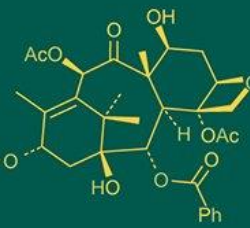
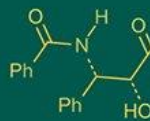
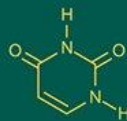
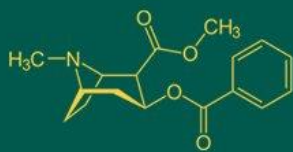


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Effect of biostimulants on pod morphology and development of soybean (*Glycine max* L.) under charcoal rot disease conditions caused by *Macrophomina phaseolina*

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Abstract

Charcoal rot, caused by *Macrophomina phaseolina* (Tassi) Goid, is a major soil-borne disease that severely affects soybean productivity by disrupting vascular flow, reducing photosynthetic efficiency, and limiting pod formation and filling. The present investigation was undertaken during Rabi 2023-24 and 2024-25 to evaluate the effect of biostimulants and stress modulators on pod morphology in soybean under charcoal rot conditions. The experiment was conducted in a factorial completely randomized design comprising two varieties—JS 20-29 (susceptible) and JS 20-98 (moderately resistant)—and twelve treatments, including untreated control, *Trichoderma* seed treatment (10 g kg⁻¹ seed), fungicide (Penflufen 13.28% w/w + Trifloxystrobin 13.28% w/w F8 @ 1 ml kg⁻¹ seed), plant extract (PE @ 50-200 ppm), *Macrophomina* fungal extract (MFE @ 50-100 ppm), and salicylic acid (SA @ 100-200 ppm). Results revealed that pod morphological traits were significantly influenced by treatments and varieties. The highest pod length (34.18 mm), girth (10.52 mm), and width (4.93 mm) were recorded in the non-inoculated control (T₁), which was statistically at par with *Trichoderma* (T₃) and fungicide (T₄) treatments, indicating effective disease suppression and physiological maintenance under stress. The lowest pod length (28.21 mm) and girth (8.54 mm) were observed in *Macrophomina phaseolina* fungal extract @ 100 ppm (T₁₀), confirming pathogen-induced inhibition of pod growth. Between varieties, JS 20-98 outperformed JS 20-29 in pod length (32.78 mm vs. 28.24 mm) and girth (9.36 mm vs. 8.94 mm), demonstrating greater tolerance and adaptability to pathogen stress. The application of *Trichoderma* and salicylic acid proved effective in mitigating disease severity by improving physiological balance and assimilate translocation during pod development. Thus, integrating biocontrol agents and resistance inducers represents a promising, sustainable approach for maintaining pod morphology and yield stability of soybean under *Macrophomina*-infested environments.

Keywords: Soybean (*Glycine max* L.), charcoal rot, *Macrophomina phaseolina*, biomodulators, *Trichoderma*, salicylic acid, pod morphology

Introduction

Soybean (*Glycine max* L.) is one of the most important oilseed-legume crops of the world, valued for its high protein (40%) and oil (20%) content, as well as its role in sustainable agriculture through biological nitrogen fixation. In India, soybean contributes significantly to oilseed production, especially in the central zone encompassing Madhya Pradesh, which provides favorable conditions for both crop growth and disease development. Among the numerous biotic stresses affecting soybean, charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid has emerged as one of the most devastating soil-borne diseases, leading to substantial yield losses in warm and drought-prone regions. The pathogen attacks the root and basal stem tissues, causing microsclerotia formation that obstructs vascular flow, resulting in premature senescence, poor pod filling, and shriveled seeds. Yield losses ranging from 10 to 60% have been reported depending on varietal susceptibility, soil moisture, and temperature (Mengistu *et al.*, 2011; Rahman, 2021) [5, 8].

