal rot is most prevalent [8]. In Pakistan, it is common in Sindh and Punjab regions [9]. Most of its symptoms appear after midseason during the reproductive stage of crop. Infected plants have smaller leaves than healthy ones. The leaves turn yellow, then wilt and turn brown in color but remain attached with petiole. In some cases, black patches appear on the stem, and pods have black powder-like substance instead of seeds [10]. The charcoal rot causes black discoloration of roots and sometimes lack of feeder roots in chickpea plant. Sometimes, the seeds of chickpea in pods are black in color or in the black powder form. This black color is due to black sclerotia of fungus *M. phaseolina.* The sclerotia are black in color, having smooth surface, 50-60 μm in diameter, round to oblong in shape, uniformly reticulate and originate from lump of hyphal cells [11].

The fungal disease can be controlled by various methods, for instance physical, chemical, and biological methods. In the physical method, the soil is treated with solar energy or heat for specific time period to remove the contamination or disease-causing residues. This method is efficient for removing disease-causing agents from upper layer of soil. The contamination present in deeper soil remains untouched. This physical method is low cost and time-consuming [12]. Other methods are also used, for instance soil fumigation, rotation of crops, cleaning the farming equipment, and burning the crop residues. Some cultural practices are also helpful in managing soil-borne diseases, for instance drainage, tillage, irrigation, and altering of soil pH. These practices may change the date and depth of seeding, thinning, pruning, and plant spacing [13].

The agricultural revolution cannot be carried out in a sustainable way by using chemicals that are hazardous for health. Plant growth and development require both macro and micro-nutrients. The provision of these nutrients in low concentration effects plant yield and chances of disease exposure may also increase [14]. Farm yard manure (FYM) is the best source of macro and micro-nutrients which are fundamental for plant growth. FYM is composed of organic waste, peat and compost. It increases the health, physiochemical and biological properties of soil. This soil is much better for root development. FYM also have an ability to control lot of fungal diseases, in which charcoal rot is also included [15]. Therefore, this study aims to explore the efficacy of organic farming practices in managing charcoal rot of chickpea, providing an environmentally sustainable alternative to chemical control. By enhancing soil health and promoting beneficial microbial activity, organic methods may offer long-term disease suppression. This approach supports sustainable agriculture and improves crop resilience under stress-prone conditions [16]. Farmyard manure (FYM) was selected in this study because it is a widely used and readily available organic amendment that improves physical properties of soil, enhances microbial activity, and increases organic matter content [17]. FYM is known to promote beneficial soil microorganisms that can suppress soil-borne pathogens, such as *M. phaseolina*, the causal agent of charcoal rot. Additionally, FYM improves nutrient availability and plant vigour, which indirectly enhances disease tolerance in chickpea. Its low cost, sustainability, and common use in organic farming systems make it an appropriate and practical choice for evaluating disease management under organic conditions [18].

2. MATERIALS AND METHODS

2.1. Growing Chickpea Plant

Chickpea seeds were procured from Bahawalpur. These seeds were soaked overnight before sowing.

2.1.1. Infestation of Pathogenic Fungi in Soil. The pathogenic fungi, that is, *M. phaseolina* (accession number: FCBP-PTF-1156) was procured from First Fungal Culture Bank of Pakistan, Institute of Agriculture Sciences, University of the Punjab, Lahore, Pakistan. The culture was sub-cultured in Malt Extract Agar (MEA) medium followed by mixing in double distilled water for the preparation of fungal spore suspension. Furthermore, the pre-sterilized soil was infested with fungal spore suspension (10 mL).

2.1.2. Soil Amendment with Farmyard Manure (FYM). FYM was procured from nursery, Bahawalpur, Pakistan. The FYM was sun dried and about 0.5%, 1%, 1.5%, and 2% of FYM was added followed by thoroughly mixing in soil.

2.2. Experiment

The experiment was carried out with six different treatments, that is, control (-ve), control (+ve), *M. phaseolina* (MP) + 0.5% FYM, MP + 1% FYM, MP + 1.5% FYM, and MP + 2% FYM. While negative control receives only water, in positive control the plant is infected with charcoal rot pathogen. The whole experiment is conducted in completely randomized design for 15 days. After 15 days, various morphological alterations in plants, physiological (total green pigment (TGP), soluble sugar (SS)), biochemical (total protein amount (TPA), catalase (CAT) and peroxidase (POX) activities) and growth [shoot, root length (RL) and biomass (fresh and dry)] attributes are studied.

2.2.1. Total Green Pigment (TGP). To create an extract, 10 mL of 80% ethanol (80 mL ethanol + 20 mL distilled water) was added to about 0.5 g of the chickpea plant's shoot. The mixture was then centrifuged at 3000 rpm for 10 minutes. Furthermore, at 645 and 663 nm, the absorption was observed.

2.2.2. Soluble Sugar (SS). After adding 10 mL of 80% ethanol, the chickpea plant shoot (0.5 g) was crushed and centrifuged for 10 minutes at 3000 rpm. In addition, 1 mL of 5% folin phenol and 5 mL of 96% H_2SO_4 were added and carefully mixed and incubated for 10 minutes at 20 ^oC. Additionally, the absorbance was observed at 490 nm.

