Plant Archives Vol. 26, Supplement 1, 2026 pp. 1613-1619 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-1.216

INFLUENCE OF BIOSTIMULANTS ON PHENOLOGICAL DEVELOPMENT OF SOYBEAN UNDER *MACROPHOMINA PHASEOLINA* STRESS

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(Date of Receiving : 28-09-2025; Date of Acceptance : 05-12-2025)

ABSTRACT

Charcoal rot, caused by *Macrophomina phaseolina*, adversely affects soybean growth and reproductive development, leading to reduced yield. A two year pot experiment was conducted to evaluate the impact of different biostimulant treatments on soybean phenology under pathogen stress. Twelve treatments were tested, including two controls (non-inoculated and inoculated), Trichoderma seed treatment (10 gkg^{-1} seed), a fungicide (Penflufen 13.28% w/w + Trifloxystrobin 13.28% w/w @ 1 ml kg^{-1} seed), plant extracts at 50, 100, 150, and 200 ppm, Macrophomina fungal extract at 50 and 100 ppm, and salicylic acid at 100 and 200 ppm. These treatments were tested against two varieties i.e., JS 20-29 (susceptible) and JS 20-98 (moderately resistant). The analysis of phenological traits revealed that Trichoderma, higher concentrations of plant extracts (150–200 ppm), and salicylic acid treatments significantly accelerated germination, leaf development, branching, flowering, and fruiting compared to untreated inoculated controls. Fungicide treatment also improved vegetative growth and reproductive transitions, while lower concentrations of plant and fungal extracts had moderate effects. These results demonstrate that biostimulant applications can effectively mitigate the adverse effects of *M. phaseolina*, enhance early vigor, and promote timely progression through phenological stages, highlighting their potential for integrated disease management and improved soybean productivity.

***Keywords* :** Soybean, Charcoal rot, *Macrophomina phaseolina*, Phenology.

Introduction

Soybean (*Glycine max* L.) is one of the most important leguminous crops globally, serving as a major source of protein and oil for human consumption, livestock feed, and industrial applications. Its productivity, however, is severely constrained by biotic and abiotic stresses, among which charcoal rot, caused by the soil-borne fungus *Macrophomina phaseolina* (Tassi) Goid., is particularly devastating. This pathogen is known for its wide host range and ability to survive in soil and plant debris as microsclerotia, enabling it to persist under adverse environmental conditions causing substantial yield losses, especially under high temperature and moisture-stress conditions (Wyllie, 1969; Gangopadhyay *et al.*, 1970). Charcoal rot affects both vegetative and reproductive growth stages, leading to delayed germination, reduced branching, impaired flowering, and ultimately lower pod formation and seed quality. The pathogen-induced disruption of assimilate allocation can result in prolonged phenological stages, which further exacerbate yield losses in susceptible cultivars (Dhingra and Sinclair, 1975 and Bellaloui, 2008).

Understanding the phenological responses of soybean under disease pressure is critical for designing effective management strategies. Phenological traits such as days to germination, leaf stage progression, branching, flowering, and fruiting not only reflect the overall growth performance but also provide insights into the plant's ability to withstand stress and allocate resources efficiently (Pearson *et al.,* 1984; Rahman, 2021). Varietal resistance plays a central role in mitigating the effects of charcoal rot. Moderately resistant varieties often maintain more uniform and timely developmental progression compared to susceptible varieties, although early stage growth may be slightly delayed due to the activation of defense mechanisms (Huilgol *et al.,* 1980; Mengistu *et al.,* 2011).

In addition to genetic resistance, the application of biostimulants has emerged as an eco-friendly strategy to enhance plant vigor and reduce the negative impact of *M. phaseolina*. Biocontrol agents such as *Trichoderma* spp., along with plant extracts and signaling molecules like salicylic acid, have been reported to promote early vegetative growth, enhance branching, and advance reproductive transitions by improving nutrient acquisition, modulating hormonal balance, and inducing systemic resistance (Khaledi, 2016, Luna *et al.,* 2017 and Poveda, 2022). These interventions are particularly valuable under disease stress, as they support timely progression through phenological stages, which is crucial for optimizing yield and maintaining crop uniformity.

