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Effect of natural farming practices on physiological and biochemical traits of cabbage (*Brassica oleracea* var. *capitata*) under mid-hill conditions of Himachal Pradesh

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ABSTRACT

Natural farming helps in improving sustainability; and it is imperative to know at which concentration of its inputs the crops respond best. Therefore, the field experiment was conducted to evaluate the effect of natural farming practices on cabbage (*Brassica oleracea* L. var. *capitata*) in the mid-hill region of Himachal Pradesh during two consecutive seasons (2023–24 and 2024–25). Six treatments, consisting of a control and five concentrations of *jeevamrit* (10%, 20%, 30%, 40% and 50%) were evaluated for physiological parameters including chlorophyll content, carotenoids, photosynthetic rate, transpiration rate, leaf area index as well as stomatal traits and biochemical characteristics such as total phenolics, flavonoids and antioxidant activity. The results revealed that all doses of *jeevamrit* significantly improved these parameters over control. Among various treatments, 40% dose recorded maximum chlorophyll content, higher transpiration rates, secondary metabolite accumulation and enhanced antioxidant activity. A reduction in values at 50% suggested that the response had surpassed the optimum concentration. Principal Component Analysis (PCA) and correlation analysis showed strong positive associations between phenolic compounds, flavonoids and antioxidant capacity (DPPH), as indicated by high correlation coefficients (r = 0.97–0.99) and close vector alignment in the PCA biplot. Phenolic and flavonoid levels were significantly correlated with antioxidant capacity at optimal levels, suggesting enhanced physiological performance and tolerance to stress conditions. In general, these results indicated that 40% *jeevamrit* is the most effective concentration for improving physiological performance and biochemical quality of cabbage under natural farming conditions.

Keywords: Antioxidants, Biochemical quality, *Jeevamrit*, Physiological attributes, Sustainable agriculture

Natural farming is a chemical-free agroecological system integrating crops, trees and livestock. Inspired by Masanobu Fukuoka (1975) and promoted in India as Zero Budget Natural Farming (ZBNF), it addresses the ecological and economic limitations of conventional agriculture by eliminating synthetic inputs and reducing farmer indebtedness (Khadse *et al.* 2018, Khan *et al.* 2022). The practice relies on four core pillars: *jeevamrit* to enhance soil nutrient, *bijamrit* for protection against pathogen, *acchadana* to conserve moisture and *waphasa* to optimise the soil microclimate (Mukherjee *et al.* 2022). By using local cows to make natural fertilisers, the system creates a self-sustaining cycle that brings the soil back to life. Evidence from adoption in Himachal Pradesh confirms that applying these principles improves soil health and significantly lowers cultivation costs, building long-term climate resilience (Chandel and Verma 2021).

To further validate these benefits at a crop-specific level, it is essential to understand how the crop responds to these natural inputs. For the study, cabbage (*Brassica oleracea* L. var. *capitata*), a widely cultivated winter vegetable, was undertaken. It is valued for its compact edible head and rich nutritional and antioxidant composition. Global production of cabbage reached about 72 mt from 2.3 mha in 2022 (FAOSTAT 2024). India ranks second, producing 9.8 mt during 2023–24, with major cultivation in West Bengal, Odisha, Madhya Pradesh, Gujarat, Assam and Bihar. Himachal Pradesh contributes 128.59 thousand tonnes (APEDA 2024). Cabbage is rich in dietary fibre, vitamins C and E, and bioactive compounds such as quercetin and glucosinolates with antioxidant and anticancer properties (Rajapriya *et al.* 2017, Wang *et al.* 2022). Parameters such as the rate of plant photosynthesis, loss of water through

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transpiration, vitamins uptake and stomata functioning inform us about the plant condition (Taiz *et al.* 2015). The secondary compounds like phenols and flavonoids can be increased with natural inputs, which increase the antioxidant capacity of the plant (Zhang and Tsao 2016). To ensure agroecosystems remain sustainable, it is indeed important to know how cabbage responds on the basis of physiological and biochemical mechanisms within natural farming conditions. This study examined the effect of *jeevamrit* on these characteristics and crop quality traits.