Under charcoal rot stress, physiological and biochemical processes such as photosynthesis, assimilate partitioning, and pod development are severely affected due to oxidative damage and impaired translocation. The reduction in pod length, girth, and width under diseased conditions directly influences the sink strength and overall yield. Management of *M. phaseolina* is challenging due to its wide host range, persistent sclerotia, and limited efficacy of conventional fungicides in soil environments. Therefore, eco-friendly and sustainable alternatives such as biostimulants, bioagents, and induced resistance compounds are increasingly being explored to improve plant health, physiological balance, and productivity under disease stress (Poveda, 2022; Amrate *et al.*, 2023) [6, 1].

Among biological approaches, *Trichoderma* spp. have gained prominence as effective biocontrol agents due to their multiple mechanisms, including mycoparasitism, antibiosis, enzyme secretion, and induction of systemic resistance (Purkayastha *et al.*, 2006; Deshmukh *et al.*, 2021) [7, 3]. Seed treatment or soil application of *Trichoderma harzianum* and *T. viride* has been shown to suppress *M. phaseolina* infection and promote root growth, enhancing the plant's physiological resilience. Likewise, salicylic acid (SA) acts as a vital signaling molecule in the regulation of systemic acquired resistance (SAR) and mitigation of biotic and abiotic stresses by enhancing antioxidant enzyme activity, osmolyte accumulation, and maintenance of chlorophyll and water status (Kaur *et al.*, 2012; Bellaloui, 2021) [4, 2]. In addition, plant extracts (PE) and fungicidal seed protectants are also known to provide partial disease suppression by reducing initial inoculum and improving seedling vigor.

Pod morphology parameters such as pod length, girth, and width are critical determinants of seed number and seed size, serving as integrative indicators of reproductive health and assimilate distribution during pod filling. Any reduction in these traits under stress conditions reflects disruption in photosynthate mobilization and vascular continuity. Hence, evaluating these parameters provides insight into the physiological effectiveness of treatments aimed at mitigating the adverse effects of charcoal rot.

Keeping these aspects in view, the present investigation was undertaken to study the effect of biostimulants on pod morphological characteristics of soybean under charcoal rot disease inoculated conditions. Two soybean varieties—JS 20-29 (susceptible) and JS 20-98 (moderately resistant)—were evaluated under a range of treatments including *Trichoderma* seed treatment, fungicide, plant extracts, *Macrophomina* fungal extract, and salicylic acid at different concentrations. The study aimed to elucidate treatment-wise differences in pod length, pod girth, and pod width and to identify effective management strategies that sustain pod development and yield potential under *M. phaseolina*-infested conditions.

Materials and Methods

The present study was conducted under pot culture conditions during the Rabi seasons of 2023-24 and 2024-25 to evaluate the effect of biostimulants and chemical treatments on pod morphological traits of soybean under charcoal rot disease caused by *Macrophomina phaseolina*. The experiment was laid out in a factorial completely randomized design (FCRD) comprising two soybean varieties, JS 20-29 (susceptible) and JS 20-98 (moderately

resistant), and twelve treatments, each replicated three times. The treatments included T₁: non-inoculated control (without growth modulator), T₂: inoculated control (without growth modulator), T₃: *Trichoderma harzianum* seed treatment at 10 g kg⁻¹ seed, T₄: fungicide (Penflufen 13.28% w/w + Trifloxystrobin 13.28% w/w F8) applied @ 1 ml kg⁻¹ seed, T₅: plant extract (PE) @ 50 ppm, T₆: PE @ 100 ppm, T₇: PE @ 150 ppm, T₈: PE @ 200 ppm, T₉: *Macrophomina* fungal extract (MFE) @ 50 ppm, T₁₀: MFE @ 100 ppm, T₁₁: salicylic acid (SA) @ 100 ppm, and T₁₂: SA @ 200 ppm. The pathogen inoculum was prepared by multiplying *M. phaseolina* on sterilized sorghum grains and incorporated into the potting mixture at sowing to ensure uniform infection in inoculated treatments. Certified seeds of both varieties were surface sterilized, treated as per the respective treatment, and sown in pots containing a mixture of soil and farmyard manure in a 3:1 ratio. Foliar sprays of PE, MFE, and SA were applied at 20 and 45 days after sowing, while uniform irrigation and standard cultural practices were maintained for all pots. At physiological maturity, three pod morphological traits were recorded on randomly selected pods from each replication, including pod length (measured from base to tip using a digital Vernier caliper), pod girth (measured at the mid-point), and pod width (measured at the broadest part of the pod). The data were subjected to statistical analysis and differences among treatment means were compared using the critical difference (CD) at 5% level of significance.

