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Effect of bio-mediated sulphur management and sowing techniques on leaf area and leaf area index of Indian Mustard (*Brassica juncea* L.)

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Abstract

A field experiment was conducted during the *Rabi* (winter) seasons of 2023-24 and 2024-25 at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India, to study the effect of sowing techniques and bio-mediated sulphur management on leaf area and leaf area index (LAI) of Indian mustard (*Brassica juncea* L.) cv. RH-749. The experiment was laid out in a split plot design replicated thrice, with four sowing methods (broadcasting, point seeding, stripe seeding and ridge and furrow method) in main plots and five sulphur nutrient levels (control, and 100, 90, 80 and 70% recommended dose of sulphur, each combined with *Bacillus subtilis* seed biopriming) in sub plots. Leaf area per plant and LAI were recorded destructively at 30, 60 and 90 days after sowing (DAS) using a leaf area meter. Pooled analysis of two years revealed that the ridge and furrow method (S_4) produced significantly higher leaf area per plant (182.64, 356.62 and 267.20 cm² at 30, 60 and 90 DAS, respectively) and LAI (0.29, 0.57 and 0.40). Among nutrient levels, 100% recommended dose of sulphur with *Bacillus subtilis* (I_1) produced significantly higher leaf area (192.80, 366.96 and 275.61 cm²) and LAI (0.31, 0.59 and 0.41) over the untreated control (I_0). The interaction of ridge and furrow sowing with 100% RDS + *Bacillus subtilis* (S_4I_1) recorded the maximum leaf area (225.16, 455.07 and 340.80 cm²) and LAI (0.35, 0.72 and 0.51) at all growth stages. The results indicate that precise seed placement combined with adequate, biologically primed sulphur nutrition favours canopy development in Indian mustard.

Keywords: *Bacillus subtilis*, bio-mediated sulphur, Indian mustard, leaf area index, sowing methods and split plot design

Introduction

Indian mustard (*Brassica juncea* L.) belongs to the family *Cruciferae* and comprises a diverse group of plants grown as vegetables, fodder, condiments and sources of edible oil worldwide. Among the oleiferous Brassica species, commonly referred to as rapeseed-mustard, Indian mustard (*Brassica juncea* (L.) Czern.) is the most widely cultivated species in India because of its wider adaptability and comparatively greater tolerance to the major diseases, pests and abiotic stresses affecting Brassicas, and it continues to be bred and conserved for these traits through India's rapeseed-mustard genetic resource programmes. Rapeseed-mustard is currently India's largest oilseed crop, with a production of 13.2 million tonnes in 2023-24, accounting for 30.3% of the country's total oilseed area and 33.2% of oilseed production, at an average productivity of about 1,443 kg ha⁻¹ (ICAR-Directorate of Rapeseed-Mustard Research, 2024) [6]. Canopy development, most commonly quantified through leaf area and leaf area index (LAI), is a key determinant of light interception, photosynthetic efficiency and consequently, dry matter accumulation and yield in field crops. LAI, defined as the total one sided leaf area per unit ground area, was first formalized by Watson (1947) [16] and remains the standard descriptor of canopy expansion in agronomic research.

In Indian mustard, the method of sowing strongly influences plant stand, seed placement depth and early root shoot development, all of which shape subsequent canopy growth. Broadcasting, the most common practice in India owing to its simplicity, often results in uneven seed distribution and crowding, whereas line sowing and ridge-furrow methods place seed at a uniform depth and

spacing, improving stand establishment, soil aeration and root proliferation relative to conventional flat or broadcast sowing, as demonstrated for oilseed Brassica by Arshad *et al.* (2026) ^[1]. Sulphur is essential for chlorophyll synthesis, enzyme activation and overall vegetative growth in *Brassica juncea*, and its exogenous, adequate supply has been shown to improve growth and photosynthetic efficiency of the crop (Shah *et al.*, 2022) ^[11]. Judicious, biologically mediated sulphur management, for instance through seed biopriming with plant growth promoting rhizobacteria such as *Bacillus subtilis*, has further been reported to enhance rhizosphere microbial activity, sulphur mineralization and nutrient uptake in Indian mustard (Singh *et al.*, 2023) ^[12]. Combining an appropriate sowing technique with adequate, bio-primed sulphur nutrition may therefore offer a practical route to improving canopy development and, in turn, productivity of Indian mustard. Keeping these facts in view, the present investigation was undertaken with the specific objective of evaluating the effect of different sowing techniques and bio-mediated sulphur

management, applied singly and in combination, on the leaf area and leaf area index of Indian mustard (*Brassica juncea* L.) under the agro-climatic conditions of the middle Gangetic plains.

