BMC Plant Biology

RESEARCH Open Access

The potential biofortification role of *Actinopolyspora* sp. JTT-01 in enhancing the yield and tissue chemical composition of caraway plants

Ahmed M. Mahmoud[1*], Mohamed S. Sheteiwy[2*], Ali El-Keblawy[3], Zaid Ulhassan[4], Maha H. Khalaf[1], Hussein S. Mohamed[5], Mohammad K. Okla[6], Amal Mohamed AlGarawi[6], Ahmed M. El-Sawah[7], Enas S. Ahmed[1] and Ahmed M. Reyad[1]

Abstract

The need for improving plant production, nutritional value, and medicinal applications has become increasingly important due to the growing global population. The caraway (*Carum carvi* L) plant has been recognized for its broad range of nutritional and therapeutic uses. Consequently, this study aimed to increase caraway seeds' nutritional and biological value. To achieve this, the *Actinopolyspora* sp. JTT-0 strain, isolated from the medicinal plant *Tephrosia purpurea*, was investigated for its potential biofortification role to enhance caraway yield and quality. Our results revealed significant improvements ($p < 0.05$) in various physical parameters, such as seed yield, pod length, and bulk density, in the treated seeds compared to the controls. Along with the yield increase, there were notable elevations in primary metabolites such as total sugars, proteins, and amino acids. Furthermore, secondary metabolites, including essential oils (EOs), alkaloids, steroids, phenols, and vitamins (e.g., tocopherol and ascorbic acid), also showed significant increases. Notably, the EO constituents showed varying levels of enhancements, with the highest increases in β-pinene (186.2%) and carvacrol (49.2%). Moreover, the treated seeds exhibited improved biological activity, as evidenced by their anti-oxidant (anti-lipid peroxidation and DPPH assays) and anti-microbial properties compared to the controls. The study reported a positive biofortification effect of the *Actinopolyspora sp.* JTT-01 strain on enhancing caraway seed's quality and yield. However, additional field trials are needed to evaluate the commercial biofertilization capacity of this strain for caraway and other plants.

Keywords Actinobacteria, Caraway seeds, Endophytes, Essential oils

*Correspondence:
Ahmed M. Mahmoud
ahmed.mahmoud@science.bsu.edu.eg
Mohamed S. Sheteiwy
msalah@uaeu.ac.ae

Full list of author information is available at the end of the article

Introduction

Caraway (*Carum carvi* L) is an aromatic plant of the Apiaceae family native to Asia, Europe, and Africa. It is a spice grown for centuries and is renowned for its wide range of nutritional and medicinal benefits. Caraway seeds are a valuable source of nutrition, containing lipids, vitamins, minerals, essential oils (carvacrol, α-pinene, and p-cymene), proteins, phenolic acids, flavonoids, alkaloids, fiber, terpenoids, and numerous other nutraceutical compounds [1–5]. This diverse composition makes caraway seeds highly valuable for both culinary and medicinal applications. Caraway EOs have been shown to enhance antioxidant enzyme activity, reduce colon cancer cell proliferation and white blood cell malfunctions, exhibit anti-diabetic effects, and demonstrate strong diuretic properties without renal toxicity in rats [4–9]. Beyond their medicinal properties, caraway seeds have potential applications in agriculture and food science, including seed priming techniques for drought tolerance [10] and insecticidal properties for pest control [11].

There have been global efforts to develop sustainable agriculture for enhanced production and efficient uses of resources while improving the nutritional value of crops [12]. Economically, it is beneficial to seek innovative ways to improve agriculture without synthetic agrochemicals [13]. Organic agriculture offers better yields concerning the nutritional value of fruits and vegetables [14, 15]. Growth-promoting bacteria (e.g., endophytic bacteria) are a promising approach to organic farming as biofertilizers to plant seeds to support nutrient availability and uptake [16]. Thus, inoculation with these bacteria could serve as a renewable and clean choice to avoid the problems of synthetic fertilizers [17] and improve the quality and yield of many plants, especially those of economic value such as wheat, rice, and maize [18, 19]. Diverse bioactive endophytic actinobacterial genera such as *Microbispora*, *Micromonospora*, and *Streptomyces* have been isolated from various plant species [20]. Therefore, several bacteria were isolated from multiple medicinal plant species, including *Tephrosia purpurea*, a perennial halophyte herb from the Fabaceae family. Native to tropical and subtropical regions, *T. purpurea* contains bioactive compounds with antioxidant, anti-inflammatory, and antimicrobial properties and has traditionally been used for treating respiratory, gastrointestinal, and skin ailments [21]. As a medicinal and halophyte plant, it thrives in saline environments, making it an excellent candidate for hosting unique endophytic microorganisms with potential biofortification benefits. In this context, leaf endophytes can promote plant growth and produce beneficial metabolites, such as phytohormones (e.g., auxins) [22].

Among the isolated bacteria, *Actinopolyspora* sp., isolated from *T. purpurea*, exhibited strong antioxidant bioactivity and growth-promoting ability due to its capacity to produce phytohormones, such as auxin [22]. *Actinopolyspora* species also produce bioactive compounds with significant pharmacological effects [23]. The genus *Actinopolyspora* that belongs to the phylum *Actinobacteria*, was first described in 1975 [24–26]. In this context, actinobacteria were reported to promote plant growth through various mechanisms like phosphate solubilization, nitrogen fixation, and phytohormone production [19]. Besides, Actinobacteria are sources of antimicrobial, immunosuppressant, and anti-oxidant agents, and versatile enzymes, and plant growth hormones [27, 28]. Notable species include *Act. halophila*, *Act. iraqiensis*, *Act. alba*, *Act. erythraea*, *Act. xinjiangensis*, *Act. algeriensis*, *Act. saharensis*, and *Act. salinaria* [29]. Polyphasic approaches, including 16 S rRNA phylogenetic analysis and genomic DNA GC content, have identified novel species such as *Actinopolyspora salinaria* and *Phytoactinopolyspora mesophila* [25, 26].

Given that we isolated our bacteria from a medicinal plant, this study aimed to evaluate how *Actinopolyspora* sp. JTT-01 could improve the growth, yield, and chemical composition of medicinal plants like caraway. Although studies on the biofortification effects of actinobacteria are increasing [30, 31], the impact of *Actinopolyspora* sp. on caraway seeds remains unexplored. This study is the first to investigate this strain-plant combination, highlighting its potential to enhance caraway seed yield, nutritional value, and metabolites, contributing to sustainable agriculture by improving crop productivity and quality.

Materials and methods

Bacterial isolation

For bacterial isolation, multiple endophytic bacteria were extracted from various medicinal and stress-tolerant plants, including *T. purpurea*. The leaves of these plants were used as the primary source for bacterial isolation and were washed and disinfected for 3 min with ethanol 70% then mercuric chloride 0.1% for 5 min and finally washed 5 times with sterilized dH_2O. A physiological saline solution (2 ml) was mixed with the crushed leaves and 100 µl of this mixture was spread into yeast-extract mannitol agar (YEMA) and incubated at 30 °C for 3 days. Colonies were purified [31] and their colony characteristics were observed. A bacterial suspension in ddH_2O distilled water at OD600 was used to inoculate the surface-sterilized caraway seeds in triplicate.

Among the isolated bacteria, *Actinopolyspora* sp., isolated from *T. purpurea*, exhibited strong antioxidant bioactivity (FRAP), antioxidant production (phenolics and flavonoids), and growth-promoting ability due to its capacity to produce phytohormones (e.g., auxin) was selected (Table 1).

Table 1 The biological (ferric reducing antioxidant capacity (FRAP)), production of plant growth regulators (indole-3-acetic acid (IAA), phenolics and flavonoids, and solubilization of nutrients of Actinopolyspora sp. JTT-0

Antioxidant activity (FRAP) (µmole Trolox g – g DW)	**75.0 ± 4.5**
Total flavonoids (mg quercetin equivalent (QE) g-1 DW)	17.2 ± 2.1
Total phenols (mg gallic acid equivalent (GAE) g-1 DW)	42.7 ± 5.3
IAA (mg g-1 DW)	6.3 ± 0.7
Ammonia (mg L-1)	7.2 ± 0.7
Phosphate (mg L-1)	163.0 ± 9.7

Values are represented by means ± standard deviations of three independent replicates

Molecular identification

The DNA extracted from our strain, according to Chen et al. [32], was used for amplifying the 16S rRNA gene in a 25 µL PCR mix included $MgCl_2$ buffer (1.5 mM), 0.5 µM of each dNTP (0.2 mM), Taq DNA polymerase (0.625 U), and the genomic DNA (5 µL) with primer pairs 27F (5'-CAGAGTTTGATCCTGGCT-3´) and 1492R (5´-A GGAGGTGATCCAGCCGCA-3´) [33]. The PCR conditions were an initial denaturation at 95 °C for 5 min, 35 cycles of denaturation for 45 s at 95 °C, annealing for 45 s at 49 °C, extension at 72 °C for 90 s, and a final extension at 72 °C for 7 min. Then the PCR products were purified, sequenced, and analyzed by the GenBank database and the blast program to identify the isolate.

Plant materials and experimental setup

Seeds of caraway (*Carum carvi* L.) plants were obtained from the Agricultural Research Center, Dokki, Giza, Egypt. A pot experiment was conducted using a completely randomized design with two treatments. The treatments consisted of two biofertilization levels: one with no biofertilization and the other with biofertilization using *Actinopolyspora sp.* JTT-01. After preliminary screening among other 9 endophytes from different medicinal plants, *Actinopolyspora* sp. JTT-01 displayed significant accumulations in yield and metabolite of caraway yield and therefore was selected for this study. The biofertilization treatment involved culturing the bacterial strain in Nutrient Broth at 29 °C for 24 h, followed by centrifugation at 5000 rpm for 15 min. The pellet was washed and adjusted to a density of 10^{-6} CFU mL^{-1} (OD 600 nm = 0.6–0.7). This suspension was applied to the soil before planting and added to the pots biweekly. Seeds of caraway were uniformly chosen, sterilized for 20 min at 5% v/v with Na-hypochlorite, and then wholly cleaned with distilled water. Then they were sown in pots (60 cm X 45 cm) containing 1.5 kg of loamy soil with organic compost in a 1:1 ratio, ensuring the mixture reached 65% of soil water content. This soil was then used to cultivate caraway seeds, which were divided into two groups: one group was treated with *Actinopolyspora sp.* JTT-01, while the other group remained untreated as a control. Each treatment included 6 pots, with each pot containing 4 plants. Every 2 pots represented a biological replicate, resulting in three biological replicates per treatment. All pots were weighed daily to maintain a water level above 65% of soil water content. The pots were placed in a growth chamber with 60% humidity, 250 µmol PAR m^{-2} s^{-1}, 22/18 °C air temperature, and 16/8 hour day/night photoperiod. The experiment was repeated twice. The harvest was done 157 days, typically in its maturation or seed-ripening stage, after sowing and growth parameters were assessed. For further analyses, seeds were ground in liquid N [30].