MATERIALS AND METHODS

Experimental site: The field experiment was conducted for two consecutive years (2023–24 and 2024–25) at the Experimental Farm of Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (30.86°N, 77.16°E; at an elevation of 1,275 m amsl), Himachal Pradesh. The soil was sandy loam with near-neutral *p*H and moderate fertility status. The cropping season spanned from November to March, with average maximum temperature of 20.40–28.80°C and minimum temperature of 2.00–11.60°C. Relative humidity ranged from 6.8–65.8% and total rainfall from 4.80–42.0 mm. Six treatments were taken i.e. T_1, Control; T_2, 10% *jeevamrit*; T_3, 20% *jeevamrit*; T_4, 30% *jeevamrit*; T_5, 40% *jeevamrit*; and T_6, 50% *jeevamrit* with three replications. Laboratory analyses were performed in the Department of Basic Sciences, and Department of Soil Science and Water Management of the university.

Physiological parameters: Photosynthetic rate, transpiration rate and stomatal conductance were measured between 10:00 AM and 12:00 PM using a LI-COR 6400XT under standard conditions (PAR ~1000 µmol/m²/sec, CO_2 ~400 ppm and temperature 25 ± 2°C). Three replicates per treatment were used with four fully expanded leaves selected per replicate. Photosynthetic rate was expressed in µmol/m²/sec, transpiration rate in mmol/m²/sec and stomatal conductance in mol/m²/sec. Chlorophyll content was estimated using the method of Kizhedath and Suneetha (2011). Leaf samples were extracted in 80% acetone and absorbance recorded at 645 and 663 nm. Chlorophyll a, b and total chlorophyll were calculated using standard equations. Leaf Area Index (LAI) was calculated using Watson's (1947) formula:

$$\text{LAI} = \frac{\text{Total functional leaf area}}{\text{Total ground area occupied by the plant}}$$

Stomatal length, width and density were recorded using a Nikon Eclipse E200 microscope at 40× magnification with density expressed as Nos./mm², following Shah *et al.* (2019).

Biochemical parameters: Total phenolic content was determined using the Folin-Ciocalteu method (Singleton and Rossi 1965) and expressed as gallic acid equivalents (GAE) based on a gallic acid standard curve. Total flavonoid content was estimated using the aluminum chloride colorimetric assay. Antioxidant activity was assessed via DPPH (2, 2-Diphenyl-1-picrylhydrazyl) radical scavenging assay following Williams *et al.* (1995), calculated using the formula:

$$\text{Antioxidant activity (\%)} = \frac{\text{AbsControl-AbsSample}}{\text{AbsControl}} \times 100$$

Where Abs, Absorbance at 517 nm.

Carotenoid content was determined following Kira *et al.* (2015) using the formula:

$$\text{C (mg/g)} = \frac{(7.6 \times \text{OD480}) - (1.49 \times \text{OD510}) \times \text{V}}{1000 \times \text{W}}$$

Where V, Final volume of supernatant (mL); W, Weight of the leaf sample (g).

Statistical analysis: Two-year experimental data were subjected to Analysis of Variance (ANOVA) to determine treatment effects using Microsoft Excel and IBM SPSS Statistics 22. To further explore the interrelationships between physiological and biochemical traits, Principal Component Analysis (PCA) was performed using PAST version 4.11. The PCA was utilised to reduce data dimensionality and visualise multivariate patterns and trait distributions through biplots. Only components with eigen values greater than 1.0 were considered significant for explaining the total variance in the dataset.

RESULTS AND DISCUSSION

Physiological parameters: Photosynthetic activity in cabbage leaves exhibited a progressive enhancement with increasing concentrations of *jeevamrit*, peaking at 40% treatment (T_5) across all growth stages (Table 1). At 30 days after treatment (DAT), the photosynthetic rate ranged from 4.27 ± 0.03 µmol/m²/sec in the control (T_1) to 8.41 ± 0.05 µmol/m²/sec in T_5, with highly significant differences among treatments ($p \leq 0.001$). Similar trends were observed at 60 DAT (T_1: 7.85 ± 0.06; T_5: 11.87 ± 0.08 µmol/m²/sec) and 90 DAT (T_1: 6.61 ± 0.37; T_5: 10.24 ± 0.58 µmol/m²/sec) representing an increase of ~49–55% with all showing significant variation ($p \leq 0.001$). Transpiration rates followed a similar trend, increasing with *jeevamrit* concentration up to T_5. At 30, 60 and 90 DAT, T_5 recorded 5.34 ± 0.05, 10.05 ± 0.05 and 9.75 ± 0.08 mmol/m²/sec transpiration rates, respectively, with statistically significant differences across treatments ($p \leq 0.001$). Stomatal conductance increased markedly with *jeevamrit* application, reaching its maximum value under T_5 at all stages (Table 1). LAI values also increased significantly with increasing *jeevamrit* concentration, with T_5 producing the greatest leaf expansion throughout the crop cycle ($p \leq 0.001$).