Results

A significant variation in pod length was observed among the different treatments imposed under charcoal rot disease conditions in soybean. The maximum pod length was recorded in pots treated with T₁ (Control, non-inoculated), which measured 34.18 mm, indicating the beneficial effect of disease-free conditions on pod elongation and development. This treatment was statistically at par with T₄ (Fungicide; 31.98 mm) and T₃ (*Trichoderma harzianum*; 31.61 mm), both of which contributed to better pod formation through their protective and growth-promoting effects. T₁₁ (Salicylic acid @ 100 ppm) also recorded comparatively higher pod length (30.88 mm), suggesting its role in enhancing pod growth even under pathogen-induced stress. On the contrary, the minimum pod length was recorded in T₁₀ (*Macrophomina* fungal extract @ 100 ppm; 28.21 mm), which was statistically comparable with other lower-performing treatments. This reduction could be attributed to higher pathogen pressure and compromised assimilate translocation towards developing pods. Among the varieties, JS 20-98 exhibited longer pods (32.78 mm) than JS 20-29 (28.24 mm), indicating that varietal resistance played a vital role in maintaining reproductive growth under charcoal rot infection.

Pod girth also exhibited noticeable differences among the treatments. The highest value was obtained in T₁ (10.52 mm), which was statistically superior to all other treatments, reflecting the maximum pod filling and development in the absence of disease stress. The next best performance was observed in T₁₂ (Salicylic acid @ 200 ppm; 9.40 mm) and T₁₁ (Salicylic acid @ 100 ppm; 9.33 mm), suggesting that exogenous application of salicylic acid might have stimulated defense-related physiological mechanisms, leading to better pod formation. In contrast, T₁₀ (*Macrophomina* fungal extract @ 100 ppm; 8.54 mm) and

T₈ (Plant extract @ 200 ppm; 8.69 mm) recorded the lowest girth, probably due to higher pathogen-induced cellular damage and restricted nutrient movement. Between the two genotypes, JS 20-98 recorded a higher mean pod girth (9.36 mm) compared to JS 20-29 (8.94 mm), further confirming its moderate tolerance and better pod filling ability under charcoal rot infection.

A similar trend was observed for pod width, which was also significantly influenced by different treatments. The highest mean pod width was recorded under T₁ (4.93 mm), followed by T₄ (Fungicide; 4.44 mm) and T₁₁ (Salicylic acid @ 100 ppm; 4.38 mm), signifying that both chemical and biostimulant treatments helped preserve pod structural integrity against pathogen-induced deterioration. The lowest pod width was noted in T₅ (Plant extract @ 50 ppm; 4.09 mm) and T₂ (Inoculated control; 4.17 mm), indicating that insufficient protection under diseased conditions adversely affected pod development. Between varieties, JS 20-29 recorded a slightly higher pod width (4.34 mm) than JS 20-98 (4.30 mm); however, this difference was negligible and statistically non-significant.



Fig 1: Comparison of the effect of Charcoal Rot on pod morphology of disease inoculated pots in susceptible (JS 20-29) and moderately resistant (JS 20-98) varieties under various biostimulant treatments.

Table 1: Effect of different biostimulants and varieties on Pod length, girth and width.