Physical parameters measurements

Seed yield was determined by collecting and weighing all mature seeds from each plant. Pod length was measured using a ruler. Bulk density was calculated by weighing a known volume of seeds using a graduated cylinder and dividing the mass by the volume to obtain the density (g/cm^3).

Minerals content determination

Seeds were dried in the oven and 0.1 g was extracted in a mixture of HNO_3 and H_2O (5:1 v/v) in an oven. To detect the concentrations of minerals (K, P, Ca, Mg, Na, Fe, Zn, Cu, and N) in the extract, mass spectroscopy (ICP-MS, Bremen, Germany) was used [34].

Nutrient analyses

The sugar content in the dry seeds was determined by the method of Hatanaka and Kobara [35]. Seeds were ground in liquid nitrogen and subjected to acid hydrolysis (0.2 M sulfuric acid). After boiling the sample, the solution is neutralized, and filtered, and the resulting sugars are quantified using the spectrophotometric methods at 600 nM. For lipid content extraction, a mixture (2:1, v/v) of chloroform and methanol was used as described in Bligh and Dyer [36]. Ethanol was used to precipitate the fiber content which was then determined as described in the "Official Methods of Analysis" from 1995 [37]. The total content of protein, alkaloids, and saponins was also measured. Total proteins were quantified using the modified semimicro-Kjeldahl method [38]. Samples were digested in sulfuric acid, with nitrogen converted to ammonium sulfate, followed by distillation, trapping, and titration. The nitrogen content was used to calculate protein levels using a 6.25 conversion factor, providing reliable protein estimates. The total alkaloid content was determined using a Bismuth nitrate pentahydrate calibration curve [39]. Methanolic extracts at pH 2-2.5 were mixed with Dragendroff's reagent and centrifuged at 5000 rpm for 10 min. The precipitate was washed twice with methanol, treated with disodium sulfide solution, and centrifuged again for 10 min at 5000 rpm. The residue was

dissolved in concentrated nitric acid, diluted with Milli-Q water, and mixed with 3% thiourea solution. Absorbance was measured at 435 nm against a blank of nitric acid and urea. Saponin extraction and quantifications were performed according to [40]. Briefly, ground, dried seeds were extracted in petroleum ether, shaken for 4 h, and the solvent removed using a vacuum rotary evaporator. Saponin was then extracted from the residues in 80% aqueous methanol. The extract was filtered and stored at 4 °C. A spectrophotometric method was employed for quantification, utilizing vanillin as a reagent. The absorbance of the reaction mixture was measured at 544 nm using a UV–Vis spectrophotometer (Shimadzu UV-160 A PC, Shimadzu Corporation, Kyoto, Japan). A standard curve was constructed using known concentrations of crude soya saponin, and the saponin content of the samples was expressed as mg soya saponin/100 g sample.

Analysis of amino acid profile

For obtaining the individual amino acids, fresh caraway seeds (0.5 g) were homogenized in a mixture of ethanol and water in MagNALyser equipment. A centrifugation was done at 15,000 rpm for 25 min and the supernatant was then dried and redissolved in chloroform. The aqueous phase was separated and filtered with a 0.22 µm filter paper. The amino acid concentrations were determined in a HPLC system (Waters Acquity UPLC-tqd) supported with a BEH amide 2.1 × 50 column [41], and the composition of amino acids was determined by the corresponding peak area of each standard.

Vitamin analysis

The reversed-phase HPLC was used to analyze the tocopherols. For ascorbate, it was combined with hexane and metaphosphoric acid in the MagNALyser (Roche, Vilvoorde, Belgium), and then centrifuged at 4 °C and 14,000 g for 20 min. Also, for separating thiamine and riboflavin, a reverse-phase C18 column with ethyl alcohol and water (mobile phase) was used as previously described [42]. The HPLC system from Agilent Technologies (Santa Clara, CA, USA) was used for the separations.

Determination of total phenolic and flavonoid contents

Fresh seeds were dried in air and 100 mg was homogenized in 1 ml of ethanol (80%). Then, centrifugation was done for 20 min at 4 °C. Ethanol (80%) was also used for the flavonoid content extraction and the microplate method with quercetin (as a standard) was used for determining the total flavonoids [43]. For flavonoid content, a modified $AlCl_3$ colorimetry with quercetin (standard) was used. For the total phenolic content, the Folin-Ciocalteu assay, with gallic acid (standard), was used [44].

Analysis and determination of the EO levels and metabolism

To extract the EOs, fresh seeds were air dried, and 15 g was distilled in steam for 3 h using a Clevenger-type apparatus [45]. This method allows for efficient extraction of volatile compounds from the seeds. Concentrations were measured in percentages using the GC/MS analysis [46]. The contents of the seed's EOs were determined by a thermoquest GC–MS instrument (EI mode at 70 eV), equipped with a DB-1 fused silica capillary column (60 m × 0.25 mm id., film thickness 0.25 mm). The temperatures of the detector and injector were 300 °C and 250 °C, respectively. The temperature started at 40 and increased to 250 °C at 4 °C min $^{-}$1, and finally kept constant for 10 min at 250 °C. The carrier gas (He) was used at a flow rate of 1.1 mL min^{-1}. The EOs were identified and compared, upon their mass spectra, with those in the NIST library [47]. Details on the retention time (RT), Kovats index (KI), and % of each compound in the essential oil are presented in Supplementary Table 1.

Assessment of biological activities

Seeds were ground and 50 mg of the powder was added to 10 mg of the investigated strain and extracted in ethyl alcohol (80% v/v) 3 times in a MagNALyser (Roche, Vilvoorde, Belgium) device. Then, centrifugation at 14.000 rpm for half an hour at 4 °C and the supernatant was used for the anti-oxidant and anti-microbial assays as below:

In-vitro anti-oxidant activity

To investigate the anti-oxidant properties of caraway seed extracts, 300 mg of seeds was added to 3 ml of ethyl alcohol (80%) till homogenization. The extracts were then mixed with DPPH dissolved in 95% ethyl alcohol (0.5 mL of 0.25 mM) [48]. The mix was then incubated for half an hour at 30 min, and 2 ml of ddH_2O was added. The absorbance (517 nm) was then measured with a microplate (Synergy Mx, Promega, Madison, WI, USA) reader [49]. A trolox calibration curve (0 to 650 µM) was utilized to calculate the anti-oxidant activity. For lipid peroxidation, the MDA reaction, in the treated and control seed samples, was detected. In the assay, MDA in the sample reacted with thiobarbituric acid (TBA) to form an MDA-TBA adduct, which can be quantified calorimetrically (OD = 532 nm) or fluorometrically (Ex/Em = 532/553 nm) [50].

Anti-microbial assay

Ethanolic extracts of caraway seeds were used to test their activity against some bacterial and fungal pathogens using the disc diffusion method and the Muller-Hinton agar medium. Suspensions from each pathogen (10^6 CFU/ml) were distributed evenly onto the medium.

Disinfected filter paper discs loaded with seed extracts (5 µg/disc) were settled gently on the inoculated agar surface and discs with ethanol were used as controls. Media with discs were incubated for 24 h at 37˚C and the formation of inhibition zones around the discs was observed and measured. For anti-fungal activity, the well diffusion method in which wells in the agar were loaded with the seed extracts and plates were incubated at 30 ˚C, and the inhibition zones around the wells were measured to determine the anti-fungal activity of the extracts [51].

Statistical analysis

The mean analysis of the collected data was performed by calculating the value of each variable across replicates. Data points from each treatment group were averaged to obtain the mean values. Multiple software applications were employed to examine the data, including R software, Microsoft Excel 365, SPSS, and GraphPad Prism, version 8.4.2. The normality and homogeneity of variances were evaluated using the Kolmogorov-Smirnoff and Levene's tests, respectively using SPSS, version 20.0 (IBM Corporation, Armonk, NY, USA). The analysis was conducted using R software (Vienna, Austria), which was loaded with the required data package [46]. By incorporating the "factoextra" and "FactoMineR" packages in R, it was possible to analyze a dataset containing continuous variables using principal component analysis (PCA) [52]. Radar plots, with statistical significance defined as $p < 0.05$ and non-statistical significance defined as $p > 0.05$.