In this experiment, LAI was highest under T_5 (2,315.13 and 2,405.21 at 60 and 90 DAT, respectively) followed by T_6 and T_4, indicating that *jeevamrit* at 40% significantly promoted canopy development (Table 1). This increase (more than 2.4 folds over control at 60 DAT: 928.60) clearly explained the enhanced photosynthetic surface area under T_5. This improvement can be attributed to enhanced nutrient uptake, stimulation of beneficial microbial populations and increased availability of growth-promoting substances that encouraged leaf expansion. Similar outcomes have been

Table 1 Effect of *jeevamrit* treatments on physiological attributes of cabbage at different growth stages

Treatments	Photosynthetic rate (µmol/m²/sec)			Transpiration rate (mmol/m²/sec)			Stomatal conductance (mmol/sec)			Leaf area index		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T_1	4.27 (±0.03)^{e}	7.85 (±0.06)^{e}	6.61 (±0.37)^{e}	3.76 (±0.03)^{e}	7.59 (±0.04)^{b}	5.41 (±0.06)^{f}	0.105 (±0.002^{f}	0.633 (±0.015^{e}	0.298 (±0.003^{e}	307.01 (±1.12)^{e}	928.60 (±21.54)^{e}	945.97 (±11.00)^{f}
T_2	5.56 (±0.04)^{d}	9.24 (±0.09)^{d}	7.98 (±0.45)^{d}	4.47 (±0.02)^{d}	9.82 (±0.09)^{a}	7.68 (±0.07)^{e}	0.190 (±0.004^{e}	1.600 (±0.017^{d}	0.718 (±0.005^{d}	504.75 (±6.64)^{d}	1388.74 (±101.26^{d}	1426.92 (±4.11)^{e}
T_3	6.34 (±0.01)^{c}	9.52 (±0.05)^{c}	8.56 (±0.34)^{c}	4.57 (±0.03)^{d}	9.95 (±0.10)^{a}	8.72 (±0.02)^{d}	0.22 (±0.002^{d}	1.710 (±0.024^{c}	0.763 (±0.008^{c}	607.76 (±4.23)^{c}	1538.62 (±61.18^{cd}	1620.56 (±14.69)^{d}
T_4	7.18 (±0.03)^{b}	10.60 (±0.05)^{b}	9.53 (±0.36)^{b}	4.87 (±0.01)^{c}	9.98 (±0.06)^{a}	9.15 (±0.01)^{c}	0.24 (±0.002^{c}	1.843 (±0.014^{b}	0.885 (±0.006^{b}	653.74 (±3.14)^{b}	1676.44 (±61.10)^{c}	1694.72 (±0.78)^{c}
T_5	8.41 (±0.05)^{a}	11.87 (±0.08)^{a}	10.24 (±0.58)^{a}	5.34 (±0.05)^{a}	10.05 (±0.05)^{a}	9.75 (±0.08)^{a}	0.28 (±0.005^{a}	2.100 (±0.007^{a}	0.933 (±0.006^{a}	707.48 (±3.91)^{a}	2315.13 (±10.74)^{a}	2405.21 (±16.63)^{a}
T_6	7.12 (±0.06)^{b}	10.84 (±0.13)^{b}	9.41 (±0.43)^{b}	4.99 (±0.02)^{b}	10.00 (±0.07)^{a}	9.32 (±0.07)^{b}	0.26 (±0.004^{b}	1.838 (±0.023^{b}	0.873 (±0.008^{b}	662.19 (±4.11)^{b}	2008.34 (±64.67)^{b}	2206.88 (±19.18)^{b}
Mean	6.48 (±0.67)	9.99 (±0.66)	8.72 (±0.72)	4.67 (±0.25)	9.56 (±0.46)	8.34 (±0.75)	0.22 (±0.03)	1.620 (±0.239)	0.745 (±0.109)	573.82 (±69.00)	1642.64 (±232.17)	1716.71 (±247.16)
p	***	***	***	***	***	***	***	***	***	***	***	***

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; DAT, Days after treatment. Data represent the mean of three replicates ± standard error; Values in parenthesis depicts the standard error of mean; Italic subscript letters in the column specify statistically significant difference between the means according to Duncan's multiple range test ($p \leq 0.05$).

Treatment details are given under Materials and Methods.

