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Harnessing microalgal inoculants for integrated damping-off management and growth promotion in *Phaseolus vulgaris*

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Abstract

Background The common bean (*Phaseolus vulgaris* L.) is a legume of significant nutritional and economic value. Fungal diseases, especially damping-off, greatly reduce its yield and threaten sustainable production. The study aimed to evaluate the efficacy of algal inoculants individually and in combinations as a biological control approach for managing seedling damping-off diseases and enhancing the growth of common bean crops.

Results The virulence of four *Rhizoctonia solani* isolates were tested on common bean (cv. *Nebraska*) under greenhouse conditions, and all isolates caused damping-off with varying degrees. The species isolated from Beni-Suef was the most virulent, causing the highest pre-emergence damping-off (66.6%) and lowest plant survival (26.6%), and was therefore selected for further studies. Soil inoculation with microalgal inoculants (MI) based on *Desmodesmus abundans* NA1 (*D. abundans* NA1), *Nostoc paludosum* NA2 (*N. paludosum* NA2), and *Tildenella torsiva* NA3 (*T. torsiva* NA3), individually or combined, significantly suppressed damping-off in common bean and enhanced plant growth ($P < 0.05$). The most effective treatment was the 20% triple microalgal inoculant (MI) combination, which reduced pre-emergence damping-off by 1.7-fold and entirely suppressed post-emergence damping-off. At the same time, it increased plant survival by 14-fold compared to the control. A remarkable 33-fold increase in overall yield per feddan was also noted, along with notable improvements in yield parameters such as increases of 1.7-fold in pods per plant, 3.1-fold in seeds per plant, 3.6-fold in seed weight per plant, and 1.6-fold in 100-seed weight. To our knowledge, This study is the first to investigate the combined application of *D. abundans* NA1, *N. paludosum* NA2, and *T. torsiva* NA3, determining their innovative role in controlling damping-off disease and acting as biofertilizers that improve the growth and yield of common bean plants.

Conclusion These results highlight the potential of algal bioformulations as promising sustainable biocontrol and biofertilization agents in legume cultivation.

Keywords Damping-off, Microalgal inoculant, *Phaseolus vulgaris*, Biocontrol, And biofertilizer

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Background

Common beans (*Phaseolus vulgaris* L.) are economically and nutritionally important legume crops that supply essential nutritive proteins and energy. Their importance is particularly pronounced in developing countries where they serve as fundamental nutritional resource (Porch et al. 2013). Bean cultivation faces several challenges from fungal infections, particularly damping-off disease, which can cause yield losses ranging from 20% to complete harvest failure under certain environmental conditions (Degu et al. 2023). Seeds and emerging seedlings are highly susceptible to this pathological condition, especially in wet soils with bad drainage systems, where the disease is mainly attributed to pathogenic species such as *Rhizoctonia*, *Fusarium*, *Pythium*, *Alternaria*, and *Macrophomina* (Sendi et al. 2020). Infested plants typically exhibit stem constriction, collar discoloration, and dehydration symptoms. In severe cases, complete seedlings may die, leading to considerable stand losses and reduced crop yield (El-Gammal et al. 2025). Conventional control of these fungal diseases has relied on chemical fungicides, but intensive use of such chemicals poses serious risks for human health and environmental stability. Accordingly, biological control agents (BCAs) gained increasing attention as eco-friendly sustainable alternatives (Udeigwe et al. 2015).

Microalgae have emerged as promising biological control agents due to their ability to produce diverse bioactive metabolites and hydrolytic enzymes (Premkumar et al. 2018). As photosynthetic microorganisms, soil microalgae can adapt to harsh environments through several mechanisms, including the production of secondary bioactive metabolites with antimicrobial and antioxidant activities, such as phenolics, flavonoids, vitamins, and pigments (Chew et al. 2017). These antimicrobial metabolites suppress pathogenic microbes by disrupting the cytoplasmic membrane, and inhibiting protein synthesis (Swain et al. 2017). Furthermore, microalgae are able to colonize and persist in the plant rhizosphere, which acts as an indirect mechanism to stimulate their antagonistic effects against plant pathogens. This is achieved through the release of antimicrobial compounds and hydrolytic enzymes which, in turn, enhance the activity of plant defense enzymes (Gupta et al. 2013). Accordingly, the inoculation with these organisms has become a promising technology as it triggers plant defense enzymes and regulates key metabolic processes. It also boosts the production of transporters and chelating agents, leading to improved plant immunity, growth, and crop yield. In this regard, previous studies have confirmed the antifungal activity of *Desmodesmus* sp., against major soil-borne fungal pathogens (Eckstien et al. 2024). Similarly, *Nostoc* spp. have been strongly suggested as effective biocontrol agents against *Fusarium oxysporum*, a pathogen

that causes severe damage to tomato plants (El-Sheekh et al. 2022). Likewise, Ghazal et al. (2025) highlighted the potential of *Anabaena* sp. and *Nostoc* sp., in controlling damping-off disease, stimulating defense-related enzymes and promoting hydrolytic enzymes in lupine plants.

In addition to their biocontrol potential, soil microalgae play a crucial role in enhancing soil fertility and increasing crop yield. In particular, *Desmodesmus abundans* NA1, *Nostoc paludosum* NA2, and *Tildenella torsiva* NA3 have been recognized for producing various compounds that promote plant growth, such as auxins, gibberellins, and cytokinins (Mohamed et al. 2025). Additionally, species such as *Anabaena* and *Nostoc* contribute to soil fertility by fixing atmospheric nitrogen and solubilizing phosphorus, thereby enhancing nutrient availability. Moreover, microalgae improve plant growth by producing bioactive compounds such as polypeptides, amino acids, polysaccharides, and siderophores. These produced compounds, along with the enhanced microbial activity in the rhizosphere and the maintenance of soil organic carbon levels, collectively contribute to improved soil fertility (Renuka et al. 2018). Therefore, microalgae have emerged as promising biofertilizers. Their effectiveness has been demonstrated in a wide range of crops, including tomato (Suchithra et al. 2022), onion (Dineshkumar et al. 2020), and maize (Dineshkumar et al. 2019). Their application has been associated with enhanced plant growth, nutrient uptake, and overall crop productivity. In support of this, *Anabaena fertilissima* and *Tildenella torsiva* have proven to be a promising biostimulants (Abdelwahab et al. 2024). Similarly, the potential of *Nostoc* sp. as a sustainable biological fertilizer and an alternative nitrogen source for rice plants has been previously reported by Oco et al. (2024). Thus, the application of *D. abundans* promoted growth and metabolic parameters in common bean crops (Lopes et al. 2023).