Results and discussion

Molecular identification of the isolate

The 16 S rRNA gene sequencing is a reliable and precise tool for identifying and classifying endophytic actinobacteria and valuable insights into the diversity and phylogenetic relationships of actinobacteria, enhancing the comprehensive understanding of their taxonomy [53]. The 16 S rRNA gene sequence BLAST analysis revealed that the isolate was related to *Actinopolyspora* species. Phylogenetic tree construction based on 16 S rRNA similarity confirmed the identification as *Actinopolyspora* sp., specifically clustering with *Actinopolyspora* sp. VITSDK2 EU551237 with 100% similarity (Fig. 1). Actinobacteria are significant plant endophytes identified in various medicinal plants. For example, Passari et al. [54] identified 42 endophytic actinobacteria, mostly *Streptomyces*, from 7 medicinal plants. Also, Qin et al. [55] conducted a comprehensive study using both cultivation and culture-independent methods and identified 312 actinobacteria affiliated with 21 genera, including rare ones. *Actinopolyspora* sp. has been previously isolated from some medicinal plants such as *Mentha arvensis*, *Aloe vera*, *and Ocimum sanctum* [56]. The findings suggest that medicinal plants harbor unexplored actinobacteria with

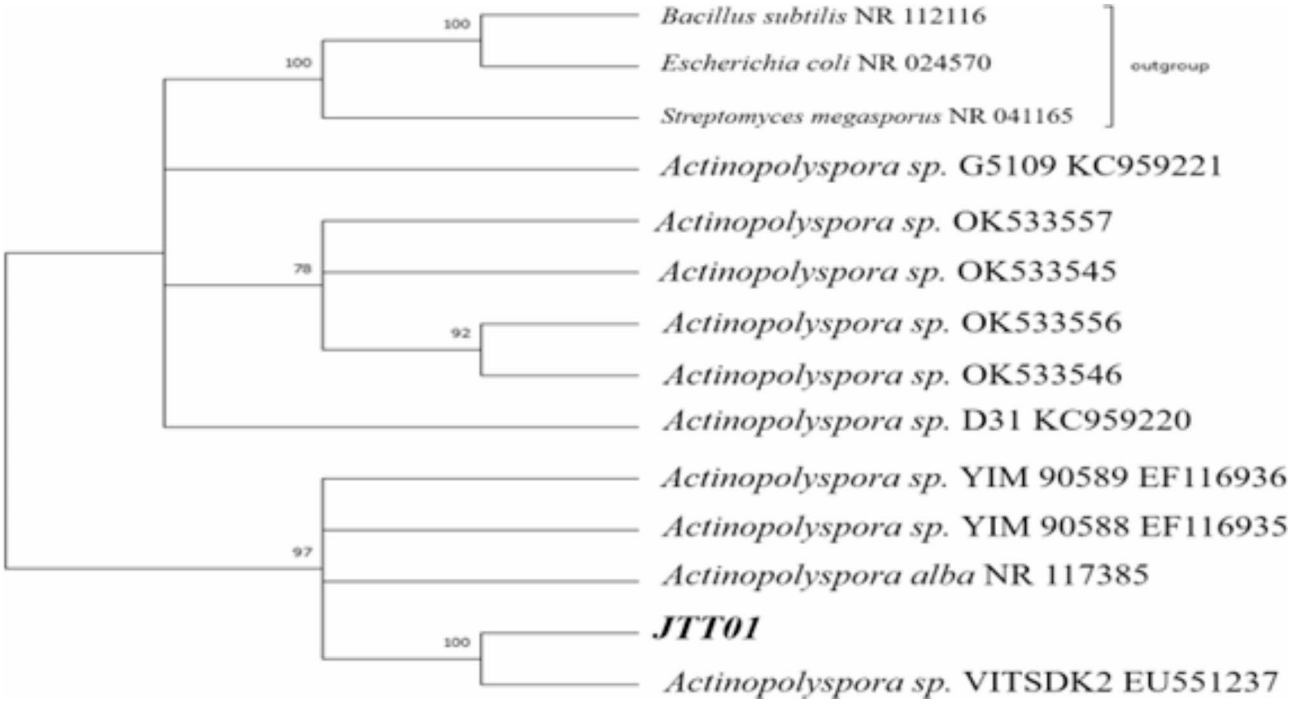

Fig. 1 Neighbor-joining-based phylogenetic tree of the isolate *Actinopolyspora* sp. JTT-01 based on the 16 S rRNA gene sequences. The bootstrap values for each node were determined from 100 replicates. *Escherichia coli*, *Streptomyces megaspores*, *Actinomadura hibisca* JCM 9627, and *Bacillus subtilis*, were used as the outgroup for this analysis

promising bioactive properties, offering new opportunities for bioprospecting and natural product discovery.

Yield and physical properties of the *Actinopolyspora sp* JTT-01-treated caraway seeds

The statistical (radar) plot (Fig. 2) demonstrates the mean values of the investigated seed parameters in *Actinopolyspora*-treated seeds and controls. Our results showed that *Actinopolyspora* sp JTT-01 significantly increased ($p \leq 0.05$) the bulk density, pod length, and seed yield by 60, 51.6, and 67.8%, respectively, and caused nonsignificant increases ($p > 0.05$) in length, mass, width, and thickness of the treated seeds compared to controls. Likewise, earlier studies have consistently shown that inoculating seeds with actinobacteria can promote the growth of various plant species [57]. Like our findings, seeds treated with *Nocardiopsis* sp. exhibited enhanced biomass accumulation, improved seed germination, and increased shoot and root length [31]. Besides, increases in seed yield and dry weight of aniseeds were observed after treatment with an actinobacterial *Actinomycetota* sp. JW0824 strain compared to untreated controls [30]. This enhancement is achieved through several mechanisms, including the production of growth hormones, improved nitrate uptake, and phosphate solubilization [58]. Consistently, *Actinopolyspora* sp. JTT-01 exhibited strong growth-promoting ability due to its capacity to produce phytohormones, such as auxin [22]. In addition to auxin production, this strain demonstrated a significant ability to synthesize phenolic compounds and flavonoids and showed effective phosphate solubilization activity, enhancing phosphorus availability to plants, a critical nutrient for root development and overall plant growth (Table 1). These attributes make *Actinopolyspora* sp. JTT-01 a promising candidate for biofertilization and sustainable agriculture, offering a multi-faceted approach to improving crop productivity.

The impact on seed mineral, ash, and crude fiber contents of *Actinopolyspora* sp. JTT-01-treated caraway seeds

For mineral content, our results revealed that seeds treated with *Actinopolyspora* sp. JTT-01 strain accumulated most minerals (K, Ca, N, Fe, Mg, Na, Cu, and Zn) higher than controls (Fig. 3). A clear separation is observed for most minerals towards the *Actinopolyspora* sp. JTT-01-treated caraway seeds along the PCA vertical axis with 53.3% of the total variation (Fig. 3). The improved mineral availability leads to enhanced chlorophyll and photosynthesis which results in improved primary metabolism as reported herein in this study. Research has reported that actinobacterial inoculation increases the availability of minerals in the soil and several plant species [59–62]. The enhanced mineral accumulation after *Actinopolyspora* sp. JTT-01 treatment might be correlated to enhanced mineral availability in soil by our strain through, modified soil pH, and improved exchange capacity of cations [63, 64]. In a similar vein, El-Tarabily et al. [65] found that *Micromonospora endolithica* enhanced phosphate solubilization, leading to increased soil levels of magnesium, iron, zinc,

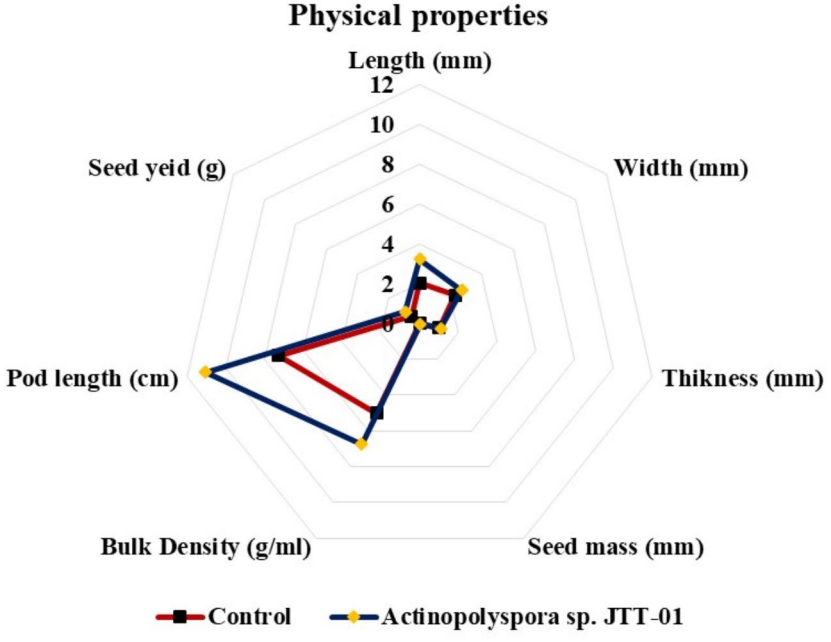

Fig. 2 Radar plot for yield and physical properties of the caraway seeds treated with *Actinopolyspora* sp. JTT-01 and their corresponding untreated controls. Values are Mean ± SE