2.2.3. Total Protein Amount (TPA). About 0.5 g of shoot of chickpea plant was crushed with 0.1 M phosphate buffer (mixed 0.1 M K_2HPO_4 = 8.7 g in 500 mL distilled water and 0.1 M KH_2PO_4 = 6.8 g in 500 mL distilled water). Moreover, it was centrifuged at 3000 rpm for 10 minutes. After that, the supernatant (0.1 mL) was mixed with 0.9 mL distilled water and 1mL of Reagent C [for preparation of Reagent C mix 50 mL of Reagent A and 1mL of Reagent B (Reagent A = 2% Na_2Co_3 in 0.1 N NaOH, Reagent B = 0.5% $CuSO_4$ in 1% Potassium Sodium Tartrate)]. It was further shacked for 10 minutes followed by adding 0.1mL of Reagent D (Reagent D = mix equal ratio of Folin phenol and distilled water). After that, the mixture was incubated at 20 ^oC for 30 minutes. Furthermore, absorbance was noted at 660 nm.

2.2.4. Peroxidase (POX). In order to create 0.5 mL of supernatant solution, 0.5 g of the chickpea plant's shoot was crushed with 0.1 M phosphate buffer and centrifuged at 3000 rpm for 10 minutes. In the supernatant solution, the reaction was initiated by adding 1 mL of phosphate buffer, 1 mL of pyrogallol, and 1 mL of 0.01 M H_2O_2, and incubation began at 25 ^oC. Additionally, 1 mL of 2.5 NH_2SO_4 was added, and the incubation was halted after 5 minutes. At 500 nm, absorbance was then measured.

2.2.5. Catalase (CAT) Activity. 0.5 g of chickpea plant's shoot was crushed with 0.1 M phosphate buffer and was centrifuged at 3000 rpm for 10 minutes. The experiment was carried out by mixing 1 mL of supernatant solution with 0.01 M H_2O_2 and was incubated at 20 ^oC for 5 minutes. Then, 10 mL of 1% H_2SO_4 was added. As the pink color developed and persisted for around 10-15 minutes, the sample was titrated against 0.05 N $KMnO_4$

and the data were recorded.

2.3. Statistical Analysis

The Least Significant Difference (LSD) and Pearson correlation of the studied parameters, based on three replicates, were analyzed using STATISTIX 8.1 software.

3. RESULTS

3.1. Morphological Alteration in Chickpea Plant

The results revealed typical symptoms, such as charcoal rot appearance on root and lower portion of shoot colour on plant that was infected by *M. phaseolina*. However, soil amendments with different doses of FYM in the presence of *M. phaseolina* exhibited no symptoms of charcoal rot disease in chickpea plant (Figure 1).

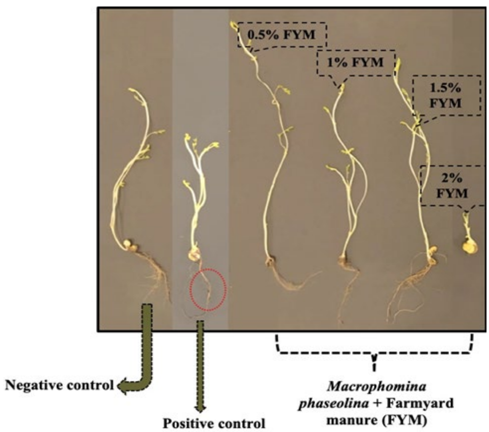


Figure 1. Impact of FYM on the Morphology of Chickpea Plant Infected by *M. phaseolina* after 15 Days of Seed Germination

3.2. Physiological and Biochemical Parameters

3.2.1. Total Chlorophyll Pigment. When infected to the charcoal rot pathogen, it was observed that the TGP of chickpea plants significantly ($p \leq 0.05$) dropped by 54% when compared to the negative control (1.23 mg/g). Whereas, the soil amendment with 0.5% to 1% of FYM enhanced the said parameter by -154% to -4% with comparison to positive control (0.56 mg/g). While, rest of the two doses of FYM, that is, 1.5% to 2% significantly ($p \leq 0.05$) reduced by 34% to 35% as compared to positive control (Figure 2).

3.2.2. Soluble Sugar (SS). When infected to the charcoal rot pathogen, it was found that the SS content of chickpea plants significantly ($p \leq 0.05$) decreased by 65% when compared to the negative control (1.43 mg/g). Whereas, the soil amendment with 0.5% to 1% of FYM enhanced the said parameter by -253% to -8% with comparison to positive control (0.49 mg/g). While, rest of the two doses of FYM, that is, 1.5% to 2% significantly ($p \leq 0.05$) reduced by 60% to 41% as compared to positive control (Figure 3).

3.2.3. Total Protein Amount (TPA). It was found that the protein amount of chickpea plant significantly ($p \leq 0.05$) increased by -312% as compared to negative control (0.48 mg/g), when infected with charcoal rot pathogen. Whereas, the soil amendment with 0.5% to 1% of FYM decreased the said parameter by 78% to 13% with comparison to positive control (1.98 mg/g). While, rest of the two doses of FYM, that is, 1.5% to 2% significantly ($p \leq 0.05$) increased by -383% to -11% as compared to positive control (Figure 4).

3.2.4. Peroxidase (POX). It was noted that the POX of chickpea plant significantly ($p \leq 0.05$) increased by -220% as compared to negative control (0.68 Units/minute/mg/g), when infected with charcoal rot pathogen. Whereas, the soil amendment with 0.5% to 1% of FYM decreased the said parameter by 71% to 9%

with comparison to positive control (10.1 Units/minute/mg/g). While, rest of the two doses of FYM, that is, 1.5% to 2% significantly ($p \leq 0.05$) increased by -249% to -10% as compared to positive control (Figure 5).