In parallel, recent studies have highlighted their ability to remediate contaminants and heavy metals from agricultural and industrial wastewater, thereby reinforcing their multifunctional ecological role (Mabood et al. 2025).

Considering all previous findings, modern agriculture encourages the use of microalgae-based products (biofertilizers, biostimulants, and biofungicides) as sustainable alternatives to reduce dependence on agrochemicals, while enhancing productivity and sustainability, and minimizing negative impacts on the agroecosystem (Niu et al. 2020).

Although microalgae have been widely studied for their individual applications as biofertilizers or biocontrol agents, their combined dual functionality remains insufficiently unexplored, particularly under field conditions.

Investigating this synergy could lead to innovative strategies that enhance crop productivity and sustainability. In addition, the ability of *T. torsiva* NA3 as biocontrol agent has not yet been studied. Accordingly, This study aims to evaluate the effectiveness of soil microalgae isolates (*D. abundans* NA1, *N. paludosum* NA2, and *T. torsiva* NA3), applied either individually or, for the first time, in combination, in controlling damping-off disease, enhancing plant development and boosting crop productivity in common bean (*Phaseolus vulgaris*) grown under field conditions heavily infested with soil-borne pathogens.

Methods

Study area

During the two growing seasons of 2021–22 and 2022–23, we conducted our experiments at the Sids Horticulture Research Station, Beni-Suef Governorate, Agricultural Research Center (ARC), Egypt. The research station is situated at latitude of 29°3'58.06" N and a longitude of 31°5'57.79" E, within a semi-arid climatic region. The initial soil analysis, conducted in accordance with A.O.A.C. (1986) guidelines, classified the experimental soil as clay with a slightly alkaline pH of 7.5, with low salinity (1.2 dS.m⁻¹), low organic matter (1.7%) and available nitrogen, phosphorus & potassium (NPK) contents were 18.0, 14.0 and 165 ppm, respectively.

Common bean seeds

Seeds of Nebraska (*Phaseolus vulgaris* L.) were obtained from Sids Horticulture Research Station, Agricultural Research Center Giza, Egypt, and are known to be susceptible to diseases caused by soil-borne pathogens. The dry seeds produced from cultivar Nebraska under field conditions.

Microalgal inoculum

Green microalga *Desmodesmus abundans* NA1 and two cyanobacterial isolates, *Nostoc paludosum* NA2 and *Tildenella torsiva* NA3, were isolated from Sids Agricultural Research Station farm (Mohamed et al. 2025). The species were assigned accession numbers: *D. abundans* NA1 (GenBank: OR053746.1), *N. paludosum* NA2 (GenBank: OR076697.1), and *T. torsiva* NA3 (GenBank: OR076693.1). Both of the cyanobacterial strains were propagated in liquid BG₁₁ medium while, the green algae cultured in Bold's Basal medium, were incubated at 30–35 °C under continuous light under continuous light with an intensity of 50 µmol photons m⁻² s⁻¹, until the stationary phase (Senousy et al. 2022). The microalgal inoculants were applied individually and in combinations at three different concentrations: 5, 10, and 20%.

Isolation and identification of the causal pathogens:-

Bean plants exhibiting damping-off symptoms were collected during the 2020–2021 growing season from El-Giza, El-Fayoum, Beni-Suef, El-Sharkia, and El-Menoufia. Diseased roots were surface-sterilized, cultured on PDA, and incubated at 25 °C. Emerging fungal colonies were purified by the hyphal tip technique (Hawker 1960) and identified as *Rhizoctonia solani* based on morphological characteristics (Sneh et al. 1992). Isolates were maintained on PDA slants at 5 °C for further use.

Evaluation the efficacy of microalgal inoculants at different concentrations on the Inhibition of *Rhizoctonia Solani* linear growth

Liquid algal cultures (15 days old) were centrifuged at 5000 rpm for 15 min, then the supernatant was filtered through Whatman No.1 paper, followed by 0.22 µm membrane filtration to obtain sterile, cell-free filtrates (Zhang et al. 2025). The effect of the resultant filtrates of *D. abundans* NA1, *N. paludosum* NA2, and *T. torsiva* NA3 at differing concentrations on *R. solani*'s radial growth was evaluated in vitro. In sterile 9 cm glass petri dishes, the filtrate of microalgae under study in concentration of (0.1%, 0.25%, 0.5% and 1.0%) were incorporated into PDA agar plates. Plates were then incubated at 20±2 °C, with untreated PDA serving as control. Each treatment was replicated three times, and mycelial growth inhibition (MGI) was calculated after 5 days according to the formula described by Ong et al. (2013).

Percentage of mycelia growth inhibition

$$(MGI)\% = \frac{DC-DT}{DC} \times 100.$$

Where: DC= average diameter of the mycelia growth in the control.

DT= average diameter of the mycelia growth in the treatment.

Total antioxidant Capacity, Phenolics, and flavonoids in the algal extracts

The antioxidant and phytochemical properties of algal extracts were evaluated using standard spectrophotometric assays (UV/Vis Analytikjena Specord D250, Germany). Antioxidant capacity was assessed by the phosphomolybdenum method, with results were expressed as ascorbic acid equivalents (Prieto et al. 1999). Total phenolics were quantified with the Folin–Ciocalteu reagent and expressed as gallic acid equivalents (Singleton et al. 1999), while flavonoid content was determined through the AlCl₃ colorimetric assay using quercetin as a reference standard (Arvouet-Grand et al. 1994).

Greenhouse studies

Pathogenicity test and relative virulence of four isolates of *R. solani*

The pathogenicity test was carried out using four isolates of *R. solani* (R1, R2, R3 and R4) to evaluate their pathogenic capability under the greenhouse conditions at the Central lab of Organic Agriculture, ARC, Giza, Egypt. A routine germination test was performed on cv. *Nebraska* bean seeds prior to planting, confirming a 100% germination rate. Seeds of cv *Nebraska* were sown in all pots at a rate of five seeds per pot with three pots were used for each replicate.

Inoculum Preparation and soil infestation

Inoculum of *R. solani* was prepared in sterilized glass bottles, each containing 250 g of corn-sand meal medium supplemented with 0.2% peptone solution (Abd-El-Moity 1985). Bottles were inoculated with equal sized agar disks (0.5 cm in diameter) taken from the edge of an actively growing culture of each *R. solani* isolate. After 12 days of incubation at 25 °C, the number of propagules per gram of each isolate was determined. Prior to soil infestation, the inoculum density was adjusted to approximately 5×10^6 CFU g⁻¹, and the propagules' viability was evaluated via serial dilution plating on PDA medium. Plastic pots (25 cm diameter) each containing 3 kg of sterilized soil were infested with the prepared inoculum at the rate of 10 g of corn-sand meal per kg soil, with each gram containing 5×10^6 CFU. Soil infestation was carried out 5 days before seeds sowing. Pots without infestations were used as control. Three replicates were included for each treatment.

