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Comparative Effect of FYM and *Azotobacter* on Morphology and Nutritional Quality of High and Low Altitude Grown Knol-khol (*Brassica oleracea* var. *Gongylodes* L.) Cultivar White Vienna

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ABSTRACT

High-Altitude (HA) environments pose unique challenges to crop cultivation due to extreme abiotic stresses. Organic agricultural treatments offer promising solutions to address these challenges and enhance crop performance and nutritional quality. Herein, we examine the comparative effects of Farm Yard Manure (FYM) and *Azotobacter* treatments on knol-khol at high *vs.* Lower Altitudes (LA), aiming to enhance resilience and nutritional value across varying altitudes. The field trial was conducted using a randomised block design as a two-factorial experiment. The first factor was treatments (T_1-FYM, T_2-*Azotobacter*, T_3-FYM+*Azotobacter*, and T_4-(control), and the second factor was cultivation locations (HA *vs.* LA). The findings revealed that the application of treatment T_3 at HA resulted in higher total soluble solids (1.38-fold), titratable acidity (0.06-fold), total carbohydrate (1.9-fold), crude protein (3.7-fold), crude fat (3-fold) and dietary fiber content (78-fold) whereas, yield (137.6-fold) and ash content (0.85-fold) content were found higher at LA. The current study emphasises the superior efficiency of the combination treatment of FYM and *Azotobacter* at HA to improve the nutritional quality of food crops compared to LA, with the added benefits of environmental sustainability and nutritional security.

Keywords: FYM; Azotobacter; Knol-Khol; Sustainable production; Nutritional Quality

1. INTRODUCTION

In recent decades, the world's population has increased exponentially, necessitating a more excellent supply of high-quality food. This requirement has been primarily met by using chemical fertilisers. Unfortunately, this practice led to a collapse in food nutritional value and a rise in various health problems[1]. Consequently, there is an increased focus on investigating agricultural practices to improve the nutritional profiles of food crops under different environmental conditions, such as high altitudes. Although, in general, research studies have established that the most efficient way to accomplish nutritional goals in food crops is through bio-organic farming, whether the same applies to high-altitude environments is a matter of intense investigation[2]. Plants that thrive in high-altitude regions are subject to various environmental factors, including temperature, humidity, daylight hours, UV radiation, wind, geology, and air pressure[3]. These abiotic stressful environmental variables majorly impact a plant's morphological and nutritional components[4]. In the Indian sub-continent, HA locations such as Leh-Ladakh have a huge unmet demand for fresh veggies due to the adversities of agriculture practices under a cold, arid climate, especially during long-harsh winters, which restrict the farming season for 4-5 months in a year. This region, which has limited resources, primarily relies on supplies from plain areas, particularly Chandigarh. However, these locations face many transportation-related challenges, which lead to inadequate nutritional quality in the food[5]. Hence, there is a need to establish a cultivation practice at HA that could achieve adequate production and nutritional composition compared to LA. Bio-organic inputs such as Farm Yard Manure (FYM) and biofertilisers (*Azotobacter)* are known to not only reduce dependency on chemical fertilisers but also provide plants with all of the nutrients needed for growth[6,7]. However, the suitability of its application in extreme environmental conditions such as high mountainous regions remains largely unexplored.

Knol-khol (*Brassica oleracea* var. *gongylodes* L.), also known as kohl rabi, belonging to the Brassicaceae family, is one of the most consumed vegetables in high altitude region of Ladakh because of its distinct flavor (mild, sweet, slightly peppery) and crunchy texture, similar to that of a broccoli stem or a radish[8]. It is

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cultivated for its edible swollen stem, although its leaves are consumed and used in various culinary preparations[9]. Nutritionally, cole crops and vegetables are an excellent source of nutritional value, as are dietary fiber, minerals, and vitamins, including A, B, and C, and low levels of calories and fat[10,11]. Due to its richness in fiber, nutritive value, and health benefits, knol-khol is one of the most popular and consumed vegetables in the HA region of Ladakh. Thus, it would be worth exploring and establishing suitable organic practices for growing nutritionally rich and chemical-free knol-khol crops at HA. This emphasises the necessity and importance of in-depth comparative research on the effect of FYM and *Azotobacter* at high and low altitude field locations, using knol-khol as a model crop.