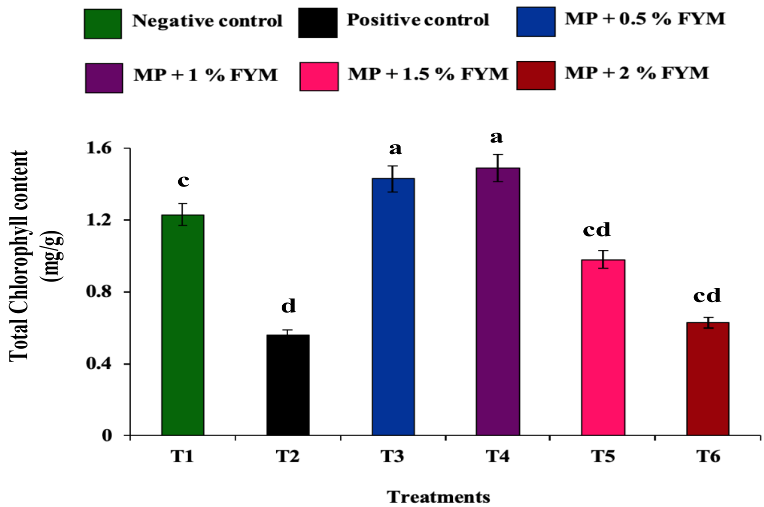


Figure 2. Impact of Farmyard Manure (FYM) on the Chlorophyll Content of Chickpea Plant Infected by *M. phaseolina* after 15 Days of Seed Germination. Standard Errors of Means of Five Replicates are Represented by Vertical Bars. Values at the Top Show Significant ($p \leq 0.05$) Difference as Determined by LSD.

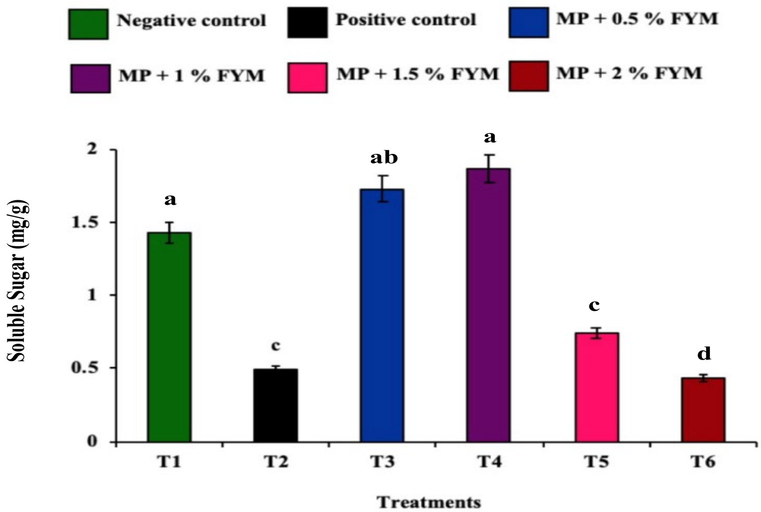


Figure 3. Impact of FYM on the SS of Chickpea Plant Infected by *M. phaseolina* After 15 days of Seed Germination. Standard Errors of Means of Five Replicates are Represented by Vertical Bars. Values at the Top Show Significant ($p \leq 0.05$) Difference as Determined by LSD

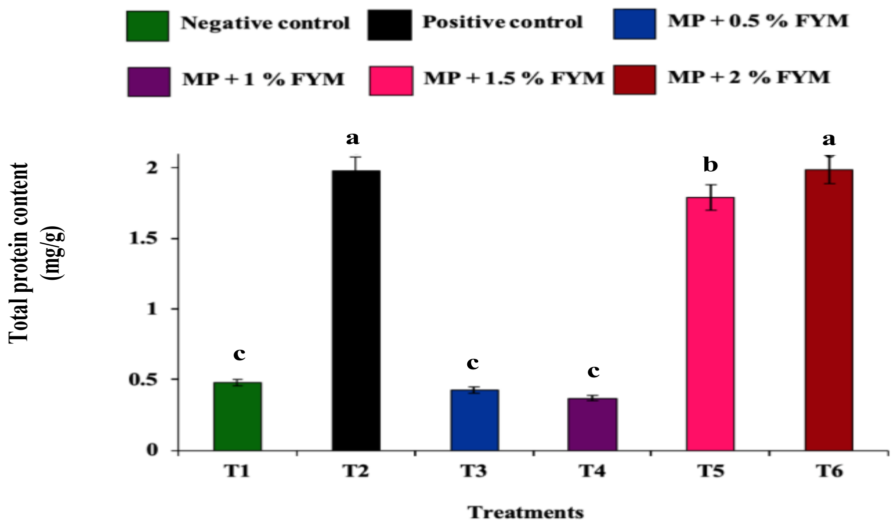


Figure 4. Impact of FYM on the Total Protein Content of Chickpea Plant Infected by *M. phaseolina* After 15 Days of Seed Germination. Standard Errors of Means of Five Replicates are Represented by Vertical Bars. Values at the Top Show Significant ($p \leq 0.05$) Difference as Determined by LSD.