Despite extensive research on disease management, studies integrating both varietal resistance and biostimulant treatments to evaluate their combined effect on soybean phenology under charcoal rot stress remain limited. Assessing how different genotypes respond to various treatments across key developmental stages can help us understand integrated disease management, aiding in the selection of resistant varieties and effective bioagents to sustain productivity under pathogen pressure.

This study, therefore, was undertaken to investigate the influence of biostimulant on the phenological progression of soybean under *M. phaseolina* stress. Key objectives included evaluating the effects on germination, leaf development, branching, flowering, and reproductive stages, and identifying treatment-genotype combinations that promote synchronized and timely development even under disease pressure.

Materials and Methods

The experiment was conducted over two consecutive rabi seasons, during the soybean growing season. Two soybean varieties differing in resistance to charcoal rot (*Macrophomina phaseolina*) were used i.e., JS 20-29 (susceptible) and JS 20-98 (moderately resistant). The experiment was laid out in a factorial completely randomized design with three replications. Twelve treatments were applied, including two controls (non-inoculated and inoculated), *Trichoderma* seed treatment (10 g kg^{-1} seed), a fungicide (Penflufen 13.28% w/w + Trifloxystrobin 13.28% w/w F8 @ 1 ml kg^{-1} seed), plant extracts at 50, 100, 150, and 200 ppm, Macrophomina fungal extract at 50 and 100 ppm, and salicylic acid at 100 and 200 ppm. Seeds were surface-sterilized, treated with respective biostimulants. The crop was sown at a seed rate of 10 seeds per pot containing sterilized soil or field plots mixed with FYM and inoculated with *M. phaseolina*, and standard agronomic practices were followed throughout the growing season. Phenological observations were recorded including days to germination, 4-leaf stage, primary branching, flower initiation, anthesis, fruit initiation, first fruiting maturity, 50% flowering and fruiting, phenological and harvest maturity. Observations were made on five randomly selected plants per pot. Data from both years were pooled and subjected to analysis of variance (ANOVA) to evaluate the effects of varieties, treatments, and their interactions, with significant differences determined using Critical Difference (CD) at 5% and Standard Error of Mean (SEm).

Results and Discussion

Table 1: Response of soybean growth and development to biostimulant treatments under charcoal rot stress.

Treatments	Days to Germination	Days to 4 Leaf Stage	Days to Branching
Varieties			
V_1 (JS 20-29)	6.25	11.13	41.00
V_2 (JS 20-98)	6.40	11.29	36.30
S.Em±	**0.07**	**0.05**	**0.26**
CD (p=0.05)	**0.40**	**0.29**	**1.46**
Treatments			
T_1 (Control without inoculum)	6.81	11.72	39.00
T_2 (Control with inoculum)	6.85	11.74	38.38

T_3 (Trichoderma)	5.83	10.74	39.04
T_4 (Fungicide)	5.97	10.78	38.22
T_5 (PE 50 ppm)	6.55	11.45	38.04
T_6 (PE 100 ppm)	6.36	11.22	38.33
T_7 (PE 150 ppm)	5.94	10.86	38.84
T_8 (PE 200 ppm)	6.86	11.75	38.38
T_9 (MFE 50 ppm)	6.40	11.27	38.08
T_{10} (MFE 100 ppm)	6.63	11.50	39.12
T_{11} (SA 100 ppm)	5.69	10.61	39.54
T_{12} (SA 200 ppm)	5.99	10.91	38.82
S.Em±	**0.17**	**0.12**	**0.63**
CD (p=0.05)	**0.48**	**0.35**	**1.77**