Hence, the current study explores environment-friendly agricultural interventions to foster the growth of plentiful, nutritionally rich food crops in challenging environmental conditions of HA that could be comparable to LA cultivation. The study covers a detailed comparative analysis of the morphological and nutritional attributes of knol-khol cultivated in high-altitude Leh, India, versus low-altitude Chandigarh, India, with a specific focus on the effects of organic biofertilisers such as FYM and *Azotobacter.*

2 MATERIAL AND METHODS

2.1 Field Trial and Site

The field experiment was conducted over two consecutive years, namely 2020-2021 and 2021-22, in open fields at both high-altitude and low-altitude locations. The high altitude trials took place at the Agriculture Research Unit (ARU), Defence Institute of High Altitude Research (Leh), India, situated at an elevation of 3340 meters (34008.2’N; 77034.3’E). Meanwhile, the low altitude trials were carried out at the Defence Institute of High Altitude Research base lab in Chandigarh, India, located at 30º41′31′′ N and 76º47′10′′ E, at an altitude of 321 meters above Mean Sea Level (MSL). Temperature and relative humidity were recorded daily at both locations using a hygro-thermometer (445,702, Extech Instruments). The average mean maximum and minimum temperature at high altitudes was 25.74±2.03 and 4.40±1.07 °C. Whereas at low altitudes was 33.34±1.65 and 10.64±1.90 °C, respectively. The maximum relative humidity was (59.77±3.10 %) and (87.11±3.22%) at HA and LA, respectively, While minimum relative humidity was recorded (14.24±2.40 %) and (62.26±1.15 %), respectively. The fertility of the HA and LA soils is examined before transplanting (Table 1).

Table 1. Soil fertility status before Transplanting

Parameters	HA	LA
pH	7.76 ± 0.17	8.63 ± 0.49
EC (ms/cm)	0.76 ± 0.28***	0.37 ± 0.02
OC (%)	0.79 ± 0.01***	0.31 ± 0.01
N (Kg/ha)	35.24 ± 1.24***	22.28 ± 5.54
P (Kg/ha)	12.42± 0.13*	10.24 ± 0.71
K (Kg/ha)	180.32 ± 5.31	196.42 ± 3.07**
S (mg/Kg)	128.41 ± 6.81	126.34 ± 5.20
Zn (mg/Kg)	5.23 ± 0.84	8.53 ± 0.72***
Fe (mg/Kg)	3.12 ± 0.41	6.38 ± 1.06***
Cu (mg/Kg)	5.02 ± 0.07	7.23 ± 0.94***
Mn (mg/Kg)	3.48 ± 0.19***	1.53 ± 0.25

Mean values in each row (between group) showed significantly different by independent t-test, ****p≤0.001; **p≤0.01 and *p≤0.05*. EC: electrical conductivity, OC: organic carbon HA: high altitude and LA: low altitude

2.2 Experimental Design

This study used the knol-khol (*Brassica oleracea* L. var. *gongylodes*) cultivar White Vienna at both high and low-altitude field locations. Crop seed was procured from Beejsheetal Research Pvt. Ltd., Mantha Road, Jalna, Maharashtra. The field trial had 12 plots in total by following a two-factorial randomised block design (2FRBD) with four treatments [T_1= FYM @ 150 q/ha; T_2 = *Azotobacter* @ 8.6 kg/ha; T_3 = FYM @ 150 q/ha+ *Azotobacter* @ 8.6 kg/ha; T_4 = control (without treatment)] replicated thrice. Each plot was 1.62 m2 (1.35 m length × 1.20 m width), and a distance of 0.5 m was maintained between both two blocks and two plots. Seed seedlings have been transplanted at a 2 to 3 true leaf stage or 15 to 18 cm height. A consistent 30 cm × 20 cm plant spacing between individual plants and rows across all experimental plots has been maintained. FYM and biofertilser were applied in each plot before transplanting the seedlings. The field underwent flooding irrigation every three days at High Altitudes (HA) and 6-7 days intervals at Low Altitudes (LA) during the initial stage of plant establishment. This was followed by a one-week interval at HA and a two-week interval at LA during later stages. Notably, no synthetic fertilisers or pesticides were employed at either location. Weed control was carried out manually, with removal occurring two to three times throughout the growing period.

2.3 Morphological Attributes

Five representative plants were chosen randomly from each plot, and the plants were tagged for further measurement. Morphological traits were measured 30, 45, and 60 Days After Transplantation (DAT). The chlorophyll (leaf) and anthocyanin content were estimated via portable meter (CCM-200 plus and ACM-200 plus, ADC Bioscientific, UK). The knob was vertically sectioned at its central point to measure both polar and equatorial diameters. The digital vernier callipers were employed to measure the horizontal distance across the broadest part of the sectioned knob, extending from one side to the other[12]. The yield (q/ha) was computed by determining the knob weight per plot and then converting it to a per-hectare basis to obtain the total yield (q/ha). After harvesting, the dried kohlrabi samples were kept at -20 °C for later examination.