Materials and Methods
Experimental site and soil

The field experiment was conducted during the *Rabi* (winter) seasons of 2023-24 and 2024-25 at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi, Uttar Pradesh, India (25°18' N, 83°31' E; 75.7 m above mean sea level), which falls in agro-climatic zone IV (middle Gangetic plains) of the Indo-Gangetic alluvial tract. The site experiences a humid sub-tropical climate with an average annual rainfall of about 1,019 mm. The soil of the experimental field was sandy loam in texture, belonging to the order Inceptisol (*Typic Ustochrept*), low in available nitrogen, medium in available phosphorus, potassium and sulphur, and slightly alkaline in reaction (Table 1).

Table 1: Physico-chemical properties of the experimental soil (0-15 cm depth)

Soil property	Value	Rating	Method used
Mechanical composition (fine sand: silt: clay,%)	57.4: 20.1: 22.3	Sandy loam	International pipette method
Bulk density (g cm ⁻³)	1.51	-	Core sampler method
Water holding capacity (%)	45.10	Moderate	Keen box method
Organic carbon (%)	0.41	Medium	Walkley and Black (1934)
Available N (kg ha ⁻¹)	245.8	Low	Subbiah and Asija (1956)
Available P ₂ O ₅ (kg ha ⁻¹)	26.34	Medium	Olsen <i>et al.</i> (1954)
Available K ₂ O (kg ha ⁻¹)	289.6	Medium	Flame photometric method
Available S (kg ha ⁻¹)	14.62	Medium	Chesnin and Yien (1951)
pH (1:2.5 soil: water)	7.20	Slightly alkaline	Glass electrode pH meter
EC (dS m ⁻¹ at 25 °C)	0.32	Normal	Solu-bridge method

Experimental design and treatments

The experiment was laid out in a split plot design replicated three times. Four sowing methods, namely broadcasting (S₁), point seeding (S₂), stripe seeding (S₃) and ridge and furrow method (S₄), were assigned to main plots, while five sulphur nutrient levels, namely control (no Sulphur) (I₀), 100% recommended dose of sulphur (RDS) + *Bacillus subtilis* (I₁), 90% RDS + *Bacillus subtilis* (I₂), 80% RDS + *Bacillus subtilis* (I₃) and 70% RDS + *Bacillus subtilis* (I₄), were assigned to sub plots, giving 20 treatment combinations replicated thrice (60 plots). The recommended dose of sulphur was 40 kg S ha⁻¹, supplied through bentonite sulphur, which was incorporated into the top 0-15 cm soil layer 10 days before sowing to allow microbial oxidation to plant available sulphate. Seeds were bioprimed with *Bacillus subtilis* culture (10 mL kg⁻¹ seed, using 1% jaggery solution as adhesive) prior to sowing. A uniform basal dose of phosphorus (60 kg P₂O₅ ha⁻¹), potassium (60 kg K₂O ha⁻¹) and half the recommended nitrogen (60 kg N ha⁻¹) was applied through diammonium phosphate (DAP), muriate of potash (MOP) and urea, respectively, at sowing, with the remaining nitrogen top dressed at 30 days after sowing (DAS). Indian mustard cv. RH-749 was sown at a seed rate of 5 kg ha⁻¹ with a spacing of 45 cm x 15 cm on 5th November in both years, in gross plots of 4.5 m x 4.0 m (net plot 3.6 m x 3.0 m).

Measurement of leaf area and leaf area index

Leaf area per plant (cm²) was estimated at 30, 60 and 90 DAS from five randomly selected plants per plot by destructive sampling using a leaf area meter and the mean value was expressed as leaf area plant⁻¹. Leaf area index (LAI) was calculated as the ratio of total leaf area per plant to the

corresponding ground area occupied by the plant canopy, following the concept originally proposed by Watson (1947) ^[16]:

$$LAI = \text{Total leaf area per plant (m}^2\text{)} / \text{Ground area occupied by the plant (m}^2\text{)}$$

Statistical analysis

Data recorded in each year were analyzed statistically following the analysis of variance (ANOVA) technique appropriate for a split plot design, as outlined by Gomez and Gomez (1984) ^[5]. Data of both years were pooled and reanalyzed following the standard procedure for pooled analysis of split plot experiments conducted over years. Wherever the F-test indicated significant differences among treatments, the critical difference (CD) was worked out at the 5% level of probability; treatment means differing by less than the CD were considered statistically at par.