Disease assessment

The percentages of pre- and post-emergence damping-off were calculated 15 and 30 days.

after sowing, respectively, while the percentage of healthy surviving plants was recorded after 45 days. All percentages of disease assessments were calculated as follows (El-Helaly et al. 1970):

$$\% \text{ pre-emergence} = \frac{\text{Number of non-germinated seeds}}{\text{Total number of sown seeds}} \times 100.$$

$$\% \text{ post-emergence} = \frac{\text{Number of dead seedlings}}{\text{number of sown seeds}} \times 100. \text{ Total}$$

$$\% \text{ survival plants} = \frac{\text{Number of survivals}}{\text{number of sown seeds}} \times 100 \text{ Total}$$

Two groups of treatments were established: the first received a soil drench application of the algal inoculant, while the control plants remained uninoculated. The second group involved seed soaking in the algal inoculant for six hours, whereas the control group contained seeds soaked in water only (uninoculated seeds). The experiment was conducted following a completely randomized design (CRD) with three replicates for each treatment, each pot comprising five seeds planted in 2.7 kg of infected soil. The treatments were as follow;

D	20%	(<i>D. abundans</i> NA1)
	10%	(<i>D. abundans</i> NA1)
	5%	(<i>D. abundans</i> NA1)
N	20%	(<i>N. paludosum</i> NA2)
	10%	(<i>N. paludosum</i> NA2)
	5%	(<i>N. paludosum</i> NA2)
T	20%	(<i>T. torsiva</i> NA3)
	10%	(<i>T. torsiva</i> NA3)
	5%	(<i>T. torsiva</i> NA3)
C	Control	Untreated seeds or/plants

Evaluating the effectiveness of various application methods of microalgal inoculants in controlling root rot in common bean plants under greenhouse conditions

A pot experiment was carried out to assess the efficacy of various microalgal inoculants, *Desmodesmus abundans* NA1, *Nostoc paludosum* NA2, and *Tildenella torsiva* NA3, for controlling damping-off disease in common bean plants. The microalgal inoculants were prepared at three different concentrations: 5, 10, and 20%, following the method described by Mutale-joan et al. (2020). *R. solani* inoculum, cultivated on sand-corn meal medium ($10^6 \times 5$ CFU g⁻¹), was used to infest the soil at a rate of 10 g/kg soil. Two groups of treatment were established: the first one obtained a soil drench application of the algal inoculant, while the control plants remained uninoculated. Where, the second group involved seed soaking in the algal inoculant for six hours, but the control group contained seeds soaked in water only (uninoculated seeds). The experiment was carried out in 2021 following a completely randomized design (CRD), with three replicates for each treatment, each pot comprising five seeds planted in 2.7 kg of infected soil.

Field experiment

The field experiment expanded upon the usage of the most successful algal inoculum application technique, which produced the best results in the greenhouse trial. It was carried out to investigate the effectiveness of microalgal inoculants, applied singly or in combination, in reducing damping off disease caused by soil-borne pathogens, as well as their potential to enhance both crop yield and quality. After preparing the experimental field

through plowing and puddling, three replicates of 120 seeds per treatment and control were sown as previously described. The control plants were grown in untreated soil, without algal inoculation. The experiment was conducted during two successive seasons (2022 and 2023), using a Randomized Complete Block Design (RCBD). The experimental field was divided into blocks to minimize the effect of environmental variation, and each block was further divided into a number of plots. Each plot measured 10.5 m² and consisted of three rows, each 70 cm wide and 5 m long. All treatments were randomly assigned to the plots, to ensure uniform representation. Seeds were sown at a spacing of 5 cm within the rows. All agronomic practices were performed in accordance with the guidelines of the Horticulture Research Institute, Agricultural Research Center (ARC). Twenty-two treatments, comprising both individual and combined micro-algal inoculants, were randomly distributed among the experimental plots. The individual inoculants were those described in the previous section, while the combinations included the following;

D+N	20%	(<i>D. abundans</i> NA1 + <i>N. paludosum</i> NA2)
	10%	(<i>D. abundans</i> NA1 + <i>N. paludosum</i> NA2)
	5%	(<i>D. abundans</i> NA1 + <i>N. paludosum</i> NA2)
N+T	20%	(<i>N. paludosum</i> NA2 + <i>T. torsiva</i> NA3)
	10%	(<i>N. paludosum</i> NA2 + <i>T. torsiva</i> NA3)
	5%	(<i>N. paludosum</i> NA2 + <i>T. torsiva</i> NA3)
D+T	20%	(<i>D. abundans</i> NA1 + <i>T. torsiva</i> NA3)
	10%	(<i>D. abundans</i> NA1 + <i>T. torsiva</i> NA3)
	5%	(<i>D. abundans</i> NA1 + <i>T. torsiva</i> NA3)
	20%	(<i>D. abundans</i> NA1 + <i>N. paludosum</i> NA2 + <i>T. torsiva</i> NA3)
D+N+T	10%	(<i>D. abundans</i> NA1 + <i>N. paludosum</i> NA2 + <i>T. torsiva</i> NA3)
	5%	(<i>D. abundans</i> NA1 + <i>N. paludosum</i> NA2 + <i>T. torsiva</i> NA3)
C	Control	Uninoculated plants

Plant analysis

Pigments contents

Three plants per plot were sampled after 35 days of growth to assess chlorophyll a, chlorophyll b, and carotenoids according to the method of Lichtenthaler (1987). Leaf tissues (50 mg) were extracted in 80% acetone, stored at freezing temperature for 48 h. The extracts were then centrifuged, and absorbance was measured at 663, 647, and 470 nm with a Spectronic 21D spectrophotometer. Pigment contents were calculated using standard equations and expressed as mg g⁻¹ fresh weight.

$$\text{Chl.a (mg/g)} = 12.25(\text{A}_{663}) - 2.79(\text{A}_{647}).$$

$$\text{Chl.b (mg/g)} = 21.50(\text{A}_{647}) - 5.10(\text{A}_{663}).$$

$$\text{Carotenoids (mg/g)} = (1000(\text{A}_{470}) - 1.82[\text{Chl.a}] - 85.02[\text{Chl.b}]) / 198.$$

Evaluation of Nitrogen, Phosphorus, and potassium in seeds

Seed samples (0.5 g) were digested with H₂SO₄–HClO₄ mixture, according to Chapman and Pratt (1961). Nitrogen content was determined by the Kjeldahl method (Jackson 1973), potassium by flame photometry (Jackson 1967), and phosphorus by inductively coupled plasma (ICP) using an Ultima 2JY Plasma spectrometer.