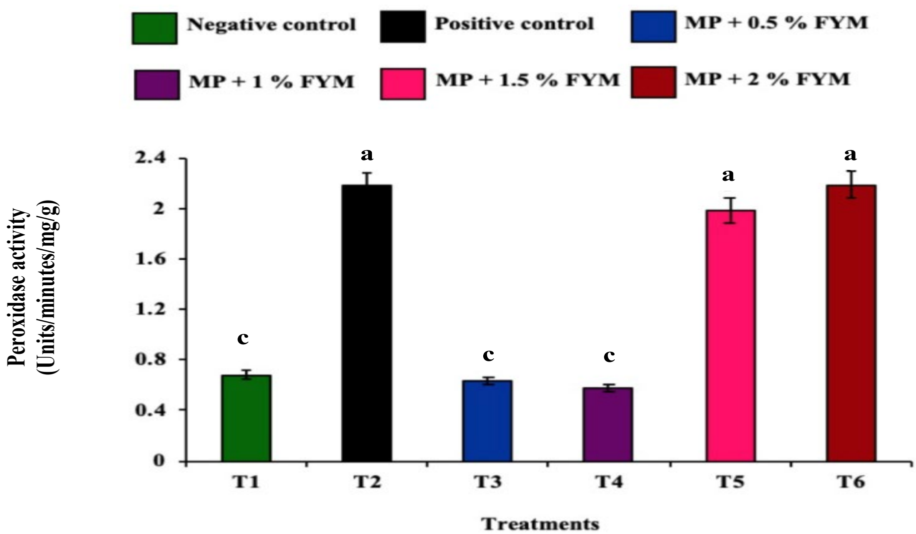


Figure 5. Impact of FYM on the POX Activity of Chickpea Plant Infected by *M. phaseolina* After 15 days of Seed Germination. Standard Errors of Means of Five Replicates are Represented by Vertical Bars. Values at the Top Show Significant ($p \leq 0.05$) Difference as Determined by LSD

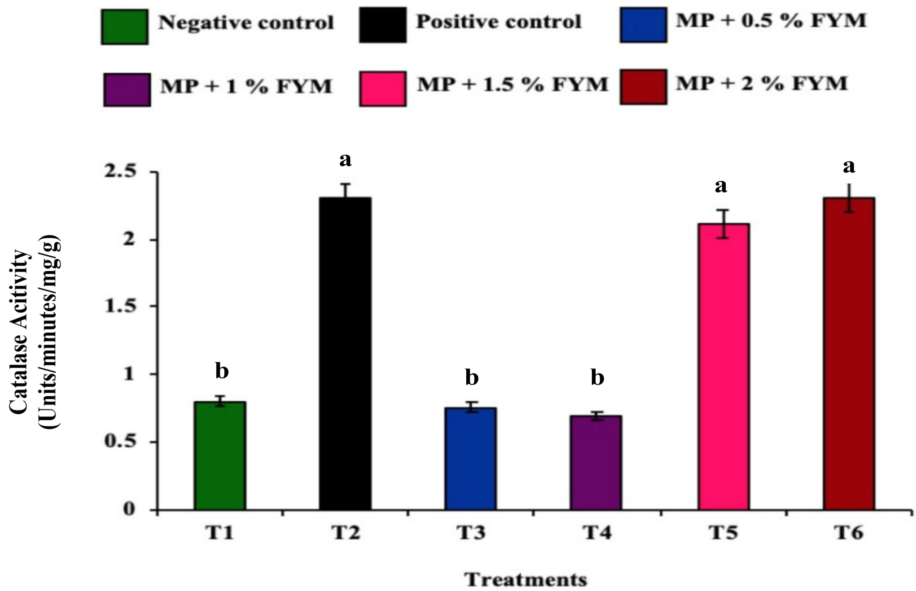


Figure 6. Impact of FYM on the CAT Activity of Chickpea Plant Infected by *M. phaseolina* After 15 Days of Seed Germination. Standard Errors of Means of Five Replicates are Represented by Vertical Bars. Values at the Top Show Significant ($p \leq 0.05$) Difference as Determined by LSD

3.2.5. Catalase (CAT). It was noticed that the CAT of chickpea plant significantly ($p \leq 0.05$) increased by -187% as compared to negative control (0.8 Units/minute/mg/g), when infected with charcoal rot pathogen. Whereas, the soil amendment with 0.5% to 1% of FYM decreased the said parameter by 67% to 8% with comparison to positive control (2.3 Units/minute/mg/g). While, rest of the two doses of FYM, that is, 1.5% to 2% significantly ($p \leq 0.05$) enhanced the CAT activity by -205% to -9% as compared to positive control (Figure 6).

3.3. Growth Parameters

3.3.1. Germination Rate. The germination rate and germination index of chickpea significantly ($p \leq 0.05$) reduced by 66% and 40%, respectively, in plants infected with the charcoal rot pathogen compared with the negative control (100% and 60%). However, soil amendment at 1.5% significantly improved these parameters by 77% and 46%, respectively, compared with the positive control. Furthermore, the Relative Injury Rate (RIR) of chickpea plants increased significantly ($p \leq 0.05$) by 0.23 compared with the negative control. Across soil amendment levels ranging from 0.5% to 2%, the germination rate decreased by 55% to 33%, the germination index decreased by 33% to 20%, while the RIR increased from 0.45 to 0.67. (Table 1).

3.3.2. Root Length (RL). It was found that the RL of chickpea plant significantly ($p \leq 0.05$) decreased by 34% as compared to negative control (15.2 cm), when infected with charcoal rot pathogen. Whereas, the soil amendment with 0.5% to

1% of FYM enhanced the said parameter by -13% to -14% with comparison to positive control (10.1 cm). While, rest of the two doses of FYM, that is, 1.5% to 2% significantly ($p \leq 0.05$) reduced the RL by 36% to 93% as compared to positive control (Figure 7).