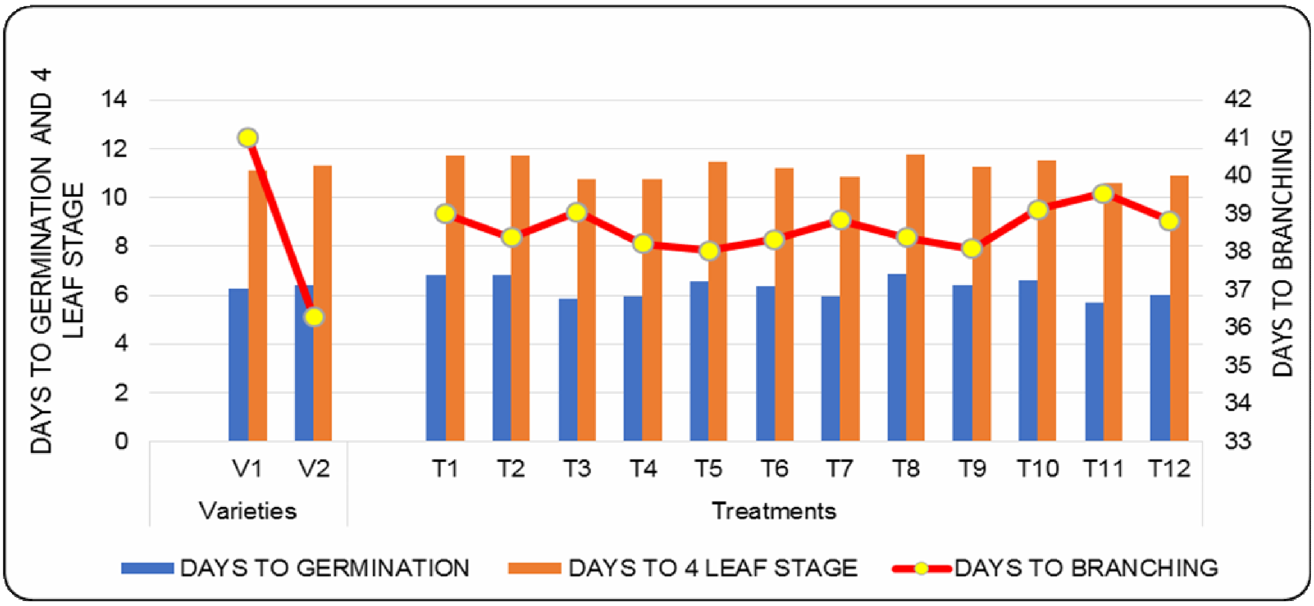

Fig. 1: Response of soybean growth and development to bio stimulant treatments under charcoal rot stress

Table 2: Effect of different biostimulants on flower progression.

Treatments	Days to Flower Initiation	Days to Anthesis	Days to 50% Flowering	Duration of Flowering
Varieties				
V_1 (JS 20-29)	42.28	46.11	47.75	9.54
V_2 (JS 20-98)	38.22	41.20	42.87	8.74
S.Em±	**0.06**	**0.06**	**0.30**	**0.01**
CD (p=0.05)	**0.34**	**0.37**	**1.72**	**0.08**
Treatments				
T_1 (Control without inoculum)	40.65	44.20	45.90	9.15
T_2 (Control with inoculum)	40.06	43.54	45.14	9.21
T_3 (Trichoderma)	41.01	44.46	46.03	9.14
T_4 (Fungicide)	39.50	42.98	44.97	9.69
T_5 (PE 50 ppm)	39.31	42.88	44.60	9.15
T_6 (PE 100 ppm)	40.10	43.55	45.18	9.32
T_7 (PE 150 ppm)	40.52	44.02	45.59	9.17
T_8 (PE 200 ppm)	40.03	43.45	45.14	9.09
T_9 (MFE 50 ppm)	41.02	44.54	46.05	9.14
T_{10} (MFE 100 ppm)	40.71	44.14	45.81	9.18
T_{11} (SA 100 ppm)	39.50	42.55	44.12	8.78
T_{12} (SA 200 ppm)	40.54	43.59	45.17	8.65
S.Em±	**0.15**	**0.16**	**0.74**	**0.04**
CD (p=0.05)	**0.41**	**0.44**	**2.08**	**0.10**