Treatments	POD Length			POD Girth			POD Width		
	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled	2023-24	2024-25	Pooled
Variety									
V1	28.05	28.43	28.24	8.92	8.95	8.94	4.34	4.35	4.34
V2	32.79	32.76	32.78	9.26	9.45	9.36	4.25	4.36	4.30
S.Em ±	0.45	0.45	0.45	0.08	0.13	0.07	0.04	0.06	0.04
CD (p = 0.05)	2.56	2.59	2.57	0.45	0.74	0.42	0.21	0.36	0.20
Treatment									
T ₁ (Control)	33.80	34.57	34.18	10.61	10.44	10.52	4.97	4.89	4.93
T ₂ (Control)	30.12	30.11	30.11	9.13	9.11	9.12	4.10	4.24	4.17
T ₃ (Trichoderma)	31.54	31.67	31.61	9.12	9.15	9.14	4.25	4.27	4.26
T ₄ (Fungicide)	31.60	32.37	31.98	8.82	9.06	8.94	4.36	4.53	4.44
T ₅ (PE 50 ppm)	29.30	28.17	28.74	8.85	8.91	8.88	4.09	4.10	4.09
T ₆ (PE 100 ppm)	30.20	29.50	29.85	8.97	9.36	9.17	4.16	4.28	4.22
T ₇ (PE 150 ppm)	30.58	30.61	30.60	9.21	9.25	9.23	4.32	4.39	4.36
T ₈ (PE 200 ppm)	29.35	29.82	29.59	8.45	8.93	8.69	4.15	4.23	4.19
T ₉ (MFE 50 ppm)	30.00	29.91	29.95	8.77	8.82	8.80	4.20	4.30	4.25
T ₁₀ (MFE 100 ppm)	27.77	28.65	28.21	8.41	8.67	8.54	4.21	4.21	4.21
T ₁₁ (SA 100 ppm)	30.67	31.09	30.88	9.37	9.30	9.33	4.39	4.37	4.38
T ₁₂ (SA 100 ppm)	30.13	30.71	30.42	9.37	9.42	9.40	4.33	4.43	4.38
S.Em ±	1.09	1.11	1.10	0.19	0.32	0.18	0.09	0.16	0.09
CD (p = 0.05)	3.09	3.13	3.11	0.55	0.89	0.51	0.25	0.44	0.25

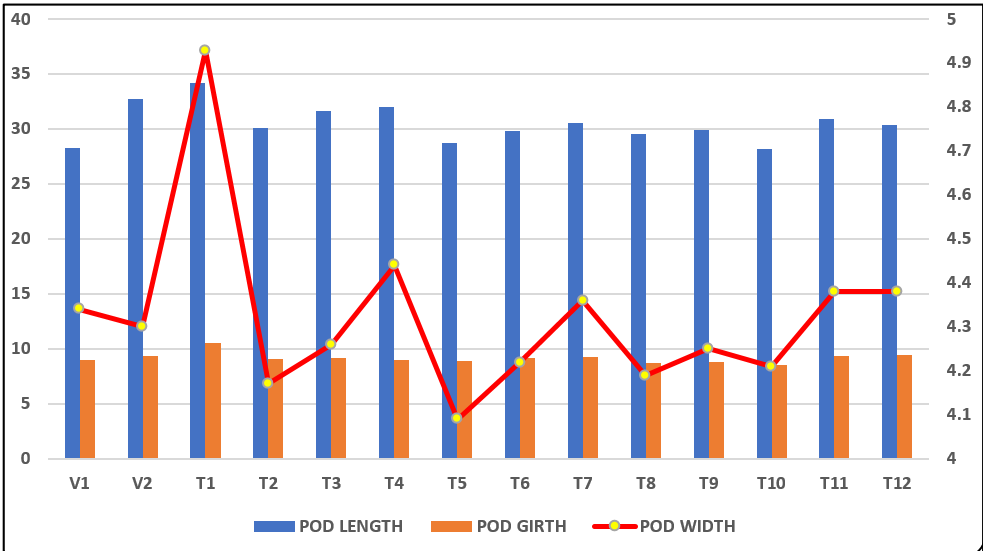


Fig 1: Effect of different biostimulants and varieties on Pod length, girth and width

Discussion

Pod morphology is an important yield component trait that reflects the plant's photosynthetic efficiency, assimilate partitioning, and physiological resilience under disease stress conditions such as charcoal rot caused by *Macrophomina phaseolina*. In the present investigation, considerable variation was observed among treatments and varieties for pod length, pod girth, and pod width under inoculated conditions.