2.4 Estimation of Total Soluble Solids (TSS) and Titratable Acidity (TA)

Approximately 10 g of fresh knob was blended, and juice was extracted to estimate TSS using a hand refractometer (ATAGO, Tokyo). Titratable Acidity (TA) was calculated as a percentage of malic acid by titrating fresh knob juice with 0.1 N NaOH up to pH 8.2[13].

2.5 Estimation of Crude Fat

The Soxhlet system was used to calculate the crude fat content of dried samples[14]. The dried kohlrabi powder (3000 mg) was extracted in three soxhlet extractors using continuous petroleum ether at a flow rate of 2-3 drops per second, followed by sample drying at 95±4 °C. The crude fat percentages were calculated using the formula:

$$\text{Crude fat (\%)} = \frac{FF - FW}{S} \times 100 \qquad \text{Eqn (1)}$$

FF= flask weight with fat, FW= flask weight without fat, S= sample weight

2.6 Estimation of Dietary Fiber

The dietary fiber of the knob was estimated according to method no. 978-10[15]. Moisture-free and defatted kohlrabi sample (2000 mg) was digested with 0.128 M (200 mL) of boiling H_2SO_4 for 35 minutes. The digested sample was filtered after the acid was discarded, and then it was washed with boiling distilled water to remove any remaining acid. To eliminate all base solubilised fractions, the sample was next subjected to a 35-minute treatment with 200 mL of boiling NaOH (0.313 M) solution. Once more, it was filtered and washed with hot distilled water. The residual remains were dried at 180°C for 95 minutes, weighed, and then ignited in a muffle furnace (Scientech laboratory equipment, India) at 560 ± 10°C for 2 hours. The dietary fiber percentage was calculated by using the following equation:

$$\text{Dietary fiber (\%)} = \frac{B - C}{A} \times 100 \qquad \text{Eqn (2)}$$

Where: A= crude sample weight, B= sample weight before ignition, C= sample weight after ignition.

2.7 Estimation of Ash Content

Method No. 942-05 was used to determine the ash content in kohlrabi samples[15]. The 5000 mg sample was put in a crucible, heated to 560 ± 10 °C in a muffle furnace for six hours, until whitish grey residues were formed. The sample was cooled before being weighed. Ash content (%) was calculated by the following formula:

$$\text{Ash (\%)} = \frac{CA - BC}{S} \times 100 \qquad \text{Eqn (3)}$$

CA= weight of crucible with ash, BC= blank crucible weight, S= sample weight

2.8 Estimation of Crude Protein

The crude protein content in knol-khol samples was analysed using a modified method with the Kjeldahl instrument (K-355, Buchi Labortechnik, Switzerland)[15]. For this, oven-dried kohlrabi sample (0.2 g) was digested through concentrated H_2SO_4 and digestion tablets until a light greenish color, which was obtained after two to three hours. Distillation was done with 32 % NaOH after digestion. The released ammonium gas was captured in a 4 % boric acid solution consisting of methyl red and bromo cresol green (indicator), generating ammonium borate that indicates nitrogen content. At last, the distillate was titrated with 0.25 M H_2SO_4 till a light pinkish color, and the volume consumed was noted. To estimate the protein content in the dried sample, nitrogen content was multiplied by the correction factor of 6.25[16].

2.9 Determination of Total Carbohydrate Content

The total carbohydrate content in the extracts was evaluated using the anthrone method with slight modifications[17]. In 100 mL of concentrated sulphuric acid, anthrone (200 mg) was dissolved and cooled by ice cooling. 400 µL of different concentrations of standard solution (Glucose; 3.9-1.000 µg/mL) and extracts were mixed with 2000 µL of anthrone reagent respectively, succeeded by placing in a water bath at 95 °C for 17 minute followed by cooling at room temperature. The absorbance of the standard and samples was calculated spectrophotometrically at 620. The findings were reported in micrograms of glucose equivalence per gram.