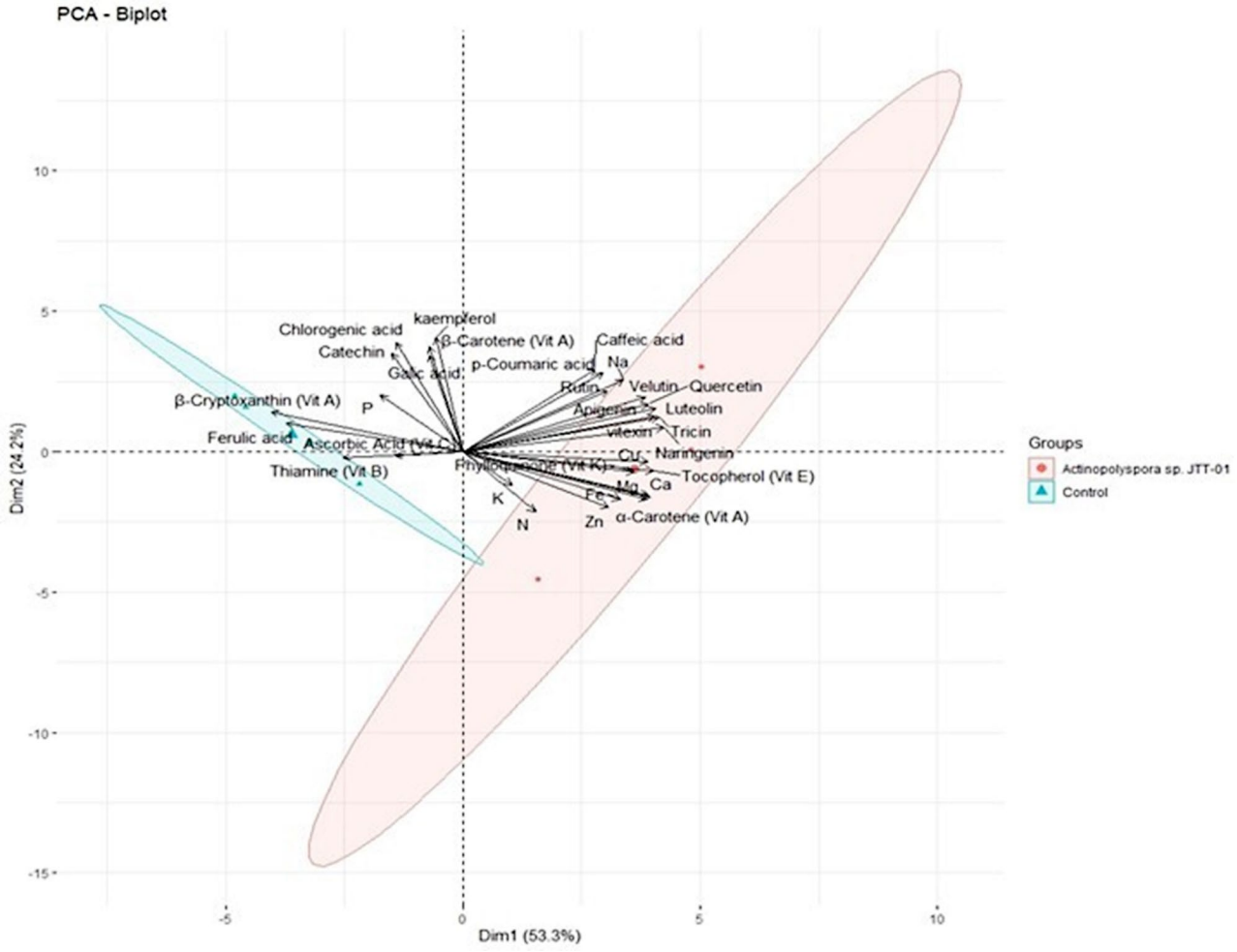

Fig. 3 Principal component analysis (PCA) of vitamin, mineral, and phenolic-flavonoid profiles of control and *Actinopolyspora* sp. JTT-01-treated caraway seeds, with 53.3% total variance described along the first axis (Dim 1) and 24.2% along the second axis (Dim 2)

and potassium. Additionally, actinobacteria can boost plant mineral accumulation by promoting root growth, improving water uptake, and enhancing nutrient absorption [66]. For example, Boondaeng [67] found that *Streptomyces lavenduligriseus* produces phytohormones like IAA, stimulating root elongation and branching, thus enhancing root surface area for nutrient uptake. Additionally, Actinobacteria can solubilize essential nutrients, particularly phosphorus and potassium, making them available to plants [68]. They also increase iron availability by producing siderophores that chelate iron [69]. Moreover, the increase in phenolic compounds reported herein (Fig. 3) in the treated caraway seeds could correlate to the increased mineral availability because of the chelating properties of phenols as previously described [70].

The relationship between increases in vitamins and minerals in *Actinopolyspora* sp. JTT-01-treated seeds was analyzed by Pearson's correlation coefficient (Fig. 4) which revealed significant positive correlations ($p < 0.05$) between some vitamin pairs (like tocopherol- α-carotene), mineral pairs (like Ca-K and NA-Mg), and vitamin-mineral pairs (like P-ascorbic acid and Na- α-carotene). Also, significant negative correlations were observed between vitamin pairs (like α-carotene-thiamine), vitamin-mineral pairs (like Cu- ascorbic acid), and mineral pairs (like P-K, Zn-P, and Mg-P). Similarly, research has reported that vitamin and mineral contents are closely correlated and mutually support each other's functions which is beneficial for plant health, growth, and development. For instance, vitamin C (ascorbic acid) was found to facilitate Fe uptake by reducing it from (Fe^{3+}) into (Fe^{2+}) [71]. Besides, Mg is a co-factor for many critical enzymes in the biosynthesis of vitamins like B6 and E. Besides, the vitamins C together with Zn were found to help plants synthesize anti-microbial compounds to enhance plant resistance against pathogens [72]. Besides, specific minerals are crucial in the biosynthesis of some vitamins. For instance, Co was essential for synthesizing vitamin B12 [73]. Furthermore, minerals like iron play a

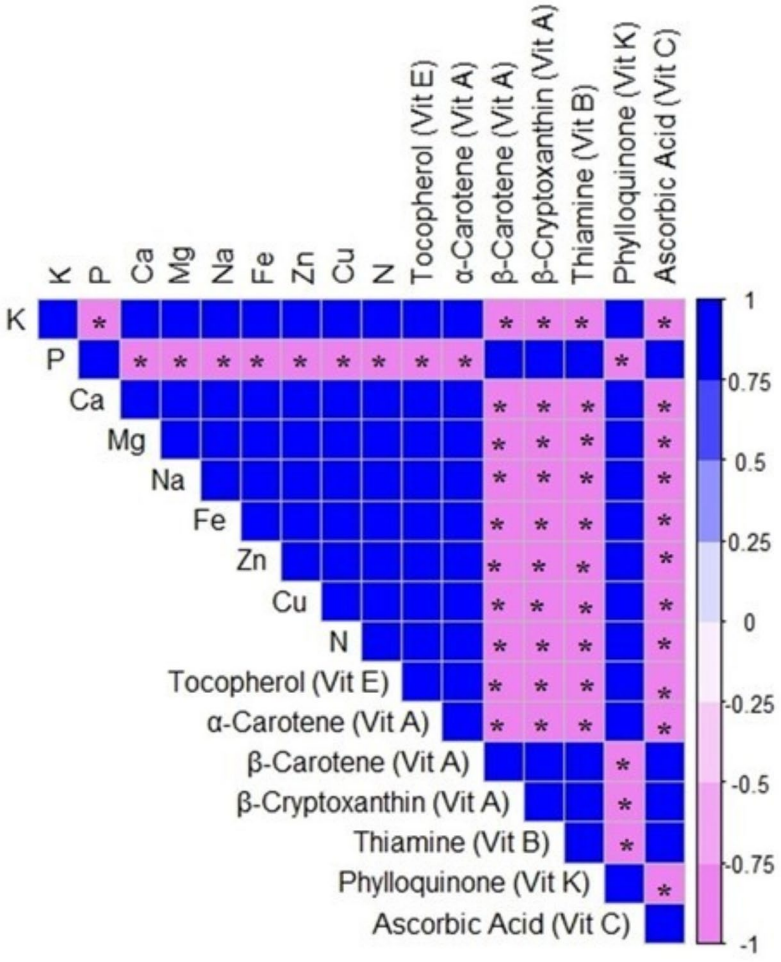

Fig. 4 The correlation coefficient of minerals, and vitamins in caraway seeds treated with *Actinopolyspora* sp. JTT-01 strain, by Pearson's correlation analysis

role in the biosynthesis of riboflavin (vitamin B2), linking mineral nutrition to vitamin production [74]. In a recent study [75], broccoli microgreens' total Vit C content was positively correlated with K levels and negatively with other minerals such as N, P, and Mg. Understanding these interactions is essential for improving effective agricultural practices to enhance crop nutritional quality.

Enhanced primary metabolites in caraway seeds after inoculation with *Actinopolyspora* sp. JTT-01

Primary metabolites like lipids, proteins, and sugars are crucial for plant nutritional value [49]. Actinobacterial inoculants, like *Actinopolyspora* sp. JTT-01 can enhance the production of these metabolites, improving plant nutritional quality [30]. Our study revealed that *Actinopolyspora* sp. JTT-01 significantly increased total sugar, protein, and lipid content in caraway seeds (Fig. 5) [76]. The enhanced sugar content could provide the plants with carbon and energy for further metabolic processes. Similarly, actinobacterial inoculation has been shown to increase sugar content in aniseeds [30]. Increased mineral availability in *Actinopolyspora* sp. JTT-01-treated seeds likely contributed to enhanced photosynthetic activity, leading to increased sugar production (Fig. 6) [76]. In addition to increased sugar levels, caraway seeds inoculated with *Actinopolyspora* sp. JTT-01 also accumulated higher levels of total proteins and lipids, possibly due to increased carbon and nitrogen availability [59, 61, 77]. Sugar-derived carbon is funneled through these pathways to drive amino acid biosynthesis through glycolysis and the tricarboxylic acid (TCA) cycle [78]. Consistently, the treated caraway seeds exhibited increased levels of several amino acids, particularly lysine, histidine, alanine, arginine, phenylalanine, threonine, glutamine, cystine, and glutamic acid (Table 2) [50]. This increase in amino acids could also be attributed to enhanced mineral (e.g.,

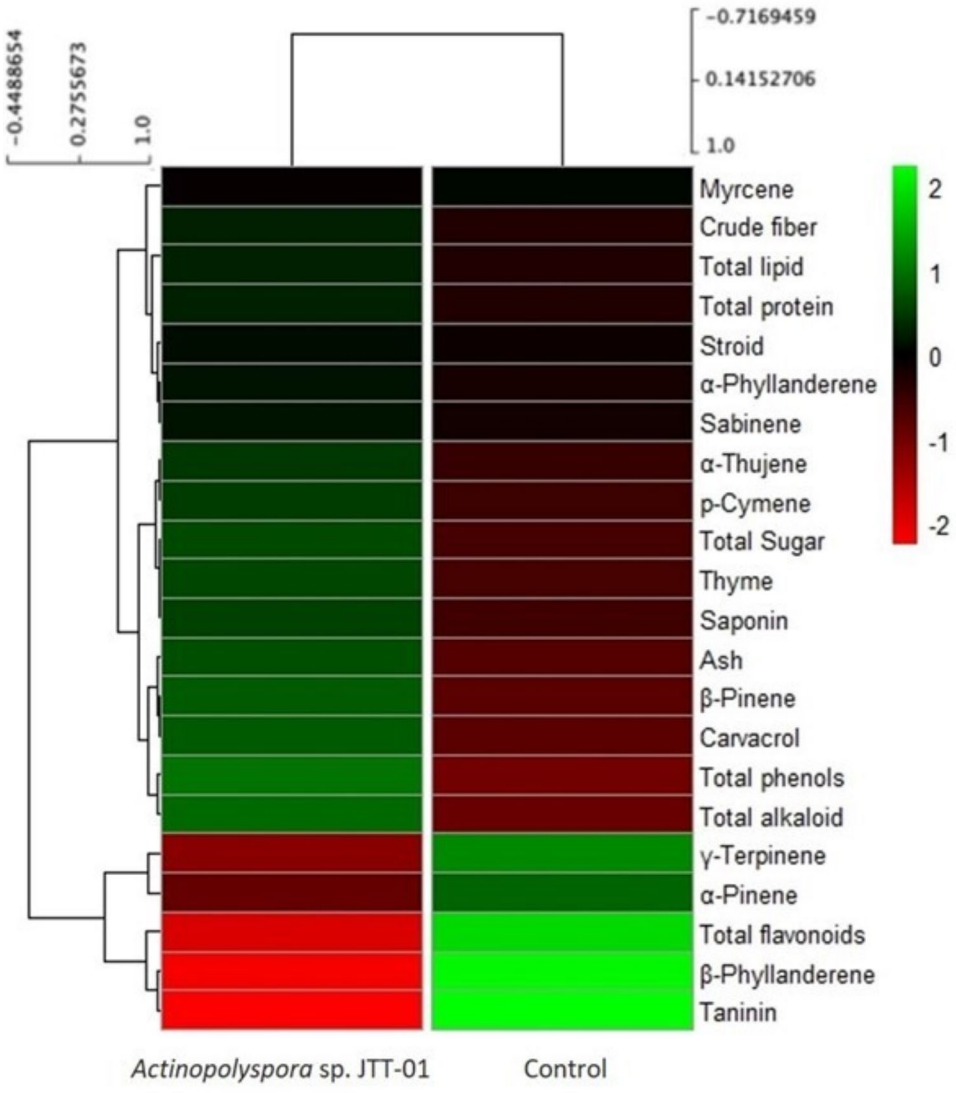

Fig. 5 Heatmap showing the changes in the profile of the investigated primary and secondary metabolites and EO constituents in caraway seeds treated with *Actinopolyspora* sp. JTT-01. Data are represented by the means of at least 3 replicates