Analysis of the results revealed that the maximum pod length was observed under treatment T₁ (control in non-inoculated pot mixture), indicating that disease-free conditions favored normal pod development. Treatments T₄ (fungicide seed treatment) and T₃ (Trichoderma seed treatment) were statistically at par with T₁, suggesting that both chemical and biological seed protectants effectively mitigated the pathogen's impact on pod elongation. The minimum pod length was recorded in T₁₀ (*Macrophomina* fungal extract @ 100 ppm), which was at par with several other pathogen-stressed treatments, indicating the suppressive effect of pathogen metabolites on pod growth.

Similarly, pod girth followed a parallel trend wherein treatment T₁ exhibited significantly higher values, which was markedly superior to all other treatments, followed by T₁₂ (salicylic acid @ 200 ppm). The improvement under T₁₂ may be attributed to the role of salicylic acid in inducing systemic acquired resistance (SAR) and enhancing physiological activity during reproductive stages (Kaur *et al.*, 2012; Poveda, 2022) [4, 6]. Conversely, treatment T₁₀ demonstrated the lowest girth, signifying that direct exposure to *Macrophomina* extract adversely affects pod filling through tissue damage and vascular restriction (Mengistu *et al.*, 2011; Rahman, 2021) [5, 8].

For pod width, the highest value was observed under T₁, which was statistically superior to all other treatments, followed by T₄ (fungicide seed treatment) and T₁₁ (salicylic acid @ 100 ppm), indicating that effective pathogen suppression and enhanced physiological integrity contributed to pod thickening. The lowest width was observed under T₅ (plant extract @ 50 ppm) and T₂ (control in inoculated pot mixture), suggesting that pathogen stress combined with inadequate biostimulant protection impaired pod development.

Between the two varieties, JS 20-98 (V₂) recorded higher pod length and pod girth compared to JS 20-29 (V₁), demonstrating the moderate resistance and better physiological adaptability of JS 20-98 under stress conditions. However, pod width was found to be marginally higher in JS 20-29 than in JS 20-98, indicating negligible varietal difference for this particular trait. The superiority of Trichoderma and fungicide treatments in maintaining pod morphological traits may be attributed to the suppression of *M. phaseolina* through antagonistic or protective mechanisms, maintaining vascular continuity and assimilate translocation to developing pods (Purkayastha *et al.*, 2006; Deshmukh *et al.*, 2021) [7, 3]. Treatments involving salicylic acid also demonstrated improved pod girth and width, highlighting its physiological role in stress signaling, antioxidative defense, and regulation of sink activity during pod filling (Bellaloui 2021; Amrate *et al.*, 2023) [2, 1].

Conclusion

The present study demonstrated that both varietal resistance and the application of biostimulants and chemical treatments

significantly influenced pod morphological traits of soybean under *Macrophomina phaseolina* infection. Among the treatments, non-inoculated control (T₁) consistently produced the highest pod length, girth, and width, followed by Trichoderma seed treatment (T₃), fungicide (T₄), and salicylic acid (T₁₁ and T₁₂), indicating their effectiveness in mitigating the adverse effects of charcoal rot on reproductive development. Conversely, *Macrophomina* fungal extract treatments (T₉ and T₁₀) and low-concentration plant extracts (T₅) resulted in reduced pod development, highlighting the detrimental impact of pathogen stress on assimilate allocation during pod filling. Between varieties, JS 20-98 exhibited superior pod length and girth compared to JS 20-29, reflecting its moderate resistance and better physiological adaptability under disease conditions, whereas pod width differences were minimal. The findings suggest that integrating biocontrol agents such as Trichoderma and resistance inducers like salicylic acid can effectively maintain pod morphology and potentially enhance yield under charcoal rot-infested environments. These strategies provide a sustainable approach for improving soybean productivity in areas prone to *M. phaseolina* infection, combining disease management with physiological resilience and reproductive efficiency.

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