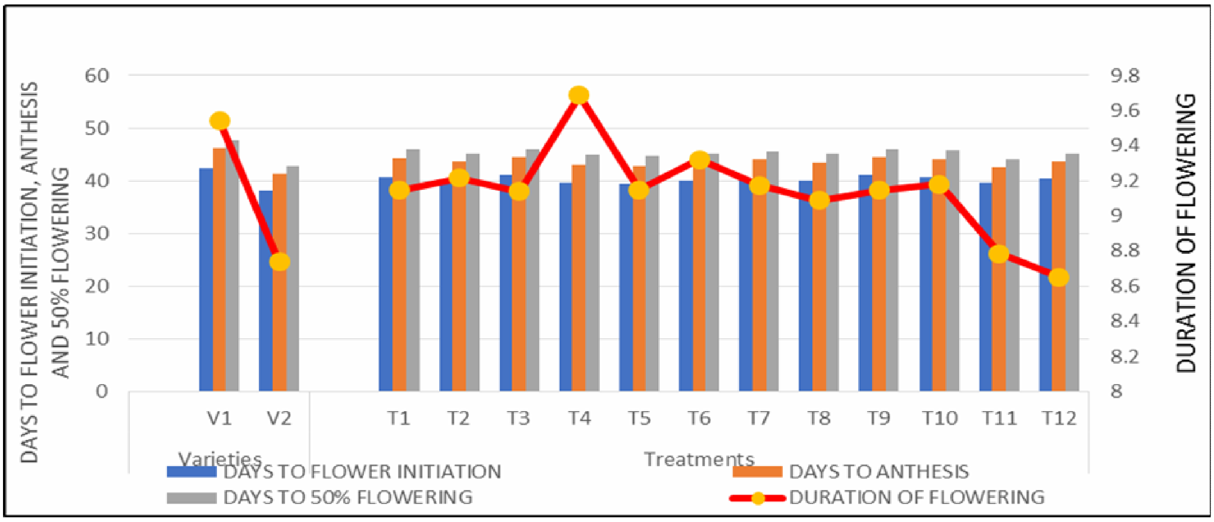

Fig. 2: Effect of different biostimulants on flower progression

Table 3: Influence of biostimulant applications on soybean reproductive progress under charcoal rot stress.

Treatments	Days to 1st Fruit Initiation	Days to 50% Fruiting	Days to 1st Fruit Maturity	Days to 50% Maturity	Duration of Seed Filling
Varieties					
V_1 (JS 20-29)	51.82	67.80	79.80	85.97	34.15
V_2 (JS 20-98)	46.95	62.94	74.93	81.17	34.21
S.Em±	**0.07**	**0.10**	**0.12**	**0.55**	**0.60**
CD (p=0.05)	**0.41**	**0.55**	**0.68**	**3.16**	**3.40**
Treatments					
T_1 (Control without inoculum)	49.91	65.79	77.66	83.84	33.94
T_2 (Control with inoculum)	49.27	65.27	77.28	83.51	34.24
T_3 (Trichoderma)	50.14	66.14	78.15	83.72	33.58
T_4 (Fungicide)	49.07	65.09	77.11	83.60	34.30
T_5 (PE 50 ppm)	48.46	64.25	75.98	81.88	33.34
T_6 (PE 100 ppm)	49.42	65.48	77.67	83.97	34.78
T_7 (PE 150 ppm)	49.66	65.68	77.70	84.02	34.40
T_8 (PE 200 ppm)	49.17	65.19	77.20	83.04	33.93
T_9 (MFE 50 ppm)	50.16	66.18	78.19	84.27	34.10
T_{10} (MFE 100 ppm)	49.89	65.90	77.89	84.73	34.84
T_{11} (SA 100 ppm)	48.29	64.29	76.31	82.80	34.51
T_{12} (SA 200 ppm)	49.19	65.21	77.23	83.46	34.27
S.Em±	**0.18**	**0.23**	**0.29**	**1.35**	**1.46**
CD (p=0.05)	**0.50**	**0.66**	**0.83**	**3.81**	**4.11**