Table 2 Effect of *jeevamrit* treatments on chlorophyll a, chlorophyll b and total chlorophyll in cabbage

Treatments	Cholorophyll a (mg/g FW)			Cholorophyll b (mg/g FW)			Total cholorophyll (mg/g FW)		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T_1	1.06 (±0.53)^{e}	1.32 (±0.01)^{e}	1.23 (±0.01)^{e}	0.623 (±0.003)^{e}	0.72 (±0.00)^{e}	0.62 (±0.00)^{e}	1.47 (±0.21)^{d}	1.79 (±0.26)^{d}	1.63 (±0.22)^{c}
T_2	1.23 (±0.61)^{d}	1.52 (±0.01)^{d}	1.44 (±0.01)^{d}	0.673 (±0.003)^{d}	0.78 (±0.01)^{d}	0.74 (±0.00)^{d}	1.66 (±0.23)^{c}	2.00 (±0.29)^{c}	1.91 (±0.27)^{b}
T_3	1.34 (±0.67)^{c}	1.63 (±0.01)^{c}	1.56 (±0.00)^{c}	0.730 (±0.007)^{c}	0.82 (±0.01)^{c}	0.78 (±0.01)^{c}	1.80 (±0.26)^{b}	2.13 (±0.31)^{b}	2.05 (±0.30)^{b}
T_4	1.46 (±0.73)^{b}	1.77 (±0.02)^{b}	1.74 (±0.00)^{b}	0.788 (±0.003)^{b}	0.89 (±0.01)^{b}	0.85 (±0.01)^{b}	1.96 (±0.29)^{a}	2.34 (±0.34)^{a}	2.25 (±0.32)^{a}
T_5	1.53 (±0.76)^{a}	1.83 (±0.02)^{a}	1.80 (±0.01)^{a}	0.830 (±0.007)^{a}	0.94 (±0.00)^{a}	0.87 (±0.01)^{a}	2.06 (±0.31)^{a}	2.41 (±0.36)^{a}	2.33 (±0.35)^{a}
T_6	1.47 (±0.74)^{b}	1.80 (±0.02)^{ab}	1.75 (±0.01)^{b}	0.793 (±0.003)^{b}	0.90 (±0.00)^{b}	0.84 (±0.00)^{b}	1.99 (±0.28)^{a}	2.37 (±0.33)^{a}	2.29 (±0.31)^{a}
Mean	1.35 (±0.67)	1.64 (±0.09)	1.59 (±0.11)	0.739 (±0.037)	0.84 (±0.04)	0.78 (±0.04)	1.82 (±0.26)	2.17 (±0.30)	2.08 (±0.29)
p	***	***	***	***	***	***	**	**	**

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; DAT, Days after treatment; FW, Fresh weight. Data represent the mean of three replicates ± standard error (SE); Values in parenthesis depicts the standard error of mean; Italic subscript letters in the column specify statistically significant difference between the means according to Duncan's multiple range test ($p \leq 0.05$).

Treatment details are given under Materials and Methods.

reported for *jeevamrit's* influence on leaf area and stomatal features, likely linked to its role in improving soil biological activity and fertility (Lakhani 2024).

Chlorophyll a content in cabbage leaves exhibited a consistent upward trend with increasing *jeevamrit* concentrations across all growth stages (Table 2). At 30 DAT, the lowest value was observed in the control [T_1: 1.06 ± 0.53 mg/g fresh weight (FW)], while T_5 recorded the highest value (1.53 ± 0.76 mg/g FW). This pattern persisted at 60 DAT (T_1: 1.32± 0.01; T_5: 1.83 ± 0.02 mg/g FW) and 90 DAT (T_1: 1.23± 0.01; T_5: 1.80 ± 0.01 mg/g FW), with highly significant differences among treatments ($p \leq 0.001$). Treatments T_4 and T_6 also showed elevated chlorophyll a levels, though consistently lower than T_5. Additionally, a similar trend was noted in chlorophyll b content, with values increasing proportionally to *jeevamrit* concentration (Table 2). At 30 DAT, T_1 recorded the lowest (0.623 ± 0.003 mg/g FW), while T_5 reached the highest (0.830 ± 0.007 mg/g FW). Treatments T_4 and T_6 maintained high values across stages. Total chlorophyll content mirrored the trends of its individual components, with T_5 consistently outperforming other treatments, confirming a 35% enhancement. This directly contributed to improved light harvesting and carbon assimilation. The current results further support earlier findings that organic inputs enhance pigment concentrations, including chlorophylls and carotenoids, which are associated with improved photosynthetic capacity and overall plant health (Khoo *et al.* 2011).