2.10 Statistical Analysis

The experimental data were replicated three times and are reported as mean ± standard deviation (SD). Statistical analyses were conducted to assess the significance of the morphological and nutritional parameters of kohlrabi data obtained from high and low altitudes. An independent t-test, one-way ANOVA, and post hoc analysis using Duncan's multiple range tests ($p \leq 0.05$) were carried out in SPSS 16.0 (SPSS Corporation, Chicago, IL). For morphological traits, a three-way ANOVA was employed to examine the relationship between altitude, treatment, and days after transplanting, including their interactions. In nutritional attributes, a two-way ANOVA was conducted to explore the interaction between altitude and treatments.

3. RESULTS AND DISCUSSION

3.1 Morphological Parameters

The growth parameters of kohlrabi, including plant height, number of leaves, leaf length with petiole, leaf width, leaf area, plant spread, leaf chlorophyll content, and leaf anthocyanin content, exhibited significant differences ($p \leq 0.05$) between the treatments and locations (Table 2).

Among the four treatments, the T_3 treatment outperformed all the other treatment groups at both locations, *i.e.*, HA and LA at 60 DAT. Various types of organic fertilisers (manures and biological nitrogen fixers, *etc.*), either alone or in combination, have improved vegetative growth compared to untreated plants[18]. Possibly, free-living N-fixing bacteria like *Azotobacter* can assimilate atmospheric nitrogen yet they also produce specific phytohormones, such as GA3, IAA, and cytokinins. These phytohormones increase plant development and enhance nutrient accessibility to plant roots by increasing nutrient dissolution[19].

However, in current investigation, among the altitudes (HA & LA), the effect of T_3 treatment at LA resulted in increased plant height (3-fold), leaf width (1.4-fold), and leaf area (134.5-fold) as compared to HA-grown kohlrabi. The observed reduced height, leaf width, and area in HA-grown plants could be adaptive features in response to abiotic stressors like cold, frost, drought, low oxygen, high wind velocity, intense UV radiations, *etc*[20]. In contrast, no significant difference was observed concerning other parameters *viz.,* number of leaves, leaf length with petiole, plant spread, leaf chlorophyll content, and leaf anthocyanin content at both HA and LA-grown kohlrabi. The most probable underlying reason for this observation could be the extended photo-period at HA, which directly influences the colored pigment contents and photosynthetic rates. These findings are comparable with those of prior investigations at HA[3,21]. This is further corroborated by a significant interaction between altitude, treatment, and days after transplanting (ALT×TRE×DT) was found on the plant height, number of leaves, leaf width, leaf area, leaf chlorophyll content, and leaf anthocyanin content (Table 3).

3.2 Yield Parameters

Altitudinal conditions and treatments showed a significant effect on kohlrabi yield attributes (Table 4), including knob equatorial diameter, knob polar diameter, knob weight per plant, and yield (q/ha).

Based on our results, among all the treatments, T_3 treatment resulted in maximum yield parameters at both locations. However, among altitudes, T_3 treatment at the LA region resulted in higher knob weight per plant (59.8-fold) and yield (137.6-fold) as compared to HA-grown kohlrabi. There is no significant variation in knob equatorial diameter and polar diameter were observed in kohlrabi grown at both locations. It could be because of superior physical indices of knol-khol, which may be attributable to superior plant growth features in LA compared to producing kohlrabi[22]. In a two-way study, the effects of altitude and treatment (ALT×TRE) on knob equatorial and polar diameter, knob weight per plant, and yield (q/ha) were significantly different. These outcomes are consistent with the earlier findings of[1,23,24].

3.3 Nutritional Quality

3.3.1 TSS, Titratable Acidity and Total Carbohydrate Content

Carbohydrates are required for various biochemical reactions and enhance energy metabolism, which plays an important function in abiotic stress management[25]. The TSS content, titratable acidity, and total carbohydrate content of mature kohlrabi significantly differed ($p \leq 0.05$) among altitude and treatments (Table 5).

Based on our study, among all the treatments, T_3 treatment resulted in the highest content of TSS, titratable acidity, and total carbohydrate at both locations. However, among altitudes, T_3 treatment at the HA region resulted in higher content of TSS (1.38-fold), titratable acidity (0.06-fold), and total carbohydrate (1.9-fold) as compared to the LA-grown sample. The interactive effects between altitudes and treatments (ALT×TRE) were found on the TSS and total carbohydrate content except the titratable acidity content of the kohlrabi sample. As explained earlier, it might be due to prolonged photoperiod-driven higher photosynthetic rate and better soil nutrient availability at high altitudes. Rokaya[26] *et al.,* (2016) and Naryal[27] *et al.,* (2020) have also made similar observations on the impact of increasing elevation on enhancing the photosynthetic rate and total sugar content in mandarin and apricot fruit. Organic manuring influences general plant health, resulting in higher carbohydrate content and organic acids[28]. Mishra[29] *et al.,* (2014) have shown that a boost in the TSS was correlated with an increase in soil nutrient levels as well as bio-organic nutrient supplements.