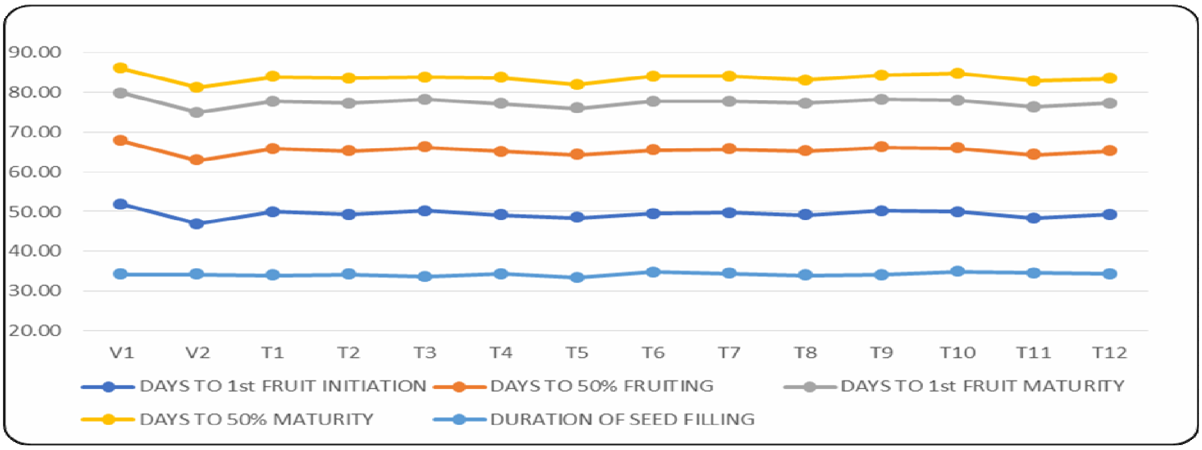

Fig. 3: Influence of biostimulant applications on soybean reproductive progress under charcoal rot stress

Table 4: Effect of biostimulant treatments on soybean maturity under charcoal rot stress.

Treatments	Days to Physiological Maturity	Days to Harvest Maturity
Varieties		
V_1 (JS 20-29)	87.11	99.57
V_2 (JS 20-98)	99.89	108.80
S.Em±	**0.76**	**0.33**
CD (p=0.05)	**4.33**	**1.90**
Treatments		
T_1 (Control without inoculum)	96.68	104.80
T_2 (Control with inoculum)	93.36	103.35
T_3 (Trichoderma)	92.50	104.77
T_4 (Fungicide)	93.11	104.08
T_5 (PE 50 ppm)	92.56	102.98
T_6 (PE 100 ppm)	93.52	104.12
T_7 (PE 150 ppm)	93.15	104.47
T_8 (PE 200 ppm)	91.26	102.73
T_9 (MFE 50 ppm)	93.80	104.78
T_{10} (MFE 100 ppm)	93.04	104.32
T_{11} (SA 100 ppm)	94.63	105.22
T_{12} (SA 200 ppm)	94.44	104.62
S.Em±	**1.86**	**0.81**
CD (p=0.05)	**5.23**	**2.29**

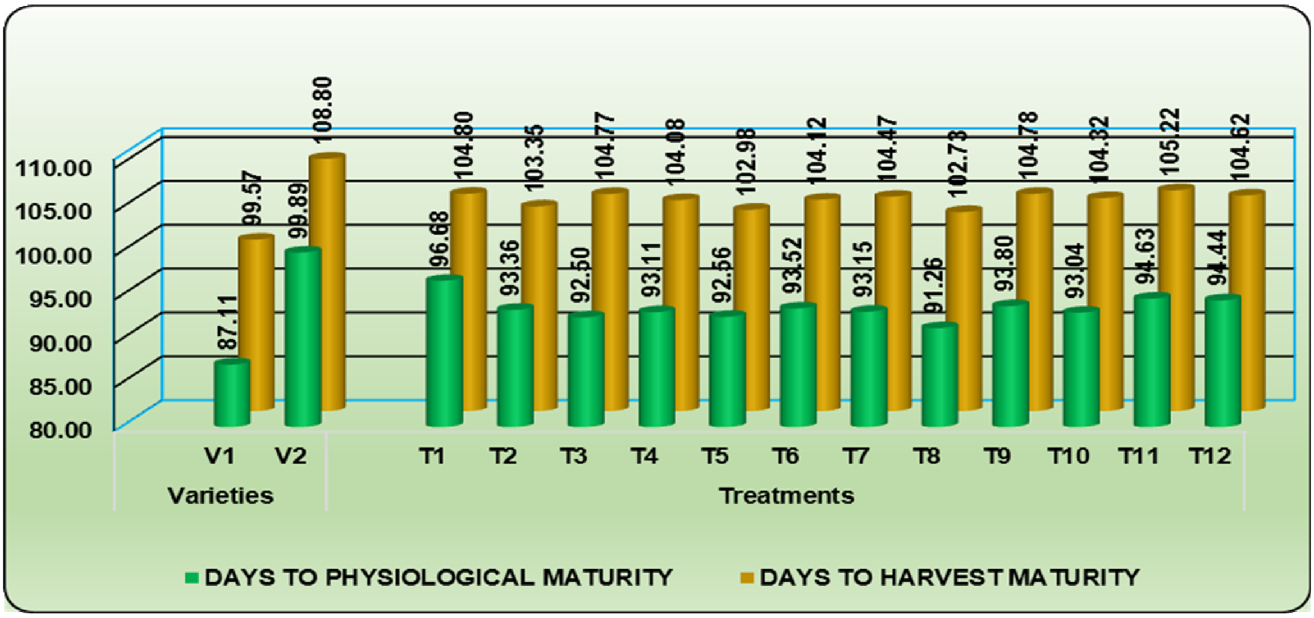

Fig. 4: Effect of biostimulant treatments on soybean maturity under charcoal rot stress