Stomatal morphology in cabbage responded positively to increasing concentrations of *jeevamrit*, particularly in terms of stomata length and width (Table 3). At 30 DAT, stomata length ranged from 8.62 ± 0.006 µm under control (T_1) to 22.71 ± 0.066 µm in T_5. This trend continued at 60 and 90 DAT with highly significant differences ($p \leq 0.001$). Treatments T_4 and T_6 also showed substantial elongation, though significantly lower than T_5. Stomata width followed a similar pattern with T_1 recording minimum value (6.04 ± 0.02 µm), while T_5 reached the maximum width (18.00 ± 0.08 µm) at 30 DAT. In contrast, stomatal density did not exhibit a consistent trend with *jeevamrit* application, although differences were statistically significant ($p \leq 0.001$). T_1 recorded the highest density at all stages (7.38 ± 0.23, 9.50 ± 0.41 and 7.50 ± 0.32/mm² at 30, 60 and 90 DAT, respectively), while T_5 showed comparatively lower values (6.88 ± 0.42, 6.25 ± 0.44 and 6.50 ± 0.31/mm²).

Among all treatments, T_5 consistently recorded the highest values, indicating that this concentration facilitated optimal gas exchange during growth. This superior performance of T_5 can be attributed to balanced nutrient availability and enhanced microbial activity, which improved stomatal regulation and enzymatic efficiency involved in photosynthesis. Notably, T_4 also showed elevated physiological performance, but remained comparatively lower than T_5, indicating that 40% is an optimum threshold beyond which no proportional gain is achieved. These observations are consistent with previous studies reporting that organic nutrient management practices can enhance nitrogen availability, improve photosynthetic efficiency, increase chlorophyll content and promote overall plant growth (Chaudhary *et al.* 2024). Lakhani (2024) similarly

Table 3 Effect of *jeevamrit* treatments on stomatal characteristics of cabbage

Treatments	Stomatal length (µm)			Stomatal width (µm)			Stomatal density (stomata/mm^2)		
	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
T_1	8.620 (±0.006)^f	15.13 (±0.21)^d	13.10 (±0.06)^f	6.04 (±0.02)^e	11.57 (±0.35)^e	11.40 (±0.07)^f	7.38 (±0.23)^a	9.50 (±0.41)^a	7.50 (±0.32)^a
T_2	13.228 (±0.075)^e	20.95 (±0.57)^c	18.04 (±0.02)^e	7.77 (±0.06)^d	13.23 (±0.26)^d	13.44 (±0.08)^e	7.13 (±0.22)^{ab}	8.13 (±0.37)^b	7.25 (±0.33) ^{a}
T_3	16.940 (±0.072)^d	21.33 (±0.99)^c	19.32 (±0.02)^d	13.06 (±0.08)^c	14.46 (±0.08)^c	14.32 (±0.01)^d	6.37 (±0.24)^b	7.38 (±0.20)^b	7.38 (±0.22) ^{a}
T_4	19.355 (±0.036)^c	25.47 (±1.19)^b	24.13 (±0.08)^b	16.30 (±0.02)^b	17.85 (±0.30)^b	16.87 (±0.04)^c	6.38 (±0.31)^b	7.12 (±0.20)^{bc}	6.50 (±0.24) ^{a}
T_5	22.708 (±0.066)^a	28.97 (±0.12)^a	27.84 (±0.04)^a	18.00 (±0.08)^a	21.32 (±0.71)^a	19.26 (±0.14)^a	6.88 (±0.42)^{ab}	6.25 (±0.44)^c	6.50 (±0.31) ^{a}
T_6	21.200 (±0.034)^b	25.34 (±0.99)^b	23.03 (±0.18)^c	16.45 (±0.13)^b	17.39 (±0.36)^b	17.71 (±0.12)^b	7.25 (±0.20)^a	7.75 (±0.21)^b	6.63 (±0.40) ^{a}
Mean	17.008 (±2.470)	22.86 (±2.35)	20.91 (±2.42)	12.94 (±2.32)	15.97 (±1.70)	15.50 (±1.37)	6.90 (±0.32)	7.69 (±0.59)	6.96 (±0.35)
p	***	***	***	***	***	***	***	***	***

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; DAT, Days after treatment. Data represent the mean of three replicates ± standard error (SE); Values in parentheses indicate the standard error of mean (SEm); Mean comparison was performed using Duncan's Multiple Range Test ($p \leq 0.05$) and values followed by different superscript letters within a column differ significantly; SEm and CD ($p \leq 0.05$)/LSD values should be provided below each parameter column.