3.3.3. Shoot Length (SL). It was found that the SL of chickpea plant significantly ($p \leq 0.05$) reduced by 21% as compared to negative control (25.4 cm), when infected with charcoal rot pathogen. However, the soil treatment with 0.5% to 1% of FYM increased the said parameter by -7% to -47% in comparison with positive control (20.2 cm). While, rest of the two doses of FYM, that is, 1.5% to 2% significantly ($p \leq 0.05$) reduced the SL by 56% to 73% as compared to positive control (Figure 8).

Table 1. Impact of FYM on the Germination Rate of Chickpea Plant Infected by *M. phaseolina*

Treatments	Germination Rate (%)	Germination Index (%)	Relative Injury Rate (RIR)
Negative control	100 ^{a}	60 ^{a}	0.00 ^{e}
Positive control	66 ^{b}	40 ^{b}	0.23 ^{bd}
MP + 0.5% FYM	55 ^{c}	33.33 ^{d}	0.45 ^{bc}
MP + 1% FYM	44 ^{d}	26.67 ^{e}	0.56 ^{ab}
MP + 1.5% FYM	77 ^{e}	46.67^{c}	0.34 ^{cd}
MP + 2% FYM	33 ^{f}	20 ^{f}	0.67 ^{a}

Note. Alphabets show significant ($p \leq 0.05$) difference as determined by LSD test.

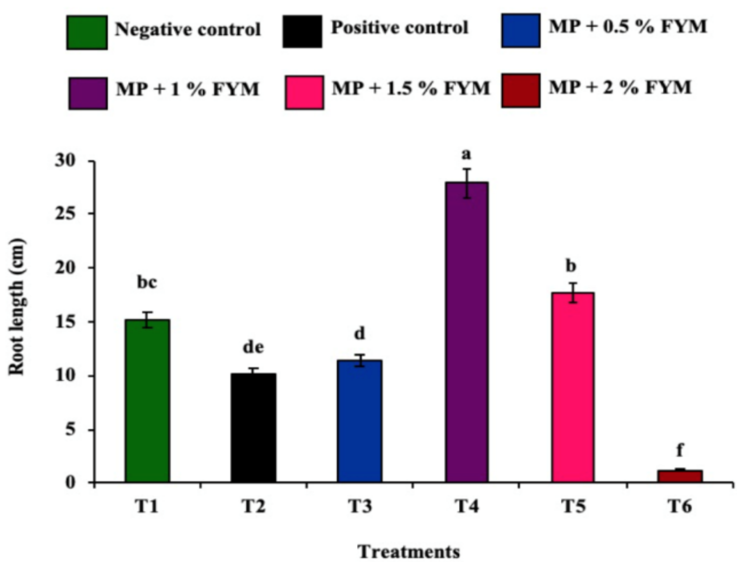


Figure 7. Impact of FYM on the RL of Chickpea Plant Infected by *M. phaseolina* After 15 days of Seed Germination. Standard Errors of Means of Five Replicates are Represented by Vertical Bars. Values at the Top Show Significant ($p \leq 0.05$) Difference as Determined by LSD

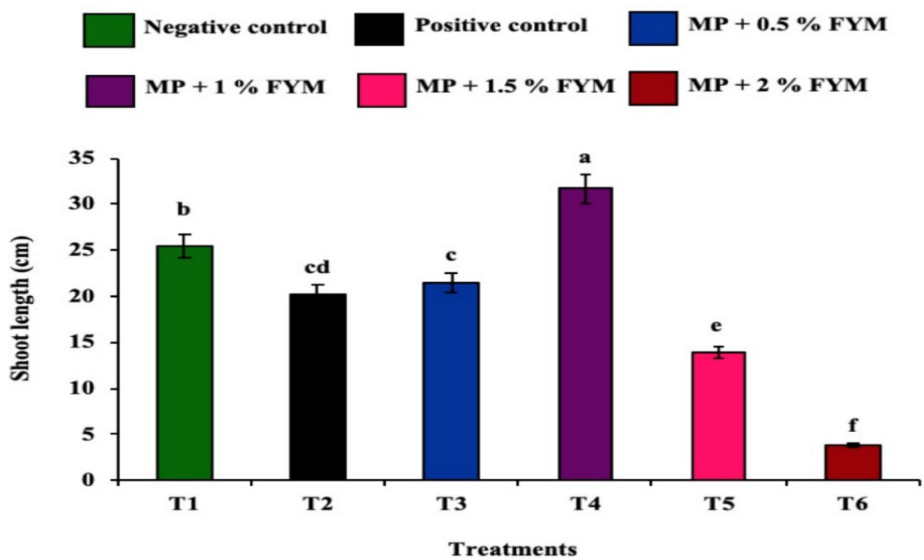


Figure 8. Impact of FYM on the SL of Chickpea Plant Infected by *M. phaseolina* After 15 days of Seed Germination. Standard Errors of Means of Five Replicates are Represented by Vertical Bars. Values at the Top Show Significant ($p \leq 0.05$) Difference as Determined by LSD.