Results and Discussion
Effect on leaf area per plant

Data on leaf area per plant as influenced by sowing methods and bio-mediated sulphur management, pooled over two years, are presented in Table 2. Among the sowing methods, leaf area per plant increased significantly and progressively from broadcasting (S₁) to ridge and furrow method (S₄) at all the three growth stages. The ridge and furrow method (S₄) recorded the maximum leaf area per plant (182.64, 356.62 and 267.20 cm² at 30, 60 and 90 DAS, respectively), being significantly superior to stripe seeding (S₃), point seeding (S₂) and broadcasting (S₁), the last recording the lowest values (146.77, 257.94 and 193.88 cm²) shown in (Fig. 1). The ridge and furrow configuration provides a precisely defined seed placement depth and creates a loosened, well aerated

root zone. These conditions promote faster root proliferation, enhanced moisture and nutrient capture and consequently greater leaf expansion. Comparable or superior improvements in canopy attributes such as higher leaf area index, optimized light interception and improved microclimatic conditions have been reported for oilseed Brassicas and various other field crops by Arshad *et al.* (2026) ^[1].

Among the bio-mediated sulphur management levels, application of 100% RDS along with *Bacillus subtilis* biopriming (I₁) recorded significantly the highest leaf area per plant (192.80, 366.96 and 275.61 cm² at 30, 60 and 90 DAS, respectively) and was significantly superior to all other nutrient levels, while the untreated control (I₀) recorded the lowest values (146.59, 257.74 and 193.68 cm²). Leaf area per plant declined progressively as the sulphur dose was reduced from 100 to 70% RDS, all remaining significantly superior to the control shown in (Fig. 2). Seed biopriming with *Bacillus subtilis* under graded sulphur fertilization has been shown to enhance rhizosphere microbial activity, sulphur mineralization and nutrient uptake in Indian mustard, which together support greater vegetative growth, as reported by Singh *et al.* (2023) ^[12].

Table 2: Effect of sowing methods and bio-mediated sulphur management on leaf area per plant (cm²) of Indian mustard at 30, 60 and 90 DAS

Treatment	30 DAS (cm ²)	60 DAS (cm ²)	90 DAS (cm ²)
Sowing methods (S)			
S ₁ - Broadcasting	146.77	257.94	193.88
S ₂ - Point seeding	166.93	300.56	226.00
S ₃ - Stripe seeding	174.00	324.95	244.30
S ₄ - Ridge and furrow	182.64	356.62	267.20
SEm±	2.50	4.94	3.68
CD (p=0.05)	7.69	15.22	11.34
Bio-mediated sulphur management (I)			
I ₀ - Control (No S)	146.59	257.74	193.68
I ₁ - 100% RDS + <i>B. subtilis</i>	192.80	366.96	275.61
I ₂ - 90% RDS + <i>B. subtilis</i>	173.27	325.08	243.59
I ₃ - 80% RDS + <i>B. subtilis</i>	169.37	314.15	236.20
I ₄ - 70% RDS + <i>B. subtilis</i>	155.89	286.15	215.15
S.Em±	1.66	3.60	2.94
CD (p = 0.05)	4.70	10.17	8.31

RDS = Recommended dose of sulphur (40 kg S ha⁻¹), DAS: Days after sowing, Values are pooled means of two years (2023-24 and 2024-25)

The interaction between sowing methods and sulphur management was also significant at all growth stages (Table 3). The combination of ridge and furrow sowing with 100% RDS + *Bacillus subtilis* (S₄I₁) recorded the highest leaf area per plant (225.16, 455.07 and 340.80 cm² at 30, 60 and 90 DAS, respectively), significantly out yielding all other treatment combinations, while broadcasting with the untreated control (S₁I₀) recorded the lowest values (120.11, 163.45 and 122.61 cm²). This synergy between improved seed bed configuration and bio-primed sulphur nutrition is consistent with the individual effects reported for ridge sowing and for *Bacillus subtilis* biopriming in Indian mustard and other oilseed Brassicas by Arshad *et al.* (2026) ^[1] and Singh *et al.* (2023) ^[12].