Vegetative characteristics, yield, and yield-related components of bean plants

At harvest, ten plants were randomly selected from each experimental plot to assess the number of branches per plant, total plant weight (g), root weight (g), and plant height (cm). Additionally, the number of pods per plant, the number of seeds per pod, the total number of seeds per plant, the seed weight per plant (g), the 100-seed weight (g), and the total seed yield per feddan were recorded.

Statistical analysis

The experiment was conducted during two consecutive seasons (2022 and 2023), with each treatment replicated three times per season, resulting in a total of six replicates. Data were expressed as means ± standard error (SE) of six replicates. Statistical analyses were performed using CoStat software version 4.2 (Cohort Software, Berkeley, CA, USA). One-way analysis of variance (ANOVA) was used to assess the significance of differences among treatments, and Duncan's multiple range test was applied for post hoc comparisons. Statistical significance was determined at $p \leq 0.05$, with six biological replicates per treatment.

Results

Isolation and identification of bean root rot pathogens

The damping-off pathogenic fungi

Samples of bean plants exhibiting symptoms of damping-off were collected from four distinct governorates. Based on their morphological, cultural, and microscopic traits, representative isolated colonies were purified and recognized as *R. solani* isolates. Four isolates of *R. solani* were isolated, representing the four governorates. The isolates were evaluated for pathogenicity against bean seeds.

Pathogenicity test and relative virulence of four *R. solani* isolates

The four isolates of *R. solani* evaluated on cv Nebraska under greenhouse conditions showed that all of them infected the roots of bean causing damping-off symptoms and reduced survived plants. It was clear that *R. solani* isolates varied significantly in their ability to cause damping-off (Table 1). All tested isolates caused damping-off ranging between 66.6 and 3.0%. *R. solani* isolate No.

Table 1 Pathogenicity test of four *Rhizoctonia Solani* isolates on damping-off incidence in bean plants under greenhouse conditions. Means with different letters (a-e) in each column are significantly different according to duncan's post hoc test at $p < 0.05$

Sources of isolates	Fungal isolates	Damping-off incidence (%)		Survival plants (%)
		Pre-emergence	Post-emergence	
El-Giza	<i>R. solani</i> No.1	3.3 ^d	13.3 ^b	83.3 ^b
El-Sharkia	<i>R. solani</i> No.2	15.0 ^c	10.0 ^{bc}	75.0 ^c
El-Fayoum	<i>R. solani</i> No.3	23.3 ^b	21.6 ^a	55.0 ^d
Beni suef	<i>R. solani</i> No.4	66.6 ^a	6.6 ^c	26.6 ^e
control		0.0 ^d	0.0 ^d	100.0 ^a
LSD 0.05		4.0	4.0	5.2

Table 2 Efficacy of microalgal filtrates at varying concentrations on *Rhizoctonia Solani* growth inhibition. Means with different letters (a-d) in each column are significantly different according to duncan's post hoc test at $p < 0.05$

Microalgal species	Concentrations (%)			
	0.1	0.25	0.5	1.0
	% of reduction in mycelial growth of <i>R. solani</i>			
<i>D. abundans</i> NA1	0.0 ^b	11.4 ^b	17.7 ^b	21.8 ^b
<i>N. paludosum</i> NA2	0.0 ^b	1.1 ^c	4.4 ^c	15.9 ^c
<i>T. torsiva</i> NA3	34.0 ^a	55.5 ^a	73.3 ^a	77.4 ^a
control	0.0 ^b	0.0 ^c	0.0 ^d	0.0 ^d
LSD 0.05	0.6	1.2	1.7	1.0

4 was the most aggressive one since it caused 66.66% pre-emergence damping-off, followed by isolate No.3 which caused 23.3%, whereas isolate No.2 occupied the third rank. On contrary, when the pathogenic isolates were compared for post-emergence damping-off, the percentage of infection caused by isolate No. 3 was 21.6% followed by isolate No.1 and No.2, being 13.3 and 10.0%, respectively. The percentage of survival plants was 26.6, 55.0, 75.0 and 83.3% for *R. solani* isolates No. 4, 3, 2 and 1 respectively. The fungus was re-isolated from the infected plants and was found to be identical with the original. The highly pathogenic *R. solani* isolate No. 4 was chosen to be used with further studies based on their pathogenic abilities.

Evaluation the efficacy of microalgal inoculants at different concentrations on the Inhibition of *Rhizoctonia Solani* linear growth

Different concentrations of the investigated microalgae assessed for their inhibiting effect against *R. solani* (Table 2). *T. torsiva* NA3 exhibits the strongest antifungal activity against *R. solani* through all tested concentrations. In particular, its inhibitory effect increases markedly with concentration, reaching a maximum of 77% at 1.0% ($p \leq 0.05$). This consistent and significant suppression, even at the lowest concentration (34.0% at 0.1%), highlights its potent biocontrol potential. In contrast, *D. abundans* NA1 showed moderate to weak inhibition, with values ranging from 11.4% to 21.8%. While it is significantly better than the control, its performance is

notably inferior to *T. torsiva* NA3, suggesting a less effective antifungal mechanism.

N. paludosum NA2 had the least impact among the three algal strains, showing negligible inhibition at the lower concentrations and only mild suppression (15.9%) at the highest dose. The control group, as expected, exhibited no inhibition across all concentrations, validating the reliability of the experimental design.

Overall, the results confirms the significant antifungal activity of *T. torsiva* NA3, highlighting it as an eco-friendly sustainable biocontrol agent against soil-borne pathogens.

Determination of total phenolic content, flavonoids, and antioxidant capacity of microalgal species utilized as bioinoculants

Total phenols of *D. abundans* NA1, *N. paludosum* NA2 and *T. torsiva* NA3 as ppm gallic acid equivalent were determined (Table 3). *D. abundans* NA1 was the only species proven to produce higher amount of total phenols (1127.3 ppm) as gallic acid equivalent. No detectable levels of total phenols were observed in *N. paludosum* NA2 and *T. torsiva* NA3.

The total flavonoid concentration, measured in quercetin equivalents, for each of the three algal species established (Table 3). It is evident that *D. abundans* NA1 was the most effective species in producing exceptionally high levels of flavonoids (4739.9 ppm), significantly surpassing the amounts produced by *N. paludosum* NA2 (543.8 ppm) and *T. torsiva* NA3 (1106.4 ppm) ($p \leq 0.05$).