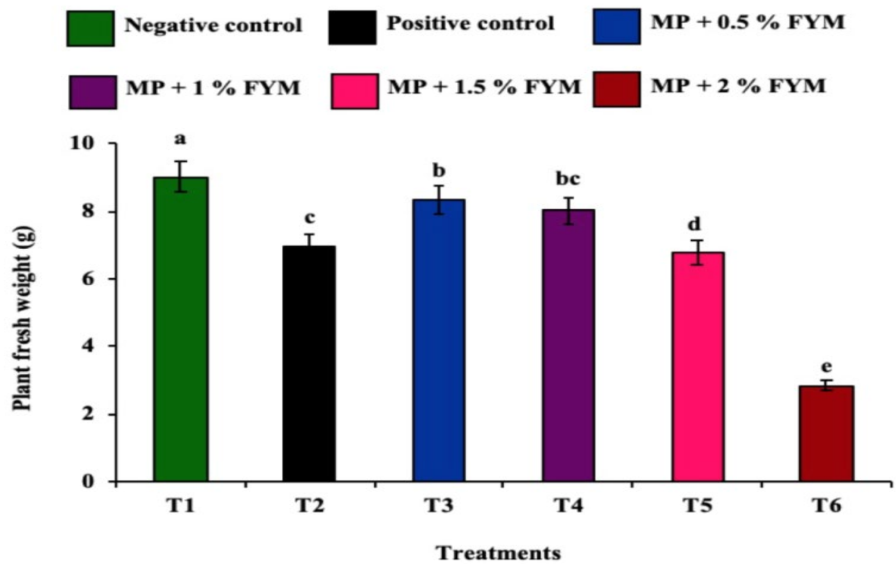


Figure 9. Impact of FYM on the Plant Fresh Weight of Chickpea Plant Infected by *M. phaseolina* After 15 Days of Seed Germination. Standard Errors of Means of Five Replicates are Represented by Vertical Bars. Values at the Top Show Significant ($p \leq 0.05$) Difference as Determined by LSD

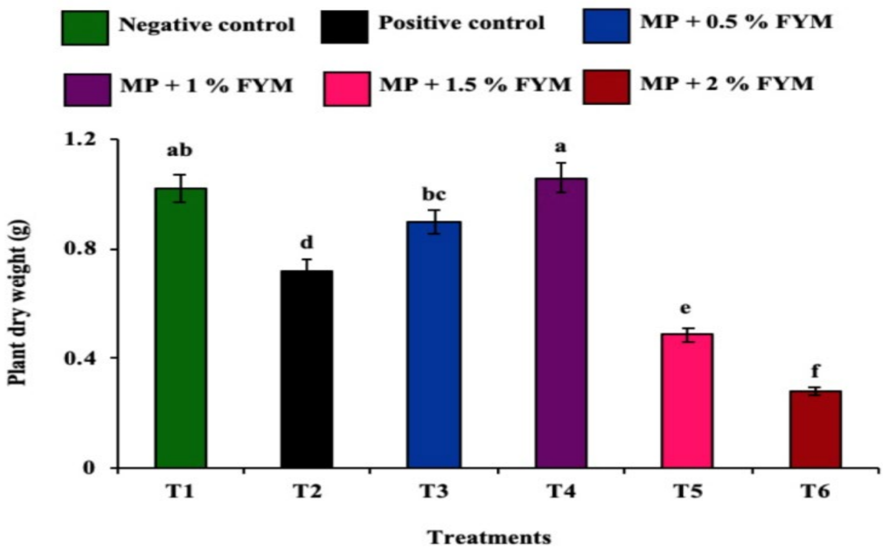


Figure 10. Impact of FYM on the Plant Dry Weight of Chickpea Plant Infected by *M. phaseolina* After 15 Days of Seed Germination. Standard Errors of Means of Five Replicates are Represented by Vertical Bars. Values at the Top Show Significant ($p \leq 0.05$) Difference as Determined by LSD.

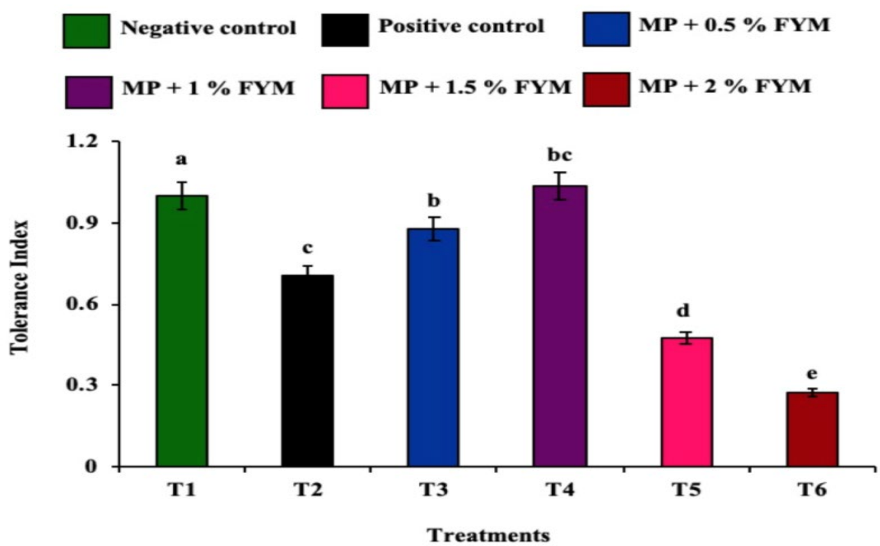


Figure 11. Tolerance Index of FYM on Chickpea Plant Infected by *M. phaseolina* After 15 days of Germination. Standard Errors of Means of Five Replicates are Represented by Vertical Bars. Values at the top show significant ($p \leq 0.05$) difference as determined by LSD

3.3.4. Plant Fresh Weight (PFW). It was noticed that the fresh weight of chickpea plant significantly ($p \leq 0.05$) decreased by 23% as compared to negative control (9.02 cm), when infected with charcoal rot pathogen. However, the soil treatment with 0.5% to of FYM enhanced the said parameter by -20% with comparison to positive control (6.99 cm). While, rest of the three doses of FYM, that is, 1% to 2% significantly ($p \leq 0.05$) reduced the fresh weight by 4% to 58% as compared to positive control (Figure 9).