nitrogen) availability or direct excretion by the strain [79], where growth-promoting microbial strains can produce amino acids or their precursors in the plant tissues.

Enhanced secondary metabolites in caraway seeds after inoculation with *Actinopolyspora* sp. JTT-01

The enhancement of secondary metabolites such as tannins, alkaloids, flavonoids, saponins after actinobacterial inoculation was reported in different plant species [30, 31]. Likewise, the current findings of this study indicated a substantial increase in most of the identified secondary metabolites when inoculated with *Actinopolyspora* sp. JTT-01. For example, the total contents of phenolics, alkaloids, saponins, and steroids were significantly increased by 121.4, 100.9, 111, and 22.5%, respectively. Consistent with our findings, inoculating maize with actinobacteria elevated the phenolic acids and other secondary metabolites in both normal and drought-stressed plants [80]. The increase in secondary metabolites following actinobacterial treatment may be attributed to enhanced photosynthesis, which supplies the necessary precursors and energy for their biosynthesis [81]. In this context, sugars and amino acids play a crucial role in providing carbon and energy for the biosynthesis of secondary metabolites such as phenolics and flavonoids. Sugars, through glycolysis and the pentose phosphate pathway, provide essential carbon skeletons and reducing power (NADPH) required for phenolic production [82]. Furthermore, the actinobacteria secreted secondary metabolites that accumulated in the treated plant [81]. The elevation in total phenolics after actinobacterial inoculations has been reported in many plants [59, 61].

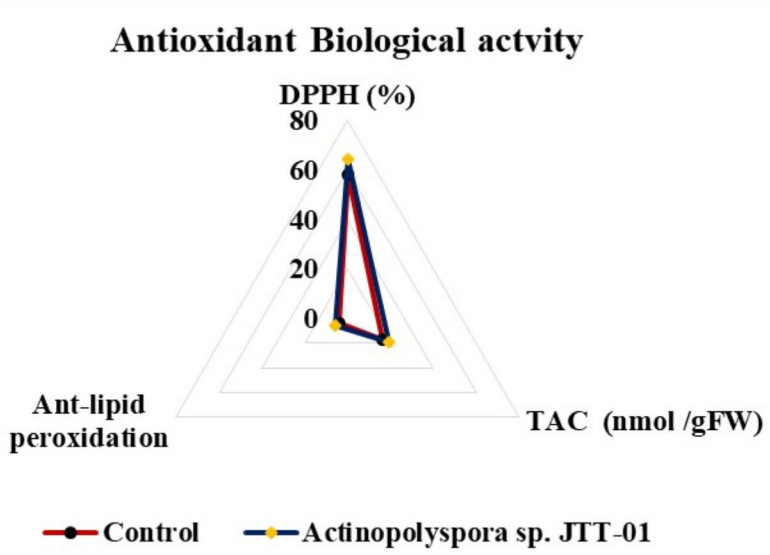

Fig. 6 Radar plot for the anti-oxidant biological activity of the caraway seeds treated with Actinopolyspora sp. JTT-01 and their corresponding untreated controls. Values are Mean ± SE

Table 2 Amino acid concentrations in uninoculated control and *Actinopolyspora* sp. JTT-01-inoculated caraway seeds

Amino acids	Control	Actinopolyspora sp. JTT-01
Lysine	1.53 ± 0.01 a	2.44 ± 0.31 b
Histidine	1.52 ± 0.25 a	1.13 ± 0.01 a
Alanine	0.54 ± 0.09 a	1.44 ± 0.18 b
Arginine	0.94 ± 0.02 a	1.17 ± 0.13 b
Isoleucine	0.07 ± 0.01 a	0.12 ± 0.01 a
Asparagine	0.53 ± 0.13 a	0.90 ± 0.10 a
Ornithine	0.17 ± 0.02 a	0.12 ± 0.02 a
Glycine	0.61 ± 0.15 a	0.75 ± 0.12 a
Phenylalanine	0.17 ± 0.04 a	0.32 ± 0.00 b
Serine	0.18 ± 0.04 a	0.29 ± 0.03 a
Proline	0.59 ± 0.15 a	0.59 ± 0.07 a
Valine	0.25 ± 0.06 a	0.29 ± 0.08 a
Aspartate	0.02 ± 0.002 a	0.02 ± 0.00 a
Cystine	0.01 ± 0.003 a	0.11 ± 0.00 b
Leucine	0.14 ± 0.03 b	0.01 ± 0.00 a
Methionine	0.008 ± 0.00 b	0.01 ± 0.00 a
Threonine	0.05 ± 0.01 a	0.07 ± 0.01 a
Tyrosine	0.51 ± 0.13 a	0.65 ± 0.07 a
Glutamine	44.37 ± 2.94 a	50.32 ± 2.59 b
Glutamic acid	38.21 ± 1.67 a	48.52 ± 5.13 b

*Values in the table represent mean ± SD of 3 independent replicates, with different letters representing significant differences between the treatments at p value < 0.05

For example, phenol and flavonoid contents were significantly affected by Actinobacterial inoculations [83, 84]. The increase in total phenolics observed with *Actinopolyspora sp. JTT-01* may be due to their ability to release these compounds into the soil or plant tissue (Table 1). Our results revealed that many components in this profile such as caffeic acid, p-coumaric acid, luteolin, apigenin, tricin, and vitexin were enhanced due to the inoculation with *Actinopolyspora* sp. JTT-01strain (Fig. 3). This appeared in the PCA analysis where these enhanced elements of the phenolic-flavonoid profile were separated towards the *Actinopolyspora* sp. JTT-01-treated caraway seeds along the PCA vertical axis with 53.3% of the total variation. The enhanced phenolic and flavonoid levels observed in the study with treated caraway seeds could boost their anti-oxidant capabilities, improve resistance to soil-borne pathogens, and increase the mineral content in both the soil and the seeds [70]. The plant contents of phenols and flavonoids were found to be significantly affected by the actinobacterial inoculation [83, 84]. Additionally, the accumulation in the total phenolics observed after inoculation with our *Actinopolyspora* sp. JTT-01 strain may be attributed to the release of such compounds into the soil by our strain. These compounds can be then taken in by roots and ultimately accumulate in various parts of the plant like seeds [81]. The observed increase in saponins, alkaloids, and phenols due to our strain could contribute to the hypocholesterolemic activity of the treated seeds [85].

Among secondary metabolites are vitamins, where our results showed that tocopherol (Vit E), α-carotene (Vit A), and phylloquinone (Vit K) vitamins were enhanced with *Actinopolyspora* sp. JTT-01, by 36.48, 46.15, and 78.57%, respectively (Fig. 3). This appeared as a clear separation for most of these enhanced vitamins towards the *Actinopolyspora* sp. JTT-01-treated caraway seeds along the PCA vertical axis with 53.3% of the total variation. Similarly, Shi [86] reported increased contents of Vit. C and Vit. B in beetroots treated with *Acinetobacter johnsonii* strain. Additionally, AbdElgawad et al. [60] found that

the vitamin content, including β- and γ-tocopherols, was elevated in the seeds of different plants with various actinobacterial strains. These actinobacteria can produce and release many types of vitamin B into the soil [87], which can be taken in by plant roots as reported in soybean roots [88]. In a similar study, Yousaf et al. [89] discovered that metabolites produced by *Acetobacter aceti*, such as quinolinic acid and p-aminobenzoate, played a significant role in promoting vitamin synthesis in barley seeds. Overall, the accumulations in some vitamins in response to our *Actinopolyspora* sp. JTT-01 strain could offer a key solution to address deficiencies in micronutrients [62].

Regarding (EOs), research has shown that actinobacterial inoculants can enhance EO biosynthesis in various plant species [90]. Similarly, actinobacteria and mycorrhizal fungi have been found to boost plant growth and EO biosynthesis in many plants [91]. In this study, caraway seeds treated with Actinopolyspora sp. JTT-01 exhibited significant increases in EOs, particularly β-pinene (186.2%), carvacrol (49.2%), thujene (80%), and p-cymene (67.8%) (Fig. 5). Previous studies also identified these components as key constituents in plant EOs [92, 93].

Observed increases in EOs can be explained by the activating key enzymes involved in the biosynthesis pathway as a recent study [30] reported that actinobacteria enhanced the accumulation of EO components like phenylpropanoids and monoterpenes by increasing phenylalanine and activating essential oil metabolic enzymes (PAL and DAHPS). The elevated EO levels after actinobacterial treatments offer a promising strategy to combat antimicrobial resistance. For example, carvacrol and thymol disrupt bacterial membranes [94], while linalool and cinnamaldehyde interfere with critical enzymatic pathways [95]. EOs from plants like *Rosmarinus* officinalis and *Lavandula angustifolia* have shown significant antibacterial activity, particularly against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* by targeting bacterial proteins [96]. Beyond direct antimicrobial effects, EOs can inhibit bacterial biofilm formation, reducing adhesion and protective layer development [97]. In a recent study by Xu et al. [98], actinobacterial inoculation has been reported to enhance EOs in chickpea seeds by improving nitrogen fixation, gene expression, and root exudate interactions. These increases may be also linked to enhanced mineral availability, vital for coenzymes such as adenosine triphosphate (ATP) and coenzyme A (CoA), which are crucial for terpenoid biosynthesis [93]. Additionally, the higher sugar content in treated caraway seeds may trigger EO synthesis, as previously reported [44].