The total antioxidant capacity of the microalgal species employed as bioinoculants was evaluated in terms of ascorbic acid equivalents and illustrated in Table 3. A significant difference ($p \leq 0.05$) was detected among the tested species. *D. abundans* NA1 exhibited the highest antioxidant capacity (15442.1 ppm), outperforming *N. paludosum* NA2 (570.0 ppm) and *T. torsiva* NA3 (1563.9 ppm).

Taken together, these results collectively indicate that *D. abundans* NA1 had a superior ability to synthesize phenolic and flavonoid compounds which could be responsible for its elevated antioxidant potential.

Table 3 Total phenols as ppm gallic acid equivalent, total flavonoids as ppm quercetin equivalent, total antioxidant capacity as ppm ascorbic acid equivalent of *Desmodemus abundans* NA1, *Nostoc paludosum* NA2 and *Tildeniella Torsiva* NA3. Means with different letters (a-c) are significantly different according to duncan's post hoc test at $p < 0.05$

Algal species	Total phenols ppm (gallic acid equivalent)	Total flavonoids ppm (quercetin equivalent)	Total antioxidant capacity ppm (ascorbic acid equivalent)
<i>Desmodemus abundans</i> NA1	1127 ^a	4739.9 ^a	15442.1 ^a
<i>Nostoc paludosum</i> NA2	0 ^b	543.8 ^c	570 ^c
<i>Tildeniella torsiva</i> NA3	0 ^b	1106.4 ^b	1563.9 ^b
LSD 0.05	31.14	87.3	60.64

Table 4 Comparative evaluation of the efficacy of algal inoculants; *Desmodemus abundans* NA1 (D), *Nostoc paludosum* NA2 (N), and *Tildeniella Torsiva* NA3 (T) applied either soil drench or by seed soaking for the management of damping-off in bean plants under greenhouse conditions at 2021 season. Means with different letters (a-g) are significantly different according to duncan's post hoc test at $p < 0.05$

Treatment	Concentration	Soil drench			Plant Soaking		
		Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survival (%)	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survival (%)
D	20%	8.3 ^e	0.0 ^f	91.6 ^a	10.00 ^f	1.66 ^f	88.3 ^a
	10%	13.3 ^d	5.0 ^e	80.8 ^b	14.1 ^e	5.8 ^e	80.8 ^b
	5%	18.3 ^c	8.3 ^{bcd}	74.1 ^c	20.0 ^{cd}	10.0 ^{bcd}	70.0 ^d
N	20%	9.1 ^e	9.1 ^{bc}	84.1 ^b	13.3 ^e	12.5 ^{ab}	70.0 ^d
	10%	24.1 ^b	5.8 ^{de}	70.0 ^c	24.1 ^b	9.1 ^{cd}	66.6 ^{def}
	5%	21.1 ^b	7.5 ^{cde}	69.1 ^e	26.6 ^b	8.3 ^{de}	65.0 ^{ef}
T	20%	15.8 ^{cd}	5.8 ^{de}	84.1 ^b	18.3 ^d	5.8 ^e	75.8 ^c
	10%	16.6 ^c	10.8 ^b	79.1 ^b	21.6 ^c	10.8 ^{abcd}	67.5 ^{de}
	5%	18.3 ^c	10.0 ^{bc}	73.3 ^c	25.8 ^b	11.6 ^{abc}	62.5 ^f
Control		32.5 ^a	13.3 ^a	54.1 ^d	32.5 ^a	13.3 ^a	54.1 ^g
LSD 0.05		2.69	1.90	3.80	2.45	2.19	3.56

Assessment of the biocontrol potential of microalgal inoculants against *Rhizoctonia Solani* in common bean plants under greenhouse conditions

The effectiveness of three algal isolates in controlling damping-off in bean plants grown in greenhouses was assessed using two different microalgal inoculant treatment techniques: soil drench and seed soaking. In comparison to the untreated control, the results shown in (Table 4) show that all algal isolates significantly ($p \leq 0.05$) decreased damping-off before and after emergence and increased the number of surviving plants. The extreme reduction in disease incidence both pre- and post-treatment was noted with isolate *D. abundans* NA1, achieving a reduction percentage 8.3% & 0.0% at a 20% concentration. Likewise, isolate *N. paludosum* NA2 exhibited its highest reduction at the same 20% concentration, recording stable values of 9.1%. Moreover, the data show that *T. torsiva* NA3 was the least effective in enhancing plant survival under the seed soaking treatment, with a survival rate of 62.5%. Thus these findings indicate that the biocontrol effectiveness of algal isolates against damping-off is concentration-dependent ($p \leq 0.05$), with soil drench proving more effective than seed soaking in reducing both pre- and post-emergence damping-off.

Evaluation of the microalgal inoculants, both individual and combined, in controlling damping-off disease of bean plants under field conditions

Individual microalgal inoculants

The application of algal inoculants significantly decreased damping-off relative to the untreated control ($p \leq 0.05$) under field conditions (Table 5). The most effective treatment was *D. abundans* NA1 (D) at 20%, which resulted in the lowest pre-emergence disease (30.8%) and the highest plant survival (68.3%). *T. torsiva* NA3 (T) at the same concentration also enhanced survival considerably (52.9%). Conversely, low concentrations of *N. paludosum* NA2 (N) were less effective, with survival rates falling below 20%. Overall, increasing the inoculum concentration enhanced disease suppression and promoted better establishment of the crop stand. In this regard, a clear positive correlation between the concentration of algal inoculants and their biocontrol potential, which may be attributed to higher production of bioactive metabolites which inhibit soil-borne pathogens and enhance early seedling development.