Table 3: Interaction effect of sowing methods and bio-mediated sulphur management on leaf area per plant (cm²) of Indian mustard at 30, 60 and 90 DAS

Treatment combination	30 DAS (cm ²)	60 DAS (cm ²)	90 DAS (cm ²)
S ₁ I ₀	120.11	163.45	122.61
S ₁ I ₁	178.47	330.76	248.67
S ₁ I ₂	155.45	285.63	214.76
S ₁ I ₃	151.54	277.97	209.00
S ₁ I ₄	128.29	231.91	174.37
S ₂ I ₀	156.45	248.61	186.88
S ₂ I ₁	183.78	341.44	256.90
S ₂ I ₂	167.61	309.74	232.89
S ₂ I ₃	164.47	303.59	228.26
S ₂ I ₄	162.33	299.39	225.10
S ₃ I ₀	158.66	309.12	232.30
S ₃ I ₁	183.79	340.58	256.07
S ₃ I ₂	180.67	334.45	251.46
S ₃ I ₃	174.34	322.05	242.14
S ₃ I ₄	172.55	318.56	239.51
S ₄ I ₀	151.16	309.77	232.91
S ₄ I ₁	225.16	455.07	340.80
S ₄ I ₂	189.35	370.49	275.25
S ₄ I ₃	187.13	353.01	265.42
S ₄ I ₄	160.40	294.73	221.60
SEm± (S x I)	5.21	10.39	7.82
CD (p=0.05)	16.06	32.01	24.10

Pooled data of two years (2023-24 and 2024-25)

Effect on leaf area index (LAI)

The trend in LAI (Table 4) closely followed that of leaf area per plant. Among sowing methods, LAI increased significantly from broadcasting (0.23, 0.42 and 0.29 at 30, 60 and 90 DAS) to ridge and furrow method (0.29, 0.57 and 0.40), with point seeding and stripe seeding being intermediate shown in (Fig. 3). Among bio-mediated sulphur management, 100% RDS + *Bacillus subtilis* (I₁) recorded the highest LAI (0.31, 0.59 and 0.41), significantly superior to the control (0.23, 0.42 and 0.29) and to the reduced sulphur treatments (I₂, I₃ and I₄). LAI increased up to 60 DAS in all treatments, coinciding with the peak vegetative and early reproductive phase, and declined thereafter at 90 DAS due to senescence of the lower canopy leaves, a pattern consistent with reports on Indian mustard under varying sowing dates, irrigation and nutrient regimes shown in (Fig. 4). A similar pattern of LAI rising to a peak and then declining sharply due to leaf senescence has been reported for Indian mustard cultivars by Sudhansu *et al.* (2021) ^[14] and LAI itself remains the standard descriptor of canopy expansion first formalised by Watson (1947) ^[16].

The interaction effect on LAI (Table 5) mirrored that recorded for leaf area per plant, with S₄I₁ (ridge and furrow sowing + 100% RDS + *Bacillus subtilis*) recording the highest LAI (0.35, 0.72 and 0.51 at 30, 60 and 90 DAS, respectively) and S₁I₀ (broadcasting + control) the lowest (0.18, 0.28 and 0.18). A similar superiority of ridge sowing over bed and flat/broadcast methods in enhancing LAI of oilseed Brassica has been reported in canola, where ridge sowing improved soil aeration, drainage and root proliferation relative to conventional flat planting. A similar superiority of ridge sowing over bed and flat/broadcast methods in enhancing LAI of oilseed Brassica, attributed to improved soil aeration, drainage and root proliferation, has been reported in canola by Arshad *et al.* (2026) ^[1].

Table 4: Effect of sowing methods and bio-mediated sulphur management on leaf area index of Indian mustard at 30, 60 and 90 DAS