Among the highest increases was in carvacrol, a monoterpenoid phenol known for its flavoring, insecticidal, antimicrobial, anticancer, and antioxidant properties [99]. The elevated EO levels could boost defensive responses, explaining the seeds' broad-spectrum antimicrobial activity. Similarly, previous research has shown that actinobacteria can increase the insecticidal and antimicrobial activities of plants by enhancing EO constituents [100]. In summary, the improved EO composition in seeds highlights the link between microbial interactions, plant growth, and bioactive metabolite production. The increased yield, biomass, bioactive metabolites, and oil composition in caraway seeds treated with *Actinopolyspora* sp. JTT-01 suggests this strain is an effective candidate for improving caraway seed growth and characteristics.

The biological activity of the inoculated caraway seeds; anti-oxidant and anti-microbial properties

The biological activity of plant seeds depends upon their bioactive secondary compounds like phenolics and flavonoids, and their anti-microbial and anti-oxidant activity as well. The anti-oxidant compounds found in seeds play a significant role in protecting against chronic diseases and oxidative stress [101]. Similarly, actinobacterial inoculation displayed a crucial role in enhancing seed antioxidant properties through modulating stress-responsive gene expression and strengthening the anti-oxidant defense system [99].

Treated caraway seeds in this study showed increased anti-oxidant properties over control through their ability to prominently quench hydroxyl, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, and lipid peroxides. Results revealed increments in the measured components of phenolics and flavonoids like caffeic acid, p-coumaric acid, quercetin, luteolin, naringenin, and tricin after inoculation with *Actinopolyspora* sp. JTT-01. *Actinopolyspora* sp. JTT-01-inoculated caraway seeds caused increased antioxidant activity by 10.3, 18.2, and 46.3% for DPPH, TAC, and anti-lipid-peroxidation assays, respectively (Fig. 6). The increased anti-oxidant activity reported herein could be ascribed to the observed increments in minerals and bioactive metabolites like in caraway seeds after inoculation as reported [59]. Anti-oxidants scavenge free radicals and prevent their accumulation which could inhibit cell damage, cancer, and other diseases. Also, caraway EOs were found to decrease the pace of lipid peroxidation products in intestinal, colonic, and caecal tissue. They enhanced the activity of anti-oxidant enzymes such as catalase and superoxide dismutase, reducing cancerous cell proliferation in rats [6]. Additionally, the induction of anti-oxidant enzymes and phenolics in chickpea leaves following inoculation with the endophyte *Streptomyces* sp. suggests a potential mechanism for enhancing plant defense mechanisms and resistance against pathogens [102]. Further research into the specific mechanisms by which actinobacteria influence the anti-oxidant

Table 3 Anti-microbial activity of uninoculated control and *Actinopolyspora* sp. JTT-01-inoculated caraway seeds

Caraway-anti-microbial activity	Uninoculated controls	Actinopolyspora sp. JTT-01-treated seed extracts
Staphylococcus saprophyticus	13.13 ± 4.98 a	21.00 ± 2.17 b
Staphylococcus epidermidis	9.41 ± 1.68 a	22.51 ± 0.27 b
Enterococcus faecalis	14.02 ± 4.88 a	16.43 ± 0.28 a
Streptococcus salivarius	15.44 ± 0.85 a	15.11 ± 2.19 a
Escherichia coli	5.61 ± 0.60 a	13.47 ± 1.02 b
Salmonella typhimurium	8.67 ± 1.25 a	17.15 ± 2.86 b
Pseudomonas aeruginosa	17.49 ± 1.81 a	19.55 ± 1.80 a
Proteus vulgaris	19.29 ± 1.66 a	19.37 ± 0.95 a
Enterobacter aerogenes	15.93 ± 1.50 b	12.27 ± 1.60 a
Serratia marcescens	5.08 ± 0.63 a	6.10 ± 0.39 a
Salmonella typhimurium	10.78 ± 1.17 a	10.41 ± 1.42 a
Candida albicans	11.66 ± 0.90 a	12.10 ± 0.77 a
Candida glabrata	5.16 ± 0.74 a	7.85 ± 0.59 b
Aspergillus flavus	10.66 ± 0.50 a	15.68 ± 1.74 b

*According to Duncan's multiple range tests, values in the table represent mean ± SD, with different letters representing significant differences between the treatments at $p < 0.05$

properties of plant seeds is warranted to fully comprehend and utilize their anti-oxidant potential for human health.

Concerning anti-microbial activity, the ability of actinobacteria to synthesize bioactive compounds with anti-microbial activities against a broad spectrum of pathogens has been reported, suggesting their role in suppressing plant pathogens and enhancing seed quality [39, 79, 103]. Our results unveiled that *Actinopolyspora* sp. JTT-01-treated caraway seeds have inhibited the growth of various pathogens including Gram-positive and Gram-negative bacteria, in addition to the fungi Candida *glabrata and Aspergillus flavus.* Among the highly inhibited pathogens, the largest zones of inhibition were recorded against *Staphylococcus epidermis* (22 mm), *Salmonella typhimurium* (17 mm), and *Aspergillus flavus* (15 mm). Also, non-significant inhibitions (than controls) were recorded against *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Candida albicans* (Table 3). Our results additionally demonstrated that the *Actinopolyspora* sp. JTT-01-treated caraway seeds exhibited higher activity against the tested Gram-positive than Gram-negative bacterial pathogens. In line with our results, the *Actinopolyspora* W20 strain, due to its volatile secondary metabolites and phytohormones, was able to antagonize most of the test phytopathogenic fungi including *Aspergillus niger*, *A. flavus*, *Alternaria brassicicola*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Phytophthora dresclea* [22]. Also, extracts of *Brassica oleracea* sprout treated with *Nocardiopsis* species displayed improved anti-microbial activity against many pathogens including *P. aeruginosa*, *(A) flavus*, *E. coli*, *S. saprophyticus*, *S. salivarius*, *and C. glabrata.* The correlation between the increased contents of both phenols and EO content and the anti-oxidant activity of caraway-treated seeds along with the increase in the anti-microbial activity of these seeds is obvious in this study. Similarly, a previous study by Vijayabharathi et al. [102] reported that actinobacterial endophytes could enhance the chickpea plant's defense and resistance against *(B) cinerea* by inducing anti-oxidant enzymes and phenolics. Phenols, alkloids, and EOs inhibit bacteria growth by disrupting cell membranes, causing leakage of vital contents [94]. They also interfere with key bacterial enzymes, hindering growth and replication. Additionally, both can disrupt quorum sensing, reducing biofilm formation and making bacteria more susceptible to treatments [97]. In conclusion, the application of actinobacterial inoculation shows promise in enhancing the anti-microbial activity of plant seeds and promoting overall plant health and productivity.

Conclusion

Seed treatment with *Actinopolyspora* sp. JTT-01 offers a promising biofortification strategy for enhancing the yield, nutritional content, and bioactivity of caraway. This approach leads to notable increases in essential oils, nutritional metabolites, and antioxidant and antimicrobial properties. These enhancements, particularly in key nutrients and bioactive compounds, suggest a potential for developing caraway products with enhanced nutritional and medicinal value. The findings of this study contribute to the growing research on endophytic actinobacteria to promote sustainable and biofortified agricultural practices. Based on our findings, to enhance *Actinopolyspora*-caraway interaction and its potential for improving agricultural sustainability and food quality, we suggest identifying genes, and gene expression changes in both caraway and JTT-01 during their interaction to understand the molecular pathways and analyzing protein profiles to identify key proteins involved in the biofortification. We also suggest conducting field trials to validate the efficacy of JTT-01 as a biofertilizer for caraway in real-world agricultural settings, optimizing the JTT-01 inoculum dosages, evaluating the most effective application methods (e.g., seed coating, soil drench), assessing long-term soil effects, and exploring the biofortification potential in other crops.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06137-1.

Supplementary Material 1

Acknowledgements
The authors extend their appreciation to King Saud University for funding this work through research supporting project (RSP2025R374), Riyadh, Saudi Arabia.

Author contributions
M.S.S. (Mohamed S. Sheteiwy) and A.E.K. (Ali El-Keblawy) conceptualized the research idea, designed the study framework, and provided critical supervision and administration. Z.U. (Zaid Ulhassan) contributed to experimental design, statistical analysis, data interpretation, and participated in manuscript revision. M.H.K. (Maha H. Khalaf) and H.S.M. (Husein S. Mohamed) carried out the microbial isolation, characterization, and bioassays, and contributed to drafting the methods section. A.M.R. (Ahmed M. Reyad) and M.K.O. (Mohammad K. Okla) were involved in the setup of greenhouse experiments, plant growth assessments, and biochemical analyses. A.M.A. (Amal Mohamed AlGarawi) contributed to the antioxidant and antimicrobial activity assays and assisted in data validation and visualization. A.M.E. (Ahmed M. El-Sawah) and E.S.A. (Enas S. Ahmed) participated in elemental and metabolite analysis, and contributed to data curation and literature review. A.M.M. (Ahmed M. Mahmoud) wrote the original draft, compiled results, and coordinated manuscript editing and formatting. All authors read and approved the final manuscript and agreed to be accountable for the accuracy and integrity of the work.

Funding
The authors extend their appreciation to King Saud University for funding this work through research supporting project (RSP2025R374), Riyadh, Saudi Arabia.