Table 5 Evaluation of the efficacy of combined microalgal inoculants *Desmodesmus abundans* NA1 (D), *Nostoc paludosum* NA2 (N), and *Tilidniella Torsiva* NA3 (T) in controlling damping-off of bean plants under field conditions. Data are presented as the means of two seasons (2022 and 2023). Means with different letters (a-h) are significantly different according to duncan's post hoc test at $p < 0.05$

Treatment	Concentration	Pre-emergence damping-off (%)	Post-emergence damping-off (%)	Survival plants (%)
D	20%	30.8 ^h	0.83 ^d	68.33 ^a
	10%	45.0 ^f	5.83 ^{bc}	49.16 ^c
	5%	48.3 ^e	8.33 ^b	43.33 ^d
N	20%	47.1 ^e	6.66 ^{bc}	46.16 ^d
	10%	65 ^c	9.16 ^b	25.83 ^f
	5%	67.5 ^b	14.16 ^a	18.33 ^g
T	20%	42.9 ^g	4.16 ^c	52.93 ^b
	10%	61.6 ^d	7.5 ^{bc}	30.83 ^e
	5%	65.8 ^{bc}	6.66 ^{bc}	27.5 ^f
control		93.3 ^a	1.66 ^d	5 ^h
LSD 0.05		1.8	2.33	2.91

Table 6 Evaluation of the efficacy of combined microalgal inoculants *Desmodesmus abundans* NA1 (D), *Nostoc paludosum* NA2 (N), and *Tilidniella Torsiva* NA3 (T) on controlling damping-off of bean plants under field conditions. Data are presented as the means of two seasons (2022 & 2023). Means with different letters (a-i) are significantly different according to duncan's post hoc test at $p < 0.05$

Treatment	Concentration	Pre-emergence Damping-off (%)	Post-emergence Damping-off (%)	Survival plants (%)
D+N	20%	35.8 ^g	5.0 ^c	59.1 ^b
	10%	39.1 ^f	5.8 ^{bc}	55.0 ^c
	5%	46.6 ^d	6.6 ^{bc}	46.6 ^e
N+T	20%	46.6 ^d	8.3 ^{ab}	45.0 ^{ef}
	10%	56.6 ^b	10.0 ^a	33.3 ^h
	5%	58.3 ^b	10.8 ^a	34.1 ^h
D+T	20%	43.3 ^e	5.8 ^{bc}	50.8 ^d
	10%	49.1 ^{cd}	9.1 ^a	41.6 ^{fg}
	5%	50.0 ^c	10.8 ^a	39.1 ^g
D+N+T	20%	30.0 ^h	0.0 ^d	70.0 ^a
	10%	31.6 ^h	1.6 ^d	66.6 ^a
	5%	35 ^g	2.5 ^d	62.5 ^b
control		93.3 ^a	1.6 ^d	5 ⁱ
LSD 0.05		2.1	2.01	3.97

Combined microalgal inoculants

The combined application of algal inoculants had a pronounced effect on both damping-off suppression and plant survival ($p \leq 0.05$) (Table 6). The mixture of *D. abundans* NA1, *N. paludosum* NA2, and *T. torsiva* NA3 (D+N+T) at all tested concentrations provided the most effective control, reducing pre-emergence damping-off to as low as 30% and completely preventing post-emergence losses at the highest concentration, resulting in the highest survival rate of 70%. Among two-species combinations, *D. abundans* NA1 and *N. paludosum* NA2 (D+N) at 20% concentration performed significantly better ($p \leq 0.05$) than *D. abundans* NA1 and *T. torsiva* NA3 (D+T) or *N. paludosum* NA2 and *T. torsiva* NA3 (N+T), maintaining pre-emergence damping-off at 35.8% and survival at 59.1%. In contrast, the untreated control exhibited severe pre-emergence losses (93.3%) and very low survival (5%). A clear concentration-dependent trend

($p \leq 0.05$) was observed across all combinations, where high inoculum levels consistently enhanced disease suppression and improved crop establishment. Accordingly, these results suggest a synergistic effect of multi-species inoculants, particularly within the triple combination, in inhibition soil-borne damping-off and supporting healthy plant development.

Effect of the microalgal inoculants as individual and combined on common bean pigmentation

The findings displayed that the amount of pigment accumulated in response to various algal treatments varied significantly ($p \leq 0.05$) as shown in Fig. (1a). The highest amounts of chlorophyll a were consistently found under *D. abundans* NA1 (D) 20% (1.3 mg g⁻¹ FW) and *N. paludosum* NA2 (N) 20% (1.2 mg g⁻¹ FW), out of all the pigments. Compared to the control and the majority of other treatments, these levels were noticeably higher.