Treatment details are given under Materials and Methods.

Table 4 Effects of *jeevamrit* treatments on biochemical quality characteristics of cabbage leaves

Treatments	Phenol (mg/g DW GAE)	Flavonoids (mg/g DW RE)	DPPH (%)	Carotenoid 30 DAT (mg/g)	Carotenoid 60 DAT (mg/g)	Carotenoid 90 DAT (mg/g)
T_1	9.93 (±0.20)*e*	3.25 (±1.63)*e*	47.85 (±0.07)*e*	0.43 (±0.01)*e*	0.53 (±0.00)*e*	0.52 (±0.01)*d*
T_2	12.50 (±0.24)*d*	6.00 (±3.00)*d*	49.65 (±0.21)*c*	0.46 (±0.00)*d*	0.58 (±0.00)*d*	0.55 (±0.00)*c*
T_3	13.64 (±0.20)*c*	8.00 (±4.00)*c*	52.81 (±0.13)*d*	0.51 (±0.00)*c*	0.62 (±0.00)*c*	0.60 (±0.00)*b*
T_4	15.17 (±0.18)*c*	9.00 (±4.50)*c*	56.78 (±0.19)*c*	0.56 (±0.00)*b*	0.72 (±0.01)*b*	0.68 (±0.01)*a*
T_5	18.32 (±0.25)*a*	12.50 (±6.25)*a*	60.28 (±0.61)*a*	0.59 (±0.01)*a*	0.75 (±0.00)*a*	0.69 (±0.01)*a*
T_6	16.58 (±0.25)*b*	10.50 (±5.25)*b*	57.76 (±0.12)*b*	0.57 (±0.00)*b*	0.71 (±0.00)*b*	0.69 (±0.00)*a*
Mean	14.36 (±1.41)	8.21 (±4.10)	54.19 (±2.29)	0.52 (±0.03)	0.65 (±0.04)	0.62 (±0.04)
p	***	***	***	***	***	***

*, $p \leq 0.05$; **, $p \leq 0.01$; ***, $p \leq 0.001$; Data represent the mean of three replicates ± standard error; Values in parentheses indicate the standard error of mean (SEm); Italic subscript letters in the column specify statistically significant difference between the means according to Duncan's multiple range test ($p \leq 0.05$). DW GAE, Dry weight gallic acid equivalent.

Treatment details are given under Materials and Methods.

noted that moderate organic inputs improved gas exchange parameters and water-use efficiency, while excessive application provided no additional benefit.

Biochemical parameters: It is evident from Table 4 that all the biochemical parameters responded positively to *jeevamrit* application, with significant differences observed across all treatments. Phenol content recorded the lowest value in T_1 [9.93 ± 0.20 mg/g dry weight (DW) GAE], while T_5 exhibited the highest concentration (18.32 ± 0.25 mg/g DW GAE), with highly significant differences ($p \leq 0.001$), representing 46% increase. Intermediate treatments showed a dose-dependent increase: T_2 (12.50 ± 0.24), T_3 (13.64 ± 0.20) and T_4 (15.17 ± 0.18 mg/g DW GAE), all significantly higher than the control (T_1). Although T_6 (50%) showed a slight decline to 16.58 ± 0.25 mg/g DW GAE, it remained superior to lower concentrations. In addition to this, flavonoid content followed a similar trend, increasing significantly with *jeevamrit* concentration. T_1 recorded the lowest value [3.25 ± 1.63 mg/g DW Rutin Equivalents (RE)], while T_5 reached the maximum (12.50 ± 6.25 mg/g DW RE), showing highly significant variation ($p \leq 0.001$). This indicated a 3.8 fold increase, clearly indicating strong metabolic activation. These effects are likely due to *jeevamrit's* rich microbial diversity and its capacity to improve nutrient acquisition, thereby activating the phenylpropanoid pathway (Singh *et al.* 2021). Similar findings were reported by Chun *et al.* (2004), who observed high phenolic and flavonoid levels in red cabbage associated with strong antioxidant activity.