3.3.2 Crude Protein Content

Proteins serve as the fundamental constituents of muscle tissue, drive nearly all biochemical processes within the body, regulate gene activity, and constitute the bulk of a cell's structural framework[30]. In the present study, T_3 treatment resulted in the highest content of crude protein at both locations (Table 5). However, among altitudes, T_3 treatment was found to have higher total protein (3.7-fold) at HA as compared to LA-grown knob. The interaction between altitudes and treatments (ALT×TRE) was also found to be significant *($p<0.05$)*. The increase in protein content with altitude could be due to N-rich compounds in the soil in highly mineralised form (NO_3^-), which boosts protein formation in plants[31]. Our findings are consistent with earlier studies on cabbage[32,33].

3.3.3 Crude Fat Content

Fats play an essential role in numerous physiological processes, including ensuring suitable energy intake and absorption of fat-soluble vitamins[34]. The crude fat content of kohlrabi differed significantly between location and treatments (Table 5). Based on our study, among all the treatments, T_3 treatment resulted in the highest content of crude fat at both locations. However, among altitudes, T_3 treatment at the HA region resulted in a higher content of crude fat (1.107-fold) as compared to the grown sample. It might be because high-altitude soil has higher levels of electrical conductivity and nitrogen content which amendment the largest concentrations of ions in the knob that encourage the synthesis of crude fat/fatty acid. Our findings correlate well with[35,36].

3.3.4 Ash Content

The ash content signifies the overall quantity of minerals in a food item. In contrast, the mineral content specifically denotes the concentration of individual inorganic components in the food, such as Ca, Na,

Table 2. Comparative effect of location and treatments on growth attributes of knol-khol cultivar white vienna