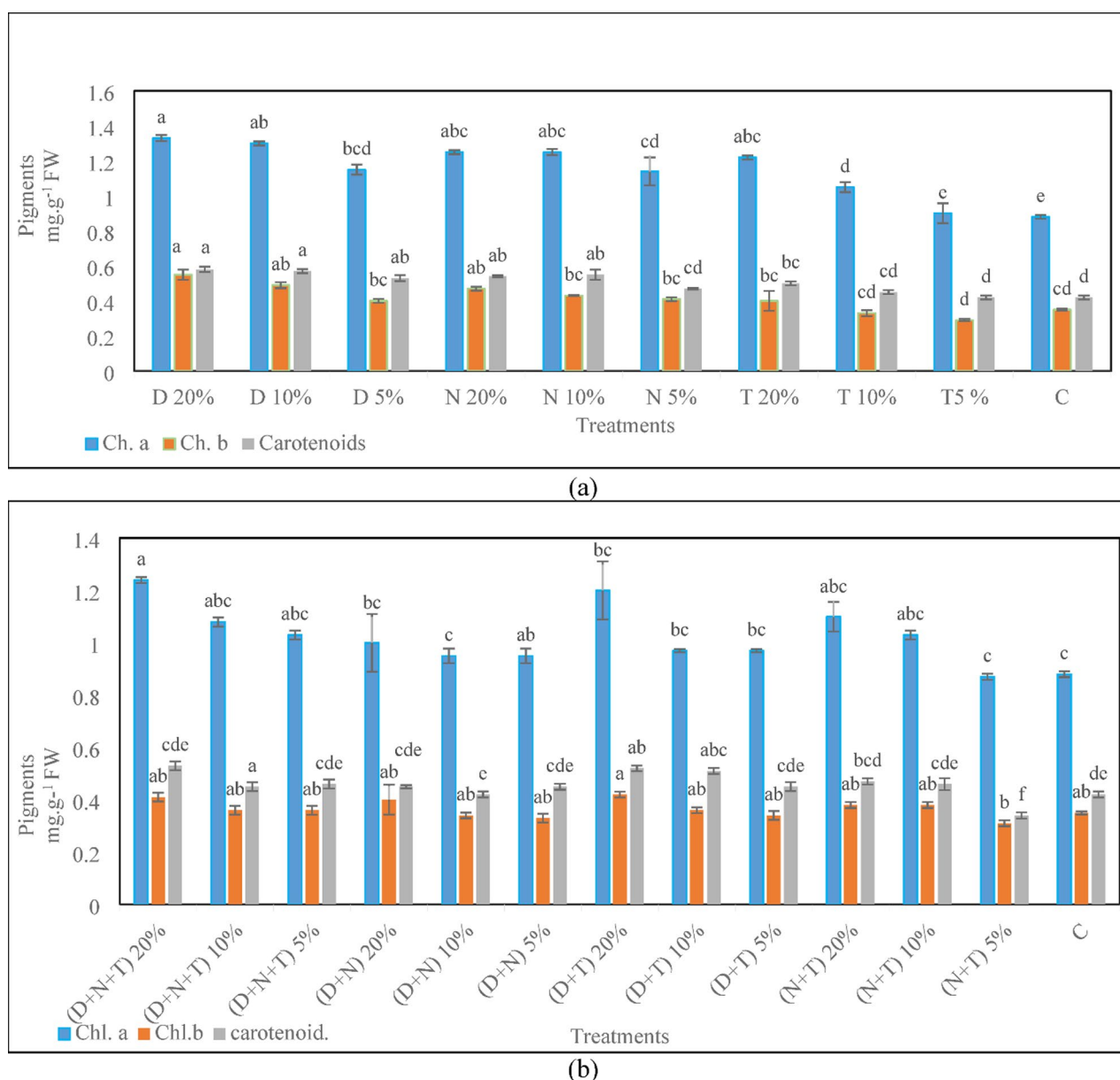


Fig. 1 Effect of the different concentrations of **(a)** individual microalgal inoculants; **(b)** combined microalgal inoculants *Desmodesmus abundans* NA1 (D), *Nostoc paludosum* NA2 (N), and *Tildenella torsiva* NA3 (T), on Chlorophyll a, b and Carotenoid content of a bean plants under field conditions. Data are presented as the means of two seasons (2022 and 2023). Means with different letters (a-f) are significantly different according to Duncan's post hoc test at $p < 0.05$

At the lowest concentrations (10% and 5%), a slow drop was noticeable, suggesting a concentration-dependent impact. The lowest values were recorded in *T. torsiva* NA3 (T) at 5% and the control (0.9 and 0.8 mg/g).

With the highest values under *D. abundans* NA1 (D) 20% (0.5 mg g⁻¹ FW) and *N. paludosum* NA2 (N) 20% (0.4 mg g⁻¹ FW), chlorophyll b showed a similar but less noticeable pattern. Carotenoids, which ranged from 0.4 to 0.5 mg g⁻¹ FW, was largely constant among regimens. Overall, algal treatments significantly increased pigment

accumulation relative to the control ($p \leq 0.05$), with chlorophyll a displaying the most distinct response.

As seen in Fig. (1b), the combined algal treatments had a significant effect on the pigment concentrations compared to control ($p \leq 0.05$), with chlorophyll a consistently appearing as the largest percentage. The triple combination (D+N+T) at 20% recorded the highest chlorophyll a level (1.2 mg g⁻¹ FW), showing a significant increase ($p \leq 0.05$), over the control (0.88 mg g⁻¹ FW). Also, chlorophyll b showed a similar pattern, peaking at 0.4 mg g⁻¹ FW under the D+N+T 20%, differing significantly

($p \leq 0.05$) from the control $0.32 \text{ mg g}^{-1} \text{ FW}$. There was minimal variation between treatments in carotenoids concentration, which ranged from 0.3 to $0.5 \text{ mg g}^{-1} \text{ FW}$. Yet, the triple mixture D + N + T 20% treatment resulted in the significant ($p \leq 0.05$) accumulation ($0.5 \text{ mg g}^{-1} \text{ FW}$), indicating that higher algal concentrations also enhance carotenoid synthesis, although to a lesser extent than chlorophylls. Overall, the findings confirm that multi-species inoculation, especially the triple inoculations at higher doses, was significantly ($p \leq 0.05$) more effective than dual combinations and induced a pronounced enhancement in pigment production, emphasizing synergistic effects that support photosynthetic capacity.

Effect of individual and combined microalgal inoculants on the growth characteristics of common bean

The effects of individual microalgal inoculants on the common bean plants' growth traits were assessed and presented in (Table 7). In comparison to the control, algal treatments significantly ($p \leq 0.05$) improved the assessed growth and yield parameters. With the tallest plants (64.2 cm), the heaviest plant biomass (99.0 g), the highest seed weight per plant (115 g), and the greatest seed yield per feddan (3703.8 kg), *D. abundans* NA1 (D) at 20% concentration produced the most significant ($p \leq 0.05$) increases among all treatments. Compared to the untreated control, which had the lowest values in every category, this indicates a notable enhancement in both vegetative and reproductive features.

While the 5% concentration still beat the control but clearly declined significantly ($p \leq 0.05$) when compared to high doses. The 10% concentration of *D. abundans* NA1(D) retained good effects, although by a smaller amount. On the other side, 20% *N. paludosum* NA2 (N) showed a significant ($p \leq 0.05$) impact on reproductive performance, particularly the number of pods (38.0 pods)

and seeds produced per plant (118 seeds). The 10 and 5% treatments produced very slight improvements, indicating that low amounts of *N. paludosum* NA2 (N) were less effective.

At 20%, *T. torsiva* NA3 (T) significantly ($p \leq 0.05$) increased the number of branches per plant (5.6) and root weight (3.9 g). But compared to *D. abundans* NA1, it contributed less to the total seed output (1782.7 kg/feddan). *T. torsiva* NA3 (T) at low concentrations, had a markedly reduced impact ($p \leq 0.05$) on the majority of measured parameters.

Collectively, the results detect a strong concentration-dependent response ($p \leq 0.05$), with the 20% applications generally outperforming 10% and 5%. *D. abundans* NA1 (D) proved the most effective treatment, consistently enhancing plant growth, reproductive output, and yield potential.

The impact of the algal combinations on the growth characteristics of common bean, is presented in (Table 8). The results indicated that simultaneous algal inoculations significantly ($p \leq 0.05$) improved plant growth and yield attributes when compared to the untreated control. The triple mixture (D + N + T) at 20% concentration, proved to be the most successful treatment, resulting in the tallest plants (70.0 cm), the highest plant biomass (118.0 g), the heaviest seed weight per plant (97.6 g), and the greatest yield per feddan (2836.7 kg), detected as a response to the triple mixture (D + N + T) at 20% concentration, proved to be the most successful treatment. These findings clearly demonstrated the synergistic effect of multi-strain inoculation, which significantly ($p \leq 0.05$) surpassing both the control and dual-species treatments. Among the dual combinations, D + T at 20% showed the greatest improvement, especially in seed yield (2773.3 kg/feddan), which was comparable to that of triple mixture. Although the 20% N + T treatment resulted in the highest

Table 7 Effect of the combined microalgal inoculants; *Desmodesmus abundans* NA1 (D), *Nostoc paludosum* NA2 (N), and *Tildenella Torsiva* NA3 (T) on growth parameters of bean plant under field conditions. Data are presented as the means of two seasons (2022 and 2023). Means with different letters (a-i) are significantly different according to duncan's post hoc test at $p < 0.05$