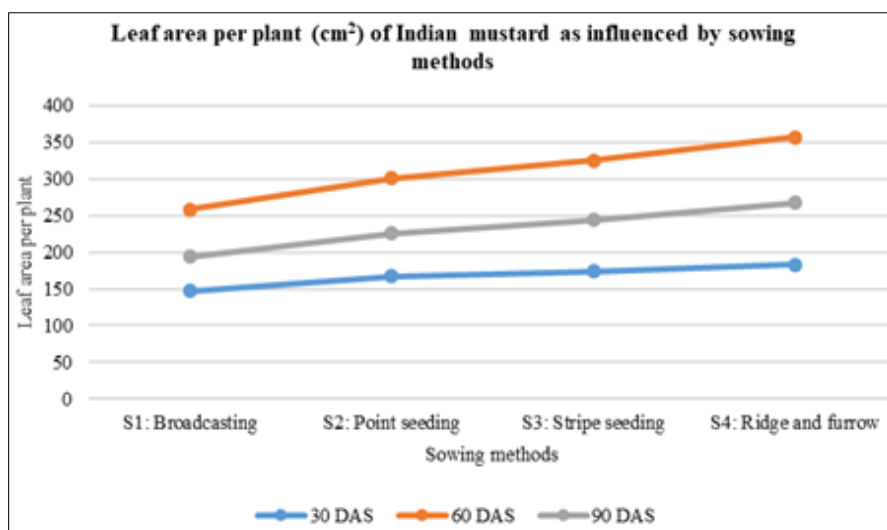
Treatment	30 DAS	60 DAS	90 DAS
Sowing methods (S)			
S ₁ - Broadcasting	0.23	0.42	0.29
S ₂ - Point seeding	0.27	0.48	0.33
S ₃ - Stripe seeding	0.28	0.52	0.36
S ₄ - Ridge and furrow	0.29	0.57	0.40
SEm±	0.20	0.01	0.01
CD (<i>p</i> =0.05)	0.61	0.04	0.02
Nutrient levels (I)			
I ₀ - Control (No S)	0.23	0.42	0.29
I ₁ - 100% RDS + <i>B. subtilis</i>	0.31	0.59	0.41
I ₂ - 90% RDS + <i>B. subtilis</i>	0.28	0.52	0.36
I ₃ - 80% RDS + <i>B. subtilis</i>	0.27	0.50	0.35
I ₄ - 70% RDS + <i>B. subtilis</i>	0.25	0.47	0.32
SEm±	0.20	0.01	0.01
CD (<i>p</i> =0.05)	0.58	0.03	0.01

RDS = Recommended dose of sulphur (40 kg S ha⁻¹), DAS: Days after sowing, Values are pooled means of two years (2023-24 and 2024-25)

Table 5: Interaction effect of sowing methods and bio-mediated sulphur management on leaf area index of Indian mustard at 30, 60 and 90 DAS

Treatment combination	30 DAS	60 DAS	90 DAS
S ₁ I ₀	0.18	0.28	0.18
S ₁ I ₁	0.29	0.53	0.37
S ₁ I ₂	0.25	0.45	0.32
S ₁ I ₃	0.24	0.45	0.31
S ₁ I ₄	0.21	0.38	0.26
S ₂ I ₀	0.25	0.40	0.28
S ₂ I ₁	0.30	0.55	0.38
S ₂ I ₂	0.27	0.50	0.34
S ₂ I ₃	0.26	0.49	0.34
S ₂ I ₄	0.25	0.48	0.33
S ₃ I ₀	0.25	0.50	0.35
S ₃ I ₁	0.31	0.54	0.38
S ₃ I ₂	0.29	0.53	0.38
S ₃ I ₃	0.27	0.51	0.36
S ₃ I ₄	0.28	0.51	0.36
S ₄ I ₀	0.22	0.49	0.34
S ₄ I ₁	0.35	0.72	0.51
S ₄ I ₂	0.30	0.59	0.41
S ₄ I ₃	0.30	0.56	0.39
S ₄ I ₄	0.25	0.51	0.33
SEm± (S x I)	0.43	0.02	0.01
CD (<i>p</i> =0.05)	0.34	0.08	0.03

Pooled data of two years (2023-24 and 2024-25)