Parameters	Treatments	30 DAT		45 DAT		60 DAT	
		HA	LA	HA	LA	HA	LA
Plant height (cm)	T_1	16.04±0.53^{b}	28.89±0.75^{b***}	24.04±0.64^{b}	31.93±0.39^{c***}	29.11±0.95^{b}	32.51±1.08^{b*}
	T_2	17.08±0.60^{b}	28.61±1.31^{b***}	23.23±0.70^{b}	30.39±0.97^{b***}	27.91±0.60^{b}	31.58±0.96^{b**}
	T_3	20.54±0.94^{c***}	31.94±0.78^{c***}	26.30±0.71^{c}	34.53±1.09^{d***}	33.00±1.22^{c}	36.05±0.91^{c*}
	T_4	14.05±0.43^{a}	23.36±0.60^{a***}	18.47±0.74^{a}	25.90±0.64^{a***}	22.52±0.91^{a}	27.51±0.10^{a***}
Number of leaves	T_1	7.89±0.51^{b}	11.89±0.26^{b***}	11.78±0.19^{c}	14.94±0.10^{b***}	15.61±0.70^{b}	16.22±0.19^{b}
	T_2	7.50±0.44^{b}	11.78±1.08^{b**}	11.11±0.48^{b}	14.33±0.88^{b**}	15.33±0.60^{b}	15.89±0.79^{b}
	T_3	8.39±0.35^{c}	13.83±0.44^{c***}	13.17±0.00^{d}	16.33±0.44^{c***}	18.94±0.42^{c}	18.39±0.10^{c}
	T_4	6.17±0.34^{a}	9.67±0.17^{a***}	9.17±0.34^{a}	12.11±0.10^{a***}	12.61±0.19^{a}	13.22±0.35^{a}
Leaf length (cm)	T_1	13.94±0.42^{b}	27.27±0.73^{b***}	21.92±0.4^{b*}	30.17±0.19^{c**}	27.45±0.72^{b}	30.84±0.65^{c**}
	T_2	14.01±0.89^{b}	26.61±1.47^{b***}	20.92±0.92^{b}	28.67±1.13^{b***}	25.89±0.97^{b}	29.34±0.87^{b**}
	T_3	18.91±0.75^{c}	30.24±0.77^{c***}	24.65±0.89^{c}	32.56±0.89^{d***}	30.55±1.33^{c}	33.28±1.12^{d}
	T_4	11.68±0.61^{a}	21.98±0.45^{a***}	16.93±0.65^{a}	24.23±0.55^{a***}	20.85±0.79^{a}	25.41±0.21^{a***}
Leaf width (cm)	T_1	5.46±0.06^{c}	9.36±0.08^{b***}	9.42±0.07^{b}	11.1±0.24^{b***}	10.77±0.26^{b}	11.62±0.19^{b**}
	T_2	5.26±0.11^{b}	9.27±0.24^{b***}	9.63±0.26^{b}	10.84±0.15^{b**}	10.63±0.31^{b}	11.41±0.26^{b*}
	T_3	6.56±0.05^{d}	10.52±0.03^{c***}	11.37±0.08^{c}	12.17±0.10^{c***}	11.34±0.12^{c}	12.74±0.14^{c***}
	T_4	4.10±0.09^{a}	7.09±0.23^{a***}	7.45±0.11^{a}	8.40±0.20^{a**}	7.87±0.21^{a}	8.95±0.26^{a**}
Leaf area (cm^2)	T_1	54.13±2.84^{b}	218.20±9.26^{b***}	146.28±4.49^{b}	291.06±7.61^{c***}	197.14±4.76^{c}	311.18±10.59^{c***}
	T_2	54.77±2.00^{b}	211.61±9.66^{b***}	140.81±4.74^{b}	269.88±9.77^{b***}	181.17±6.82^{b}	294.78±7.04^{b***}
	T_3	83.61±3.90^{c}	266.33±5.66^{c***}	188.94±5.07^{c}	348.01±3.50^{d***}	246.46±10.75^{d}	381.02±6.91^{d***}
	T_4	33.08±1.63^{a}	129.82±3.68^{a***}	90.84±4.54^{a}	180.47±4.58^{a***}	106.04±4.69^{a}	205.11±4.62^{a***}
Plant spread (cm)	T_1	13.59±0.62^{b}	25.56±0.55^{b***}	22.22±0.89^{b}	28.82±0.82^{c***}	27.18±1.31^{b}	30.47±0.70^{b*}
	T_2	13.00±0.57^{b}	24.53±0.66^{b***}	21.58±0.74^{b}	27.28±0.71^{b***}	26.13±1.05^{b}	29.32±0.61^{b**}
	T_3	18.79±0.81^{c}	28.66±0.18^{c***}	26.07±0.28^{c}	31.52±0.9^{d***}	31.90±0.84^{c}	33.07±1.49^{c}
	T_4	11.04±0.55^{a}	21.30±0.91^{a***}	18.28±0.45^{a}	23.57±0.72^{a***}	22.23±0.76^{a}	25.42±1.07^{a***}
Leaf Chlorophyll content (cci)	T_1	16.18±0.67^{b}	26.64±1.44^{b***}	26.83±0.36^{b}	28.71±0.82^{b*}	30.85±0.65^{b}	32.13±0.37^{c*}
	T_2	15.35±0.78^{b}	26.05±0.78^{b***}	26.75±0.06^{b}	27.73±0.70^{b}	30.91±0.84^{b}	31.01±0.37^{b}
	T_3	18.35±0.65^{c}	29.80±0.33^{c***}	29.13±0.15^{c}	32.41±0.56^{c}	36.16±0.60^{c}	36.10±0.37^{d}
	T_4	10.83±0.44^{a}	21.68±0.93^{a***}	20.59±0.48^{a}	25.09±0.49^{a***}	23.56±0.67^{a}	26.55±0.16^{a**}
Leaf anthocyanin content (aci)	T_1	5.87±0.20^{b}	6.48±0.26^{b*}	7.48±0.19^{b}	8.65±0.09^{c***}	8.72±0.62^{b}	10.67±0.08^{b**}
	T_2	5.87±0.37^{b}	6.41±0.19^{b}	7.39±0.22^{b}	8.28±0.11^{b**}	8.20±0.17^{b}	10.47±0.23^{b***}
	T_3	6.52±0.29^{c}	7.22±0.09^{c*}	8.12±0.10^{c}	9.86±0.32^{d***}	11.68±0.51^{c}	11.92±0.15^{c}
	T_4	5.10±0.22^{a***}	3.68±0.12^{a}	5.59±0.36^{a}	6.15±0.14^{a}	5.91±0.26^{a}	7.91±0.07^{a***}

HA: high altitude and LA: low altitude, Values presented as means ± SD, T1: FYM @ 150 q/ha, T2: Azotobacter @ 8.6 kg/ha, T3: FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T4: control. cci: chlorophyll content index, aci: anthocyanin content index, DAT: Days after transplanting.