Antioxidant activity, measured via DPPH radical scavenging was significantly influenced by *jeevamrit* treatments (Table 4). Control (T_1) exhibited the lowest value (47.85 ± 0.07%), whereas T_5 recorded the maximum value (60.28 ± 0.61%), with highly significant differences ($p \leq 0.001$). However, T_6 (57.76 ± 0.12%) showed a marginal decline from T_5 but remained significantly higher than the control. Moreover, carotenoid accumulation in cabbage leaves was positively influenced by *jeevamrit* application (Table 4). At 30 DAT, T_5 recorded the highest carotenoid content (0.59 ± 0.01 mg/g FW), while T_1 was lowest (0.43 ± 0.01 mg/g FW). This trend continued at 60 DAT and 90

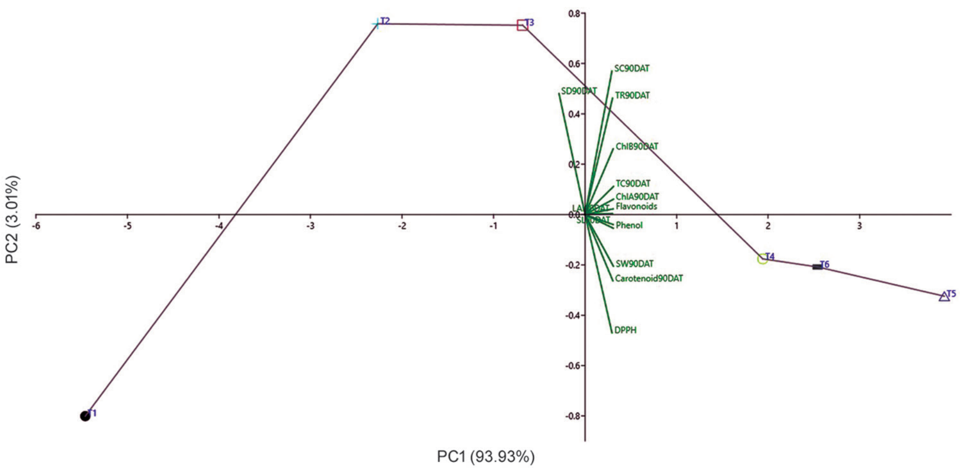

Fig. 1 PCA biplot showing distribution of cabbage treatments and physiological traits under *jeevamrit* application.

TR, Transpiration rate; SC, Stomatal conductance; LAI, Leaf area index; ChlA, Chlorophyll a; ChlB, Chlorophyll b; TC, Total chlororphyll; SL, Stomatal length; SW, Stomatal width; SD, Stomatal diameter; DAT, Days after treatment; PC, Principal component.

Treatment details are given under Materials and Methods.

DAT with highly significant differences observed across treatments ($p \leq 0.001$).

Principal component analysis (PCA) and corelation matrix: The PCA biplot (Fig. 1) illustrates cabbage treatments and physiological traits across PC1 (93.93%) and PC2 (3.01%). Treatment T_2 and T_5 were distinctly positioned, indicating strong trait influence. Most variables clustered near the origin, viz. photosynthetic rate, transpiration and chlorophyll content, represented limited variation. Treatment T_5 aligned with enhanced physiological performance, reflecting its positive effect on plant growth. Increases in stomatal length and width under T_5 (28.97 µm and 21.32 µm at 60 DAT, respectively) suggested a physiological adjustment facilitated by improved nutrient supply, microbial stimulation and phytohormonal activity that promote cell expansion. This aligns with the documented inverse relationship between stomatal size and density- A balance, critical for regulating gas exchange and water-use efficiency (Lawson *et al.* 2014, Bertolino *et al.* 2019). In the present study, stomatal density was comparatively lower in T_5 (6.25/mm² at 60 DAT) than control (9.50/mm²), confirming this inverse relationship and indicating improved stomatal efficiency rather than mere density increase. Notably, excessive organic inputs may disturb nutrient balance and impair physiological function, as noted by Vimala *et al.* (2004). This is evident in T_6 (50% *jeevamrit*), where a marginal decline was observed as compared to T_5 (e.g. photosynthesis: 10.84 vs 11.87 µmol/m²/sec at 60 DAT), suggesting that supra-optimal concentrations may lead to nutrient imbalance or osmotic effects. The work of Patel *et al.* (2024) further reinforces these findings, showing that *jeevamrit* and *beejamrit* improved physiological responses under abiotic stresses such as salinity and drought by increasing chlorophyll content, relative water content and antioxidant enzyme activities, while reducing stress markers including hydrogen peroxide (H_2O_2), malondialdehyde (MDA), proline and glycine betaine.