3.3.5. Plant Dry Weight (PDW). The results revealed that the dry weight of chickpea plant significantly ($p \leq 0.05$) declined by 29% as compared to negative control (1.02 cm), when infected with charcoal rot pathogen. Whereas, the soil amendment with 0.5% to 1% of FYM enhanced the said parameter by -24% to -18% with respect to positive control (0.72 cm). While, the rest of the two doses of FYM, that is, 1.5% to 2% significantly ($p \leq 0.05$) reduced the dry weight by 54% to 42% as compared to positive control (Figure 10).

3.3.6. Tolerance Index. The results revealed that the tolerance index of chickpea plant significantly ($p \leq 0.05$) decreased by 0.74 as compared to negative control (1.00), when infected with charcoal rot pathogen. Whereas, the soil amendment with 0.5% and 2% of FYM enhanced the said parameter by 0.71 to 0.23 with respect to positive control (0.74). While, the rest of the two doses of FYM, that is, 1% to 1.5% significantly ($p \leq 0.05$) increased by 0.79 to 1.09 as compared to positive control (Figure 11)

3.4. Correlation between Growth and Physiological Attributes

The association between growth parameters, that is, SL, RL, PFW, and PDW vs physiological traits, that is, TGP, SS, TPA, CAT, and POX activities is described in Table 2. The results indicated that the relationship between CAT × PDW, RL, SL, SS, TGP showed negative, strong to strongest, and exceedingly significant ($r = -0.58$ to -0.8; $p < 0.01$). Whereas, the association between CAT × PFW was weak, negative, and non-significant ($r = -0.4$; $p > 0.05$).

Moreover, the relationship between PDW × POX, TPA exhibited negative, strong, and exceedingly significant ($r = -0.58$; $p < 0.01$). Furthermore, the relationship between PFW × POX, TGP exhibited negative, weak, and non- significant ($r = -0.41$; $p < 0.01$). Likewise, the relationship between POX × RL, SL, SS, and TGP showed negative, strongest, and exceedingly significant ($r = -0.61$ to -0.8; $p < 0.01$). Also, the relationship between RL × TPA exhibited negative, strong, and exceedingly significant ($r = -0.57$; $p < 0.01$). Additionally, the relationship between SL × TPA exhibited negative, strongest, and exceedingly significant ($r = -0.74$; $p < 0.01$). Likewise, the relationship between SS × TPA exhibited negative, strongest, and highly significant ($r = -0.84$; $p < 0.01$). Also, the relationship between TCL × TPA exhibited negative, strongest, and exceedingly significant ($r = -0.64$; $p < 0.01$).

The results showed that the relationship between CAT × POX, TPA exhibited positive, strongest, and exceedingly significant ($r = 0.99$; $p < 0.01$). Moreover, the relationship between PDW × PFW, RL, SL, SS, and TGP exhibited positive, strong, and strongest and statistically significant to exceedingly significant ($r = 0.78$ to 0.53; $p < 0.01$). Furthermore, the relationship between PFW × RL, SL, SS, and TGP exhibited positive, strong to strongest, and statistically significant to exceedingly significant (r = 0.57 to 0.74; p < 0.01). Also, the relationship between POX × TPA exhibited positive, strongest, and exceedingly significant (r = 0.99; p < 0.01). Additionally, the relationship between RL × SL and SS exhibited positive, strong to strongest, and statistically significant to exceedingly significant (r = 0.80 to 0.52; p < 0.01). Whereas, the association between RL × TGP was weak, positive, and non-significant (r = 0.42; p > 0.05). Likewise, the relationship between SL × SS exhibited positive, strongest, and exceedingly significant (r = 0.70; p < 0.01). Whereas, the association between SL × TGP was strong, positive, and non-significant (r = 0.51; p > 0.05). Furthermore, the relationship between SS × TGP exhibited positive, strongest, and exceedingly significant (r = 0.93; p < 0.01).

Table 2. Correlation Coefficient between Growth Parameters and Physiological Attributes of Chickpea Plant Infected by *M. phaseolina* After 15 Days of Germination

	CAT	PDW	PFW	POX	RL	SL	SS	TGP
PDW	-0.58**							
PFW	-0.40^{ns}	0.78**						
POX	0.99**	-0.58**	-0.41^{ns}					
RL	-0.61**	0.57*	0.57*	-0.61**				
SL	-0.75**	0.78**	0.74**	-0.75**	0.80**			
SS	-0.8**	0.64**	0.65**	-0.8**	0.52*	0.70**		
TGP	-0.60**	0.53*	0.65**	-0.61**	0.42^{ns}	0.51^{ns}	0.93**	
TPA	0.99**	-0.58**	-0.41^{ns}	0.99**	-0.57**	-0.74**	-0.84**	-0.64**

Note. *p < 0.05, ** p < 0.01, ns (non-significant)

4. DISCUSSION

FYM was used to prevent the chickpea seed disease known as charcoal rot caused by *M. phaseolina*. *M. phaseolina* effects the growth as well as physiology of plant. The total chlorophyll content in plants was increased by the availability of nutrients in the soil, such as NO_3, H_2PO_4, and some trace elements (Zn, Fe, Cu, Mn and Mg) [19]. These nutrients improved the biochemical components and co-enzymes which enhanced the photosynthetic and metabolic activities. As a result, higher cell division took place which gave higher or maximum yield [20]. In the current study, it was noticed that the application of 0.5% FYM enhanced the green pigment by -154%. Whereas, the soil amendment with 1% to 2% reduced the said parameters.