Values in columns different lowercase letters (small alphabet) indicate significantly different; P≤0.05, Duncan's multiple range test between treatments.

Mean values in each row (between groups) showed significant differences by independent t-test (***p≤0.001; **p≤0.01 and *p≤0.05).

Table 3. Three-way ANOVA for location, treatment, and days after transplanting and their interactions on morphology parameters of knol khol cultivar white vienna

Source	df	*F*	Source	df	*F*
		Plant Height			**Plant spread**
ALT	1	1543.73***	ALT	1	1145.97***
TRE	3	322.809***	TRE	3	300.023***
DT	2	502.382***	DT	2	699.503
ALT×TRE×DT	6	3.065*	ALT×TRE×DT	6	0.64
		Number of leaves			**Leaf chlorophyll content**
ALT	1	535.83***	ALT	1	1045.31***
TRE	3	257.550***	TRE	3	598.453***
DT	2	1013.13***	DT	2	1597.95***
ALT×TRE×DT	6	3.191**	ALT×TRE×DT	6	3.716**
		Leaf length with petiole			**Leaf anthocyanin content**
ALT	1	1583.16***	ALT	1	235.257***
TRE	3	301.554***	TRE	3	560.814***
DT	2	484.419***	DT	2	1116.46***
ALT×TRE×DT	6	0.77	ALT×TRE×DT	6	18.459***
		Leaf width			
ALT	1	2147.36***			
TRE	3	1167.53***			
DT	2	2528.52***			
ALT×TRE×DT	6	7.845***			
		Leaf area			
ALT	1	7781.15***			
TRE	3	1243.83***			
DT	2	1854.06***			
ALT×TRE×DT	6	6.273***			

df: Degrees of freedom, *F*: F ratio, *$p \le 0.05$; **$p \le 0.01$; ***$p \le 0.001$. ALT: Altitude, TRE: Treatment, DT: Days after transplanting

Tasble 4. Comparative effect of location and treatments on yield attributes of knol-khol cultivar white vienna

ALT	TRE	Knob equatorial diameter (mm)	Knob polar diameter (mm)	Knob weight / plant (g)	Yield (q/ha)
HA	T_1	81.76±2.37^{c**}	84.94±0.35^{c}	259.97±8.22^{b}	257.72±7.72^{b}
	T_2	73.78±0.73^{b***}	77.82±2.67^{b}	253.03±11.98^{b}	250.21±11.74^{b}
	T_3	90.64±1.89^{d}	89.35±1.24^{d}	365.03±9.62^{c}	370.99±8.90^{c}
	T_4	65.79±2.24^{a}	65.08±2.21^{a}	196.2±5.38^{a}	196.71±7.26^{a}
LA	T_1	77.24±1.13^{b}	82.34±0.56^{b}	290.6±6.12^{b***}	360.49±5.63^{b**}
	T_2	78.86±0.40^{b}	80.96±1.04^{b}	285.72±5.44^{b***}	356.58±4.33^{b***}
	T_3	89.89±0.08^{c}	90.68±1.28^{c}	424.86±11.83^{c***}	508.64±18.55^{c***}
	T_4	64.99±1.29^{a}	62.77±1.4^{a}	168.53±3.67^{a***}	204.11±1.86^{a}
ALT		NS	NS	***	***
TRE		***	***	***	***
ALT×TRE		***	*	***	***

HA: high altitude and LA: low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1: FYM @ 150 q/ha, T_2: *Azotobacter* @ 8.6 kg/ha, T_3: FYM @ 150 q/ha+ *Azotobacter* @ 8.6 kg/ha and T_4: control. ALT×TRE: interaction of altitude and treatment.

Values in columns different lowercase letters (small alphabet) indicate significantly different; $P\leq0.05$, Duncan's multiple range test between treatments.

Mean values in each column (between groups) showed significant differences by independent t-test. Two-way ANOVA was applied to visualise the interaction between altitude and treatments (***$p\leq0.001$; **$p\leq0.01$ and *$p\leq0.05$).