The phenological behaviour of soybean under the influence of *Macrophomina phaseolina* infection and various biostimulant treatments exhibited clear and statistically significant differences across growth stages, reflecting the combined effects of varietal characteristics, pathogen pressure, and treatment efficacy. During early vegetative development, days to germination varied considerably, with JS 20-29 taking 6.25 days compared to the slightly faster germination of JS 20-98 (6.39 days). Although JS 20-29 appeared slower in emergence, observations from the pathogen-influenced context suggest that moderately resistant varieties such as JS 20-98 may initially allocate resources toward defense, resulting in subtle delays during early growth, as also indicated by Gangopadhyay *et al.* (1970) and Huilgol *et al.* (1980). Treatment effects on germination were notable. Seeds treated with salicylic acid (SA) @ 100 ppm germinated earliest (5.69 days), followed by Trichoderma (5.83 days) and PE @ 150 ppm (5.94 days), whereas the inoculated control recorded the maximum germination period (6.85 days). The improved emergence in treated seeds corresponds with earlier reports that Trichoderma and plant-derived biostimulants enhance early seedling vigour by promoting antagonism against soil-borne pathogens, improving nutrient mobilisation, and stimulating defence-related biochemical pathways (Luna *et al*., 2017; Poveda, 2022). The delay under pathogen-influenced controls further supports the understanding that charcoal rot exerts early physiological stress on the seedling, leading to reduced vigour and slower metabolic activation.

Progression to the four-leaf stage followed a similar trend, with JS 20-98 advancing more rapidly than JS 20-29 under most treatments. SA @ 100 ppm (10.60 days) and Trichoderma (10.73 days) significantly reduced the time required for leaf development relative to both controls. Such accelerated vegetative progression aligns with the role of biostimulants in modulating hormonal balance, improving water and nutrient uptake, and alleviating stress effects imposed by *M. phaseolina* infection. Earlier research indicated that susceptible genotypes often exhibit delays in leaf expansion under pathogen pressure as assimilates are redirected toward defence responses (Dhingra & Sinclair, 1975; Pearson *et al*., 1984). The present findings corroborate this pattern, showing that JS 20-29 under pathogen stress consistently lagged behind JS 20-98, particularly when untreated or under high pathogen load. Conversely, the faster leaf development observed in biostimulant-treated plants reflects the capacity of these treatments to buffer biotic stress and sustain vegetative growth, as previously noted by Mengistu *et al*. (2007) and Kaur *et al*. (2012).

Branching behaviour further emphasized the interaction of genotype and treatment with stress conditions. JS 20-29 recorded 40.99 days to primary branching compared with only 36.30 days in JS 20-98, demonstrating the greater sensitivity of the former to pathogen-induced delays. Treatments such as SA @ 100 ppm (39.54 days) and Trichoderma (39.04 days) supported earlier branching, while PE @ 50 ppm recorded the shortest duration (38.03 days) among all treatments. The improved branching in treated plants suggests enhanced physiological stability under stress, likely facilitated by improved auxin signaling, nutrient acquisition, and root-zone activity mediated by microbial antagonists-mechanisms widely reported in studies on Trichoderma and plant extracts (Saleh *et al*., 2010; Poveda, 2022). The delayed branching in untreated conditions supports earlier findings that charcoal rot disrupts apical dominance and slows lateral shoot development by imposing systemic stress on carbon assimilation processes.