Data availability
The datasets used and analyzed in the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Botany and Microbiology, Faculty of Science, Beni-Suef University, Beni-Suef 62511, Egypt
[2]Department of Integrative Agriculture, College of Agriculture and Veterinary Medicine, United Arab Emirates University, P.O. Box 15551, Al Ain, Abu Dhabi, United Arab Emirates
[3]Department of Applied Biology, College of Sciences, University of Sharjah, Sharjah 27272, United Arab Emirates
[4]Institute of Crop Science, Ministry of Agriculture and Rural Affairs, Key Laboratory of Spectroscopy Sensing, Zhejiang University, Hangzhou 310058, China
[5]Chemistry of Medicinal and Aromatic Plants Department, Research Institute of Medicinal and Aromatic Plants (RIMAP), Beni-Suef University, Beni-Suef 62511, Egypt
[6]Department of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[7]Department of Agricultural Microbiology, Faculty of Agriculture, Mansoura University, Mansoura 35516, Egypt

Received: 3 September 2024 / Accepted: 21 January 2025
Published online: 25 April 2025

References

1. Laribi B, et al. Essential oils and fatty acids composition of Tunisian, German and Egyptian caraway (Carum carvi L.) seed ecotypes: a comparative study. Ind Crops Prod. 2013;41:312–8.
2. Ayyat AM. Influence of plant density and fertilization treatments on growth and productivity of caraway (Carum carvi, L.) plants. J Plant Prod. 2022;13(9):711–8.
3. Homayounpour P, Shariatifar N, Alizadeh-Sani M. Development of nanochitosan-based active packaging films containing free and nanoliposome caraway (Carum carvi. L) seed extract. Food Sci Nutr. 2021;9(1):553–63.
4. Salari Rafsanjani M, et al. Wound healing effect of Carum carvi L. on the incised skin wound in male rats: histopathology, total protein and biomechanical evaluations. Veterinary Med Sci. 2022;8(6):2726–37.
5. Samojlik I, et al. Antioxidant and hepatoprotective potential of essential oils of coriander (Coriandrum sativum L.) and caraway (Carum carvi L.)(Apiaceae). J Agric Food Chem. 2010;58(15):8848–53.
6. Mahboubi M. Caraway as important medicinal plants in management of diseases. Nat Prod Bioprospecting. 2019;9(1):1–11.
7. Erjaee H, et al. The effect of caraway (Carum carvi L.) on the blood antioxidant enzymes and lipid peroxidation in streptozotocin-induced diabetic rats. Comp Clin Pathol. 2015;24:1197–203.
8. Lahlou S, et al. Diuretic activity of the aqueous extracts of Carum carvi and Tanacetum vulgare in normal rats. J Ethnopharmacol. 2007;110(3):458–63.
9. Keshavarz A, et al. Effects of Carum carvi L.(Caraway) extract and essential oil on TNBS-induced colitis in rats. Res Pharm Sci. 2013;8(1):1.
10. Marthandan V, et al. Seed priming: a feasible strategy to enhance drought tolerance in crop plants. Int J Mol Sci. 2020;21(21):8258.
11. Yeom H-J, et al. Insecticidal and acetylcholine esterase inhibition activity of Apiaceae plant essential oils and their constituents against adults of German cockroach (Blattella germanica). J Agric Food Chem. 2012;60(29):7194–203.
12. Hassan MO, Saleh AM, AbdElgawad H. Sonchus oleraceus residue improves nutritive and health-promoting value of common bean (Phaseolus vulgaris L.): a metabolic study. J Agric Food Chem. 2018;66(9):2092–100.
13. Gu X, et al. Differential aluminum tolerance and absorption characteristics in Pinus massoniana seedlings colonized with ectomycorrhizal fungi of Lactarius deliciosus and Pisolithus Tinctorius. J Forestry Res. 2023;34(5):1523–33.
14. Gomiero T. Food quality assessment in organic vs. conventional agricultural produce: findings and issues. Appl Soil Ecol. 2018;123:714–28.
15. Li Z, et al. Rice carotenoid biofortification and yield improvement conferred by endosperm-specific overexpression of OsGLK1. Front Plant Sci. 2022;13:951605.
16. Bulgarelli D, et al. Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol. 2013;64(1):807–38.
17. Rasul M, et al. Glucose dehydrogenase gene containing phosphobacteria for biofortification of Phosphorus with growth promotion of rice. Microbiol Res. 2019;223:1–12.
18. Qu H et al. Pulsed electric field treatment of seeds modulates the endophytic bacterial community and promotes early growth of roots in buckwheat. 2023.
19. Anwar S, Ali B, Sajid I. Screening of rhizospheric actinomycetes for various in-vitro and in-vivo plant growth promoting (PGP) traits and for agroactive compounds. Front Microbiol. 2016;7:1334.
20. De Meyer SE, et al. A large diversity of non-rhizobial endophytes found in legume root nodules in Flanders (Belgium). Soil Biol Biochem. 2015;83:1–11.
21. Aneshwari RK, Vyas A, Jain V. Conspectus on Tephrosiapurpurea: an introduction. Res J Pharmacognosy Phytochemistry. 2022;14(4):281–7.
22. Gangwar M, Rani S, Sharma N. Diversity of endophytic actinomycetes from wheat and its potential as plant growth promoting and biocontrol agents. J Adv Lab Res Biol. 2012;3(1):18–23.
23. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981–2002. J Nat Prod. 2003;66(7):1022–37.
24. Gochnauer MB, et al. Isolation and characterization of Actinopolyspora halophila, gen. et sp. nov., an extremely halophilic actinomycete. Can J Microbiol. 1975;21(10):1500–11.
25. Feng X-M, et al. Phytoactinopolyspora mesophila sp. nov., isolated from a saline–alkaline soil. Int J Syst Evol MicroBiol. 2020;70(7):4179–85.
26. Duangmal K, et al. Actinopolyspora salinaria sp. nov., a halophilic actinomycete isolated from solar saltern soil. Int J Syst Evol MicroBiol. 2016;66(4):1660–5.
27. Barka EA, et al. Taxonomy, physiology, and natural products of Actinobacteria. Microbiol Mol Biol Rev. 2016;80(1):1–43.