Table 5. Comparative Effect of location and Treatments on Nutritional Attributes of Knol-Khol Cultivar White Vienna

ALT	TRE	TSS (°B)	TA (%)	Total Carbohydrate (μg/g)	Crude Protein (g/100g)	Crude Fat (%)	Dietary Fiber (%)	Ash (%)
HA	T_1	8.05±0.09^{b***}	0.23±0.01^{b**}	59.74±0.16^{b***}	16.05±0.31^{b***}	0.23±0.01^{b}	10.61±0.02^{b***}	11.55±0.02^{b***}
	T_2	8.05±0.05^{b***}	0.25±0.02^{b**}	60.12±0.17^{c***}	18.41±0.10^{c***}	0.27±0.01^{c}	10.57±0.09^{b***}	11.61±0.03^{c***}
	T_3	9.00±0.10^{c***}	0.33±0.01^{c***}	65.51±0.28^{d***}	19.40±0.12^{d***}	0.31±0.02^{d*}	11.09±0.17^{c**}	11.95±0.04^{d***}
	T_4	7.27±0.20^{a**}	0.18±0.01^{a**}	47.13±0.03^{a***}	14.72±0.20^{a***}	0.20±0.01^{a}	9.38±0.19^{a}	11.18±0.04^{a***}
LA	T_1	6.85±0.00^{c}	0.18±0.01^{b}	57.39±0.27^{b}	13.14±0.08^{b}	0.23±0.01^{b}	9.60±0.10^{b}	12.42±0.01^{c}
	T_2	6.63±0.06^{b}	0.18±0.00^{b}	57.61±0.15^{b}	14.12±0.34^{c}	0.25±0.01^{c}	9.64±0.07^{b}	12.21±0.03^{b}
	T_3	7.62±0.12^{d}	0.27±0.01^{c}	63.54±0.12^{c}	15.62±0.31^{d}	0.28±0.01^{d}	10.33±0.03^{c}	12.80±0.04^{d}
	T_4	6.42±0.08^{a}	0.12±0.02^{a}	45.32±0.13^{a}	11.63±0.14^{a}	0.21±0.01^{a}	9.14±0.04^{a}	12.01±0.03^{a}
ALT		***	***	***	***	*	***	***
TRE		***	***	***	***	***	***	***
ALT×TRE		***	NS	*	***	**	***	***

HA: high altitude and LA: low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T1: FYM @ 150q/ha, T2: Azotobacter @ 8.6 kg/ha, T3: FYM @ 150q/ha+ Azotobacter @ 8.6 kg/ha and T4: control. ALT×TRE: interaction of altitude and treatment. TSS: Total soluble solid, TA: Titratable acidity.

Values in columns different lowercase letters (small alphabet) indicate significantly different; $P\leq0.05$, Duncan's multiple range test between treatments.

Mean values in each column (between groups) showed significant differences by independent t-test. Two-way ANOVA was applied to visualise the interaction between altitude and treatments (***$p\leq0.001$; **$p\leq0.01$ and *$p\leq0.05$).

and K. In the present study, T_3 treatment resulted in the highest ash content at both locations (Table 5). However, among altitudes, T_3 treatment was found to result in higher ash (0.85-fold) at LA as compared to HA-grown knob. The interaction between altitudes and treatments (ALT×TRE) was also found to be significant ($p<0.05$). The knobs appear to absorb higher inorganic components (K, Ca, Cl, and Na) from the soil at LA, and These outcomes parallel the results reported by Kumar[32] *et al.,* (2015).

3.3.5 Dietary Fiber Content

Dietary fiber is the complex carbohydrate portion of a plant that is not readily digested, thus remains bound to the surface of the human colon, and is wholly or partially fermented in the large intestine[37]. Based on our study, among all the treatments, T_3 treatment resulted in the highest content of dietary fiber at both locations (Table 5). However, T_3 treatment at the HA region among altitudes resulted in higher dietary fiber content (78-fold) than the grown sample. This has a direct correlation with higher photosynthetic rates at HA, as explained earlier. These findings are consistent with earlier studies on butternut squash[38, 39].

4. CONCLUSION

The bio-organic potential is being investigated worldwide to minimise the consumption of chemical fertilisers and to develop eco-friendly, sustainable agricultural alternatives. In this direction, our study has elaborated that using FYM and *Azotobacter* alone or in combination significantly improves the morphology and nutritional composition of *B. oleracea* L. var. *gongylodes* vegetable cultivated at HA (Leh) *vs.* LA (Chandigarh). The study's key finding is that combining FYM and *Azotobacter* at high elevations can lead to an enrichment of knob yield, TSS, titratable acidity, crude protein, crude fat, dietary fiber, and total carbohydrate content in plants, as demonstrated in the T_3 treatment group. Therefore, organic manure and biofertiliser are being recommended for growers to produce high-quality knol-khol knobs at higher elevations to obtain maximum food and nutritional security advantages under challenging environmental conditions.

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