Reproductive development exhibited pronounced variability. JS 20-29 consistently recorded delayed flowering, with first floral initiation at 42.28 days and anthesis at 46.11 days, compared to 38.22 and 41.20 days in JS 20-98. The delay extended to 50% flowering (47.75 days) and flowering duration (9.54 days) in JS 20-29, while JS 20-98 completed these phases more rapidly (44.20 days and 8.74 days). These observations are aligned with earlier findings that susceptible genotypes under biotic stress often divert resources toward structural and biochemical defence, slowing reproductive transitions (Bellaloui, 2008; Rahman, 2021). Treatments influenced flowering patterns distinctly. SA @ 100 ppm and fungicide seed treatment advanced floral initiation and anthesis (39.50 days), whereas Trichoderma and MFE @ 50 ppm displayed slightly delayed reproductive onset (41.01–44.54 days). Variations in flowering duration, which ranged from 8.65 days under SA @ 200 ppm to 9.69 days under fungicide treatment, suggest differential modulation of reproductive physiology, likely through treatment-driven adjustments in hormonal signaling and nutrient redistribution. The maintenance of synchronised and moderately extended flowering under Trichoderma and fungal extract treatments corresponds with previous studies highlighting improved reproductive resilience under bioagent application (Gupta *et al*., 2012; Amrate *et al*., 2023).

Fruiting also reflected clear varietal and treatment effects. JS 20-29 required significantly more time to reach first fruit initiation (51.82 days), 50% fruiting (67.80 days), and first fruit maturity (79.79 days) compared with JS 20-98 (46.95, 62.94, and 74.93 days, respectively). These results reinforce the cumulative effect of pathogen stress on phenological delay in susceptible varieties, consistent with the findings of Mengistu *et al*. (2007). Seed-filling duration, however, remained comparatively stable across varieties (34.15 days in JS 20-29 and 34.21 days in JS 20-98), suggesting that once reproductive development is established, grain filling proceeds with comparable physiological efficiency irrespective of genotype. Treatment-wise, earlier fruiting was observed under SA @ 100 ppm and PE @ 50 ppm, while Trichoderma and MFE @ 50 ppm slightly delayed fruit initiation (50.14–50.16 days). Seed-filling duration varied marginally, with PE @ 50 ppm recording the shortest (33.34 days) and MFE @ 100 ppm the longest (34.84 days), indicating subtle treatment-dependent modulation of assimilate partitioning during grain development.

Differences were also evident in maturity stages. JS 20-98 attained physiological maturity earlier (87.11

days) and harvest maturity at 99.57 days, whereas JS 20-29 required 99.89 and 108.80 days, respectively. Moderately resistant varieties thus completed their life cycle more efficiently under stress, reinforcing the role of genetic tolerance in maintaining phenological stability. Among treatments, the earliest physiological maturity was recorded under PE @ 200 ppm (91.26 days), followed closely by Trichoderma (92.50 days). Uninoculated controls exhibited maximum delay (96.68 days), demonstrating that biostimulant and microbial treatments not only mitigated pathogen effects but also improved physiological efficiency. Harvest maturity followed a similar pattern, with PE @ 200 ppm and PE @ 50 ppm maturing earliest (102.73–102.98 days), whereas SA @ 100 ppm and the uninoculated control exhibited the longest crop duration. These findings reflect the capacity of exogenous biostimulants and bioagents to positively influence the timing of reproductive and terminal phenophases, likely by improving overall plant vigour, enhancing defence responses, and stabilizing metabolic activity even under disease pressure, as supported by earlier observations (Babu *et al*., 2007; Saleh *et al*., 2010; Poveda, 2022).

Conclusion

The study clearly demonstrated that soybean phenology is significantly influenced by both varietal resistance and the application of biostimulants under *Macrophomina phaseolina* stress. Resistant variety maintained a more synchronized and timely progression through germination, vegetative growth, branching, flowering, and reproductive development, whereas susceptible variety exhibited delays at multiple stages. Treatments such as Trichoderma seed application, plant extracts, and salicylic acid effectively mitigated the adverse effects of charcoal rot, enhancing early vigor, promoting branching, and advancing reproductive transitions. The results highlight that integrating resistant genotypes with appropriate biostimulant interventions can optimize developmental progression, maintain uniformity in growth stages, and reduce the disruptive impact of pathogen stress. These findings emphasize the importance of combining genetic resistance with eco-friendly treatments for sustainable management of charcoal rot in soybean, ultimately supporting improved plant performance and potential yield stability.

Acknowledgement

The authors express their sincere gratitude to the College of Agriculture, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur for providing all the necessary facilities to conduct this study.

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