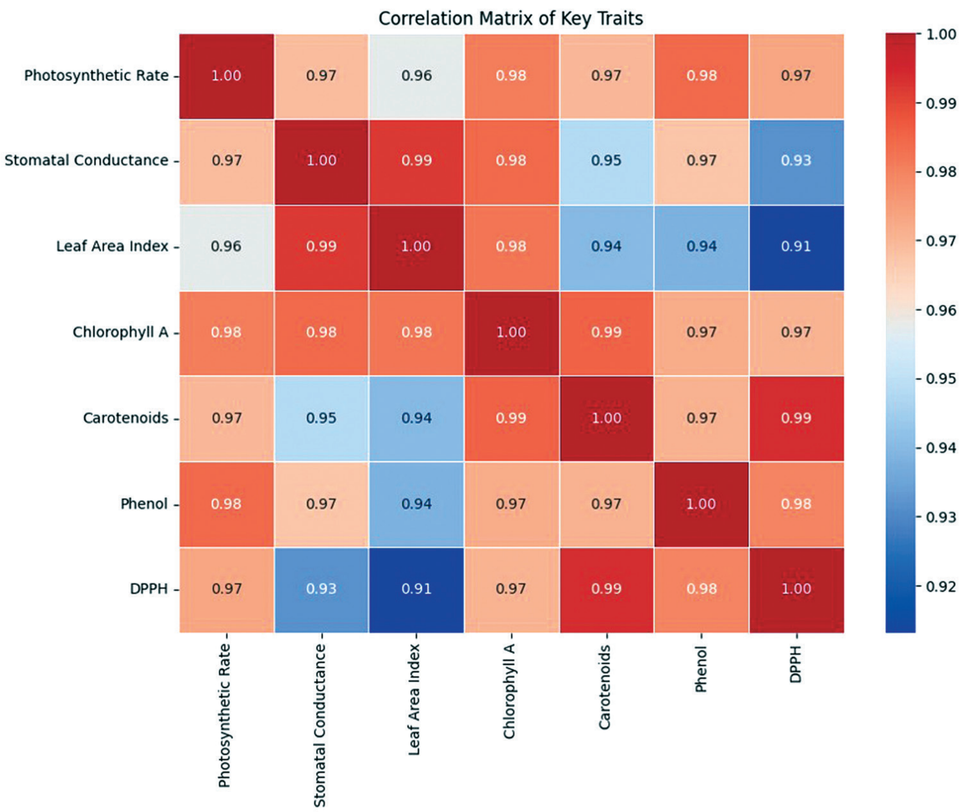

Fig. 2 Heatmap showing strong correlations among key physiological and biochemical traits in cabbage at 90 DAT.

A correlation matrix at 90 DAT evaluated relationships among major physiological and biochemical traits in cabbage. The heatmap (Fig. 2) showed strong positive correlations among photosynthetic rate, stomatal conductance, leaf area index, chlorophyll, carotenoids, phenols and antioxidant activity (DPPH %). Most coefficients exceeded 0.90, indicating coordinated responses and enhanced metabolite accumulation under *jeevamrit* treatments. Seong *et al.* (2016) also found a positive relationship between phenolic content and DPPH radical scavenging activity in Chinese cabbage, while Naguib *et al.* (2012) highlighted the role of bio-based treatments in enhancing phenolic metabolism and antioxidant defences. Statilko *et al.* (2024) similarly documented strong correlations between phenolic and flavonoid concentrations and antioxidant activity in cabbage. The higher antioxidant activity observed in T_5 (60.28%) compared to control (47.85%) further confirmed improved oxidative stress tolerance, which may be linked to enhanced secondary metabolite synthesis and improved cellular redox balance. *Jeevamrit* application significantly improved physiological and biochemical traits in cabbage.

Overall, the consistent superiority of T_5 across all parameters can be explained by its optimal balance between nutrient supply, microbial activity and plant metabolic response, whereas lower concentrations were insufficient and higher concentrations (T_6) showed marginal decline due to possible nutrient imbalance. The 40% treatment (T_5) recorded the highest photosynthetic rate, transpiration, chlorophyll, carotenoids, leaf area index, stomatal traits, phenolic and flavonoid contents and antioxidant activity. Thus, from both statistical and physiological perspectives, 40% *jeevamrit* proved to be optimal, sustainable and costeffective for enhancing physiological performance and biochemical quality in cabbage.

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