**Fig 1:** Leaf area per plant (cm²) of Indian mustard as influenced by sowing methods

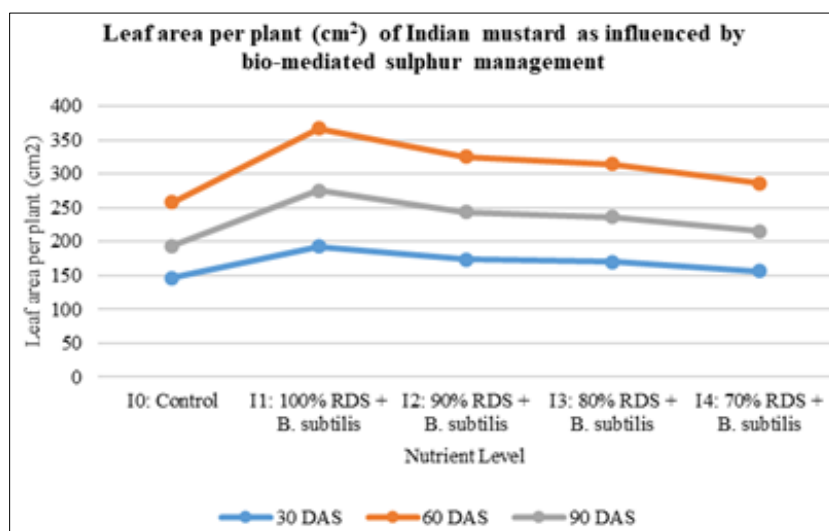


Fig 2: Leaf area per plant (cm²) of Indian mustard as influenced by bio-mediated sulphur management

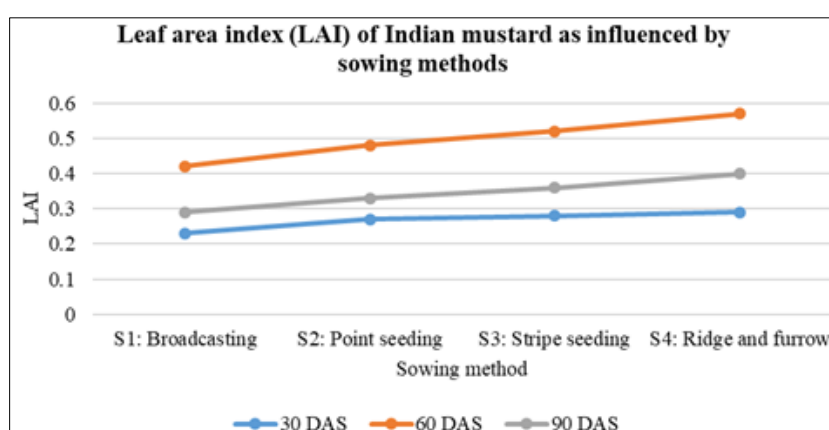


Fig 3: Leaf area index (LAI) of Indian mustard as influenced by sowing methods

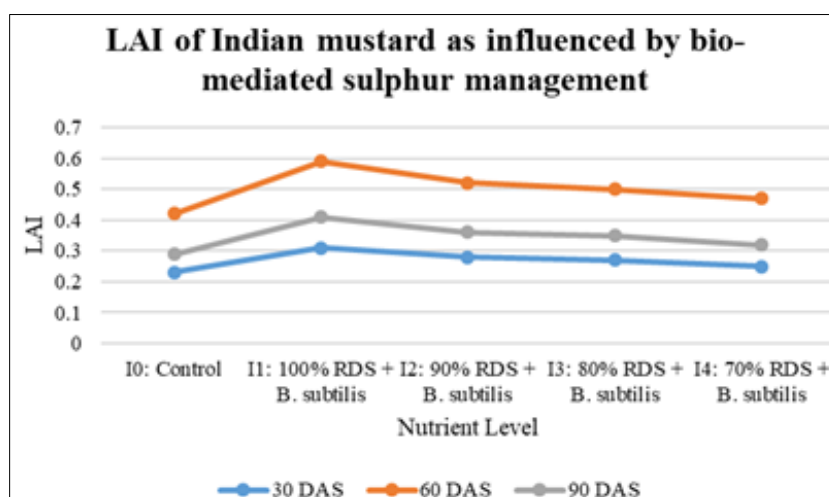


Fig 4: LAI of Indian mustard as influenced by bio-mediated sulphur management

Conclusion

From the present field experiment, it can be concluded that both sowing technique and bio-mediated sulphur management significantly influenced canopy development in Indian mustard cv. RH-749. Ridge and furrow sowing consistently produced greater leaf area and leaf area index than broadcasting, point seeding and stripe seeding, while seed biopriming with *Bacillus subtilis* combined with the full recommended dose of sulphur (100% RDS) outperformed reduced sulphur doses and the

untreated control. The interaction of ridge and furrow sowing with 100% RDS + *Bacillus subtilis* (S₄I₁) produced the maximum canopy expansion at all growth stages, indicating that this combination can be recommended for maximizing canopy development, and thereby light interception and productivity, of Indian mustard under conditions similar to the middle Gangetic plains.

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