It was observed that the soil amendment with 0.5% of FYM enhanced the said parameter by -253%. According to the findings of [21], nitrogen is the main factor that directly impacts the plants since it makes SS for the plants [22].

The protein amount increased by increasing the FYM quantity. These enzyme activities increased the physical condition of soil associated with the growth and development of roots, which, in turn, enhanced the nutrient absorption capacity of plant [23]. The host's defence mechanism and pathogen attack may be responsible for the host cell's production of several proteins and nitrogenous substances, which could lead to an increase in the TPA [24]. In this study, it was found that the protein content of chickpea plant increased by -383% in treatments (1.5%). Whereas, the soil amendment with 0.5%, 1%, and

2% of FYM decreased the said parameter.

The POX activity increased by increasing FYM quantity due to the humified organic matter which resists the microbial mineralization. The microbial activity and soil fertility are closely related to each other because the mineralization of important elements occur [25]. It was noted that the POX activity of chickpea plant increased by -249% in treatment 1.5%. While, rest of the doses of FYM with *M. Phaseolina, that is,* 0.5%, 1%, and 2% decreased the said parameters.

The CAT is intracellular enzyme which is involved in microbial oxidoreductase metabolic activity. As a result, it increases the soil aeration and soil porosity [26]. It was noticed that the CAT activity of chickpea plant increased by -205% in treatment (1.5%). While, the rest of the doses of FYM with *M. phaseolina* (0.5%, 1% and 2%) decreased the said parameters.

It was noticed that the germination rate of chickpea plant decreased in other treatments, that is, 0.5%, 1%, and 2%. However, the soil amendments with 1.5% enhanced the said parameters by 77%. These results showed that edible amount of FYM resists the effect of *M. phaseolina*. Furthermore, FYM has an ability to increase the water holding capacity and soil infiltration characteristics, decreasing the cone index and bulk density of soil which plays an important role in the germination of seeds. The water holding capacity helps retain soil moisture for a longer duration, enhances the effectiveness of FYM, and thereby supports improved seed germination [27, 28].

FYM contains organic matter which enhances the presence of micro-organisms. The activity of organic matter softens soil, improves the aeration of soil, and allows roots to anchor deeper. These micro-organisms also fix nitrogen which is essential for root growth and development. FYM is the source of nutrients (N, K, and P) which play an important role in the root growth [29]. In the current study, it was found that the RL of chickpea plant significantly ($p \leq 0.05$) decreased in treatments, that is, 0.5%, 1.5%, and 2%. Whereas, the soil amendment with 1% of FYM enhanced the said parameter by -14%.

The porosity, water holding capacity, and availability of nutrients in the soil enhanced the root growth which, in turn, increased the nutrient uptake from soil to the upper part of plant and enhanced the shoot growth of the plant [30]. The nitrogen is crucial for plant's vegetative growth, cell enlargement, cell division, and protein synthesis. Nitrogen, potassium, and phosphorus are helpful in increasing the height of the plant [31]. In the current study, it was found that the SL of chickpea plant reduced in treatments, that is,, 0.5%, 1.5%, and 2%. However, the soil treatment with 1% of FYM increased the said parameter by -47%.

It was noticed that the fresh weight of chickpea plant decreased in treatments, that is, 1% to 2%. However, the soil treatment with 0.5% of FYM enhanced the said parameter by -20%. The results revealed that the dry weight of chickpea plant declined in treatments, that is, 1% to 2%. Whereas, the soil amendment with 0.5% of FYM enhanced the said parameter by -24%. The results showed that tolerance index of FYM on chickpea plant decreased in treatments, that is, 0.5% and 2%, while increased in rest of the two doses, that is, 1% and 1.5%. The findings were noticed in the study of Akhtar et al. [16]. They noticed that FYM mitigates stress responses and enhances physiological traits in mash bean. Their study showed that 2% of

FYM improved physiological and biochemical markers under stress, indicating enhanced tolerance indices.

The correlation coefficient relationship between growth and physiological attributes of chickpea plants infected by *M. phaseolina* varied from strong to strongest, statistically significant ($p \leq 0.05$) to exceedingly significant, positive. Sometimes, it showed weak, negative, and non-significant relationship.

4.1. Conclusion

M. phaseolina infection disrupted normal plant development and physiology (chlorophyll, sugar, protein, catalase and peroxidase levels). However, the use of FYM (0.5%, 1%, 1.5% and 2%) to improve antioxidative enzyme activity was the most effective way to manage disease (charcoal rot). Furthermore, FYM enhanced the germination rate, root and shoot length as well as improved yield, weight, and total biological dry biomass.

Author Contribution

Sundus Akhtar: conceptualization, supervision, writing – review & editing. **Asma Maryyum:** methodology, data curation, validation. **Ayesha Shafqat:** data curation. **Sajida Sharif:** formal analysis. **Fahad Riaz:** formal analysis

Conflict of Interest

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

Data Availability Statement

Data supporting the findings of this study will be made available by the corresponding author upon request.

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   This comprehensive review highlights the historical and geographical background of chickpea cultivation, its nutritional composition—including proteins, fiber, and minerals—and its agricultural importance globally. It also delves into modern breeding techniques and the plant's symbiotic relationship with *Mesorhizobium* species, which enhances nitrogen fixation and plant health. The paper is a useful foundation for understanding both the nutritional and ecological significance of chickpea.

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