28. Fiedler H-P, et al. Proximicin A, B and C, novel aminofuran antibiotic and anticancer compounds isolated from marine strains of the actinomycete Verrucosispora. J Antibiot. 2008;61(3):158–63.
29. Parte AC, et al. List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. Int J Syst Evol MicroBiol. 2020;70(11):5607–12.
30. Mahmoud AM, et al. Investigating the endophyte actinomycetota sp. JW0824 strain as a potential bioinoculant to enhance the yield, Nutritive Value, and Chemical composition of different cultivars of Anise (Pimpinella anisum L.) Seeds. Biology. 2024;13(8):553.
31. AbdElgawad H, et al. Synergistic impacts of plant-growth-promoting bacteria and selenium nanoparticles on improving the nutritional value and biological activities of three cultivars of Brassica sprouts. ACS Omega. 2023;8(29):26414–24.
32. Chen X et al. Molecular phylogenetic identification of Actinobacteria. D. Dharumadurai, & Y. Jiang, Actinobacteria-Basics and Biotechnological Applications, 2016: pp. 141–74.
33. Farris M, Olson J. Detection of Actinobacteria cultivated from environmental samples reveals bias in universal primers. Lett Appl Microbiol. 2007;45(4):376–81.
34. Hamad I, et al. Metabolic analysis of various date palm fruit (Phoenix dactylifera L.) cultivars from Saudi Arabia to assess their nutritional quality. Molecules. 2015;20(8):13620–41.
35. Hatanaka C, Kobara Y. Determination of glucose by a modification of Somogyi-Nelson method. Agric Biol Chem. 1980;44(12):2943–9.
36. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Can J Biochem Physiol. 1959;37(8):911–7.
37. Baur FJ, Ensminger LG. The association of official analytical chemists (AOAC). J Am Oil Chemists' Soc. 1977;54(4):171–2.
38. Bremner J, Hauck R. Advances in methodology for research on nitrogen transformations in soils. Nitrogen Agricultural Soils. 1982;22:467–502.
39. Sreevidya N, Mehrotra S. Spectrophotometric method for estimation of alkaloids precipitable with Dragendorff's reagent in plant materials. J AOAC Int. 2003;86(6):1124–7.
40. Lai L-R, et al. Effect of lactic fermentation on the total phenolic, saponin and phytic acid contents as well as anti-colon cancer cell proliferation activity of soymilk. J Biosci Bioeng. 2013;115(5):552–6.
41. Sinha AK, et al. Regulation of amino acid metabolism as a defensive strategy in the brain of three freshwater teleosts in response to high environmental ammonia exposure. Aquat Toxicol. 2013;130:86–96.
42. Almuhayawi M, et al. Bacterial endophytes as a promising approach to enhance the growth and accumulation of bioactive metabolites of three species of Chenopodium Sprouts. Plants. 2021;10:2745.
43. Hozzein WN, et al. CO2 treatment improves the hypocholesterolemic and antioxidant properties of fenugreek seeds. Food Chem. 2020;308:125661.
44. Okla MK, et al. Laser light treatment of seeds for improving the biomass photosynthesis, chemical composition and biological activities of lemongrass sprouts. Agronomy. 2021;11(3):478.
45. Clevenger J. Apparatus for the determination of volatile oil. J Am Pharm Association (1912). 1928;17(4):345–9.
46. Wang Z, et al. Effects of light irradiation on essential oil biosynthesis in the medicinal plant Asarum heterotropoides Fr. Schmidt var. Mandshuricum (Maxim) Kitag. PLoS ONE. 2020;15(9):e0237952.
47. Habeeb TH, et al. Interactive impact of arbuscular mycorrhizal fungi and elevated CO2 on growth and functional food value of Thymus vulgare. J Fungi. 2020;6(3):168.
48. Kumar S, Stecher G, Mega KT. Molecular evolutionary genetics analysis version 7.0 for bigger datasets. 2016;33:1870–1874. https://doi.org/10.1093/molbev/msw054
49. AbdElgawad H, et al. Climate extreme effects on the chemical composition of temperate grassland species under ambient and elevated CO2: a comparison of fructan and non-fructan accumulators. PLoS ONE. 2014;9(3):e92044.
50. Almuhayawi MS, et al. Laser light as a promising approach to improve the nutritional value, antioxidant capacity and anti-inflammatory activity of flavonoid-rich buckwheat sprouts. Food Chem. 2021;345:128788.
51. Talibi I, et al. Antifungal activity of some Moroccan plants against Geotrichum candidum, the causal agent of postharvest citrus sour rot. Crop Prot. 2012;35:41–6.
52. Kassambara A, Mundt F. Extract and visualize the results of multivariate data analyses [R package factoextra version 1.0. 7]. Comprehensive R Archive Network (CRAN); 2020.
53. Sreenivasan A, Manikkam R, Manigundan K. Isolation, characterization and antimicrobial activity of endophytic actinobacteria from medicinal plants. Indian J Pharm Sci. 2022;84(5).
54. Passari AK, et al. Isolation, abundance and phylogenetic affiliation of endophytic actinomycetes associated with medicinal plants and screening for their in vitro antimicrobial biosynthetic potential. Front Microbiol. 2015;6:273.
55. Qin S, et al. Abundant and diverse endophytic actinobacteria associated with medicinal plant Maytenus austroyunnanensis in Xishuangbanna tropical rainforest revealed by culture-dependent and culture-independent methods. Environ Microbiol Rep. 2012;4(5):522–31.
56. Gangwar M, et al. Diversity and biopotential of endophytic actinomycetes from three medicinal plants in India. Afr J Microbiol Res. 2014;8(2):184–91.
57. Zhang X, et al. Effects of raised-bed planting for enhanced summer maize yield on rhizosphere soil microbial functional groups and enzyme activity in Henan Province, China. Field Crops Res. 2012;130:28–37.
58. Santoro MV, et al. Volatile organic compounds from rhizobacteria increase biosynthesis of essential oils and growth parameters in peppermint (Mentha piperita). Plant Physiol Biochem. 2011;49(10):1177–82.
59. AbdElgawad H, et al. Utilization of actinobacteria to enhance the production and quality of date palm (Phoenix dactylifera L.) fruits in a semi-arid environment. Sci Total Environ. 2019;665:690–7.
60. AbdElgawad H, et al. Actinomycetes enrich soil rhizosphere and improve seed quality as well as productivity of legumes by boosting nitrogen availability and metabolism. Biomolecules. 2020;10(12):1675.
61. Hozzein WN, et al. Exploring the potential of actinomycetes in improving soil fertility and grain quality of economically important cereals. Sci Total Environ. 2019;651:2787–98.
62. Sathya A, et al. Plant growth-promoting actinobacteria on chickpea seed mineral density: an upcoming complementary tool for sustainable biofortification strategy. 3 Biotech. 2016;6:1–6.
63. Ahmad M et al. *Potassium-solubilizing bacteria and their application in agriculture.* Potassium solubilizing microorganisms for sustainable agriculture, 2016: pp. 293–313.
64. Uroz S, et al. Mineral weathering by bacteria: ecology, actors and mechanisms. Trends Microbiol. 2009;17(8):378–87.
65. El-Tarabily KA, Nassar AH, Sivasithamparam K. Promotion of growth of bean (Phaseolus vulgaris L.) in a calcareous soil by a phosphate-solubilizing, rhizosphere-competent isolate of Micromonospora endolithica. Appl Soil Ecol. 2008;39(2):161–71.
66. Naveed M, et al. Drought stress amelioration in wheat through inoculation with Burkholderia phytofirmans strain PsJN. Plant Growth Regul. 2014;73:121–31.
67. Boondaeng A et al. *Biological conversion of agricultural wastes into indole-3-acetic acid by S. lavenduligriseus BS50-1 using response surface methodology (RSM).* 2023.
68. Wu Y-R, et al. Diversity and function of culturable actinobacteria in the root-associated of Salvia miltiorrhiza Bunge. PeerJ. 2021;9:e11749.
69. Zhao K, et al. Actinobacteria associated with Glycyrrhiza inflata Bat. Are diverse and have plant growth promoting and antimicrobial activity. Sci Rep. 2018;8(1):13661.
70. Cherrak SA, et al. In vitro antioxidant versus metal ion chelating properties of flavonoids: a structure-activity investigation. PLoS ONE. 2016;11(10):e0165575.
71. Ranganathan U, Groot SP. Seed longevity and deterioration. Seed science and technology: biology, production, quality. Nature Singapore Singapore: Springer; 2023. pp. 91–108.
72. Sano N, et al. Staying alive: molecular aspects of seed longevity. Plant Cell Physiol. 2016;57(4):660–74.
73. Assis DAd, et al. Biosynthesis of vitamin B12 by Propionibacterium freudenreichii subsp. shermanii ATCC 13673 using liquid acid protein residue of soybean as culture medium. Biotechnol Prog. 2020;36(5):e3011.
74. Fischer M, Bacher A. *Biosynthesis of vitamin B2 and flavocoenzymes in plants*, in *advances in Botanical Research*. Elsevier; 2011. pp. 93–152.
75. Kathi S, et al. Vitamin C biofortification of broccoli microgreens and resulting effects on nutrient composition. Front Plant Sci. 2023;14:1145992.
76. Nunes-Nesi A, Fernie AR, Stitt M. Metabolic and signaling aspects underpinning the regulation of plant carbon nitrogen interactions. Mol Plant. 2010;3(6):973–96.
77. Al-Alawi RA, et al. Date palm tree (Phoenix dactylifera L.): natural products and therapeutic options. Front Plant Sci. 2017;8:845.

78. Cura A, Carruthers A. Role of monosaccharide transport proteins in carbohydrate assimilation, distribution, metabolism, and homeostasis. Compr Physiol. 2012;2:863–914.
79. Siddharthan N, et al. *Actinobacterial enzymes—An approach for engineering the rhizosphere microorganisms as plant growth promotors*, in *Rhizosphere engineering*. Elsevier; 2022. pp. 273–92.
80. Selim S, et al. Actinobacterium isolated from a semi-arid environment improves the drought tolerance in maize (Zea mays L). Plant Physiol Biochem. 2019;142:15–21.
81. Solecka J, et al. Biologically active secondary metabolites from Actinomycetes. Open Life Sci. 2012;7(3):373–90.
82. Mitra D, et al. Actinobacteria-enhanced plant growth, nutrient acquisition, and crop protection: advances in soil, plant, and microbial multifactorial interactions. Pedosphere. 2022;32(1):149–70.
83. Ghorbanpour M, et al. Phytochemical variations and enhanced efficiency of antioxidant and antimicrobial ingredients in Salvia officinalis as inoculated with different rhizobacteria. Chem Biodivers. 2016;13(3):319–30.
84. Singh UP, Sarma BK, Singh DP. Effect of plant growth-promoting rhizobacteria and culture filtrate of Sclerotium rolfsii on phenolic and salicylic acid contents in chickpea (Cicer arietinum). Curr Microbiol. 2003;46:0131–40.
85. Kutchan TM. Ecological arsenal and developmental dispatcher. The paradigm of secondary metabolism. Plant Physiol. 2001;125(1):58–60.
86. Shi Y, Lou K, Li C. Growth promotion effects of the endophyte Acinetobacter johnsonii strain 3–1 on sugar beet. Symbiosis. 2011;54:159–66.
87. Strzelczyk E, Leniarska U. *Production of B-group vitamins by mycorrhizal fungi and actinomycetes isolated from the root zone of pine (Pinus sylvestris L.)*. Plant and soil, 1985: pp. 387–394.
88. Mozafar A, Oertli J. Uptake of a microbially-produced vitamin (B12) by soybean roots. Plant Soil. 1992;139:23–30.
89. Yousaf A, et al. Identification of microbial metabolites elevating vitamin contents in barley seeds. J Agric Food Chem. 2015;63(32):7304–10.
90. Çakmakçı R, et al. Effect of different plant growth-promoting Rhizobacteria on biological soil properties, growth, yield and quality of oregano (Origanum onites L). Agronomy. 2023;13(10):2511.
91. Rasouli F, et al. Influence of drought stress and mycorrhizal (Funneliformis Mosseae) symbiosis on growth parameters, chlorophyll fluorescence, antioxidant activity, and essential oil composition of summer savory (Satureja hortensis L.) plants. Front Plant Sci. 2023;14:1151467.
92. Morshedloo MR, et al. Essential oil profile of oregano (Origanum vulgare L.) populations grown under similar soil and climate conditions. Ind Crops Prod. 2018;119:183–90.
93. Alraey DA, et al. Fluctuation of essential oil constituents in Origanum syriacum subsp. sinaicum in response to plant growth promoting bacteria. J Essent Oil Bearing Plants. 2019;22(4):1022–33.
94. Aminkhani A, Sharifi R, Dorosti R. Chemical composition and antimicrobial activity of Achillea tenuifolia Lam. Essential oil at different phenological stages from Khoy. Chem Biodivers. 2019;16(12):e1900289.
95. Kuttithodi AM, et al. Chemical composition of the Cinnamomum malabatrum leaf essential oil and analysis of its antioxidant, enzyme inhibitory and antibacterial activities. Antibiotics. 2023;12(5):940.
96. Mourabiti F, et al. The antimicrobial effectiveness of Rosmarinus officinalis, Lavandula angustifolia, and Salvia officinalis essential oils against Klebsiella pneumoniae and Pseudomonas aeruginosa in vitro and in silico. South Afr J Bot. 2024;168:112–23.
97. El-Tarabily KA, et al. Using essential oils to overcome bacterial biofilm formation and their antimicrobial resistance. Saudi J Biol Sci. 2021;28(9):5145–56.
98. Xu T, et al. Revealing the underlying mechanisms mediated by endophytic actinobacteria to enhance the rhizobia-chickpea (Cicer arietinum L.) symbiosis. Plant Soil. 2022;474(1):299–318.
99. Ali T, et al. Recent advances in the pharmacological properties and molecular mechanisms of carvacrol. Revista Brasileira De Farmacognosia. 2024;34(1):35–47.
100. Gonzales J, et al. Interrelationships among seed yield, total protein and amino acid composition of ten quinoa Chenopodium quinoa willd. Cultivars from two different agroecological regions. J Sci Food Agric. 2012;92(6):1222–9.
101. Ilyasoğlu H. Characterization of rosehip (Rosa canina L.) seed and seed oil. Int J Food Prop. 2014;17(7):1591–8.
102. Vijayabharathi R, et al. Deciphering the tri-dimensional effect of endophytic Streptomyces sp. on chickpea for plant growth promotion, helper effect with Mesorhizobium ciceri and host-plant resistance induction against Botrytis Cinerea. Microb Pathog. 2018;122:98–107.
103. Bhuyan B, et al. Isolation of yeast endophytes from healthy seeds of Capsicum annuum L. and assessment of their antimicrobial activity. J Appl Biology Biotechnol. 2023;11:111–6.

Publisher's note