Treatment	Conc.	Plant Height (cm)	Plant weight (g)	Root weight (g)	Branches/plant	Pod number/plant	Seed number/pod	Seed number/plant	Seed Weight/ plant (g)	100-Seed Weight (g)	Seed weight/ feddan (kg)
D	20%	64.2 ^a	99.0 ^a	2.6 ^{cd}	4.6 ^{abcd}	30.0 ^c	5.3 ^b	129.6 ^a	115 ^a	123.6 ^a	3703.8 ^a
	10%	60.2 ^b	64.3 ^b	2.7 ^{cd}	4.0 ^{bcd}	29.0 ^c	5.0 ^b	128.0 ^a	80.0 ^b	86.6 ^b	2873.3 ^b
	5%	51.3 ^g	54.0 ^e	2.4 ^{cde}	3.6 ^{cd}	23.6 ^e	4.0 ^c	112.6 ^{bc}	56.6 ^c	67.0 ^d	1685.4 ^d
N	20%	58.1 ^c	61.3 ^{cd}	3.8 ^{ab}	5.0 ^{abc}	38.0 ^a	6.0 ^a	118.3 ^{ab}	67.6 ^{bc}	71.0 ^c	1846.4 ^c
	10%	57.1 ^d	62.3 ^c	2.8 ^c	4.0 ^{bcd}	21.0 ^f	5.0 ^b	115.3 ^{ab}	57.0 ^c	53.0 ^f	916.0 ^e
	5%	54.0 ^f	50.0 ^f	2.4 ^{de}	4.3 ^{abcd}	22.0 ^f	5.0 ^b	108.6 ^{bc}	53.3 ^c	46.0 ^h	738.5 ^f
T	20%	60.5 ^b	64.0 ^b	3.9 ^a	5.6 ^a	36.0 ^b	5.0 ^b	116.6 ^{ab}	71.3 ^{bc}	61.0 ^e	1782.7 ^c
	10%	54.8 ^e	60.0 ^d	3.7 ^{ab}	5.3 ^{ab}	27.0 ^d	5.0 ^b	111.6 ^{bc}	56.6 ^c	52.5 ^f	885.6 ^e
	5%	50.3 ^h	54.3 ^e	3.5 ^b	3.6 ^{cd}	26.0 ^d	4.6 ^b	99.3 ^c	51.9 ^c	48.3 ^g	511.1 ^g
control		41.5 ⁱ	50.6 ^f	2.2 ^e	3.3 ^d	21.6 ^f	5.0 ^b	55.6 ^d	27.4 ^d	35.2 ⁱ	85.2 ^h
LSD 0.05		0.6	1.4	0.25	0.9	1.5	0.4	10.6	12.4	1.3	69.5

Table 8 Effect of the combined microalgal inoculants; *Desmodesmus abundans* NA1 (D), *Nostoc paludosum* NA2 (N), and *Tildenella Torsiva* NA3 (T) on growth parameters of bean plant under field conditions. Data are presented as the means of two seasons (2022 and 2023). Means with different letters (a-j) are significantly different according to duncan's post hoc test at $p < 0.05$

Treatment	Conc.	Plant Height (cm)	Plant weight (g)	Root weight (g)	Branche/ plant	Pod number/plant	Seed number/pod	Seed number/ plant	Seed Weight /plant (g)	100-Seed Weight (g)	Seed weight/ feddan (kg)
D+N	20%	57.0 ^{ef}	50.0 ^h	3.1 ^d	4.0 ^{ab}	29.0 ^{cd}	5.0 ^a	119.0 ^d	54.0 ^g	49.9 ^d	1825.5 ^e
	10%	52.0 ^g	46.0 ⁱ	2.9 ^d	3.6 ^b	26.3 ^{ef}	4.6 ^a	107.0 ^f	51.0 ^h	48.8 ^{de}	1602.8 ^f
	5%	49.0 ^h	42.3 ^j	2.2 ^e	3.6 ^b	24.6 ^{fg}	4.3 ^a	98.6 ^g	48.6 ⁱ	44.6 ^f	1297.5 ^g
N+T	20%	61.0 ^c	109.6 ^b	4.5 ^a	4.6 ^{ab}	37.0 ^a	5.0 ^a	174.6 ^a	69.9 ^d	53.8 ^b	1798.2 ^e
	10%	56.0 ^{ef}	100.0 ^d	3.9 ^b	4.6 ^{ab}	29.0 ^{cd}	5.0 ^a	131.6 ^c	65.0 ^e	50.0 ^d	1237.9 ^h
	5%	50.3 ^h	93.3 ^e	3.5 ^c	4.0 ^{ab}	24.0 ^g	4.6 ^a	116.3 ^{de}	56.3 ^f	47.8 ^e	1099.2 ⁱ
D+T	20%	63.0 ^b	71.0 ^f	3.8 ^{bc}	4.6 ^{ab}	34.3 ^b	5.0 ^a	150.0 ^b	69.3 ^d	51.6 ^c	2773.3 ^b
	10%	61.0 ^c	65.0 ^g	3.2 ^d	4.0 ^{ab}	25.0 ^{fg}	5.0 ^a	115.0 ^e	56.0 ^f	49.0 ^{de}	2131.2 ^c
	5%	59.0 ^d	50.0 ^h	2.9 ^d	4.0 ^{ab}	21.0 ^h	5.0 ^a	109.0 ^f	53.0 ^g	48.3 ^e	1892.8 ^d
D+N+T	20%	70.0 ^a	118.0 ^a	4.6 ^a	5.6 ^a	38.0 ^a	5.0 ^a	172.0 ^a	97.6 ^a	56.0 ^a	2836.7 ^a
	10%	57.6 ^{de}	105.0 ^c	4.0 ^b	5.6 ^a	31.0 ^c	4.6 ^a	151.0 ^b	87.4 ^b	48.8 ^{de}	2082.2 ^c
	5%	55.3 ^f	99.0 ^d	3.5 ^c	4.0 ^{ab}	27.3 ^{de}	4.6 ^a	1340.0 ^c	74.0 ^c	47.8 ^e	1655.9 ^f
Control		41.5 ⁱ	50.6 ^h	2.2 ^e	3.3 ^b	21.6 ^h	5.0 ^a	55.6 ^h	27.4 ^j	35.2 ^g	85.2 ^j
LSD 0.05		1.4	2.9	0.2	1.1	1.7	0.6	2.7	1.8	0.8	53.0

Table 9 Effect of the individual microalgal inoculants; *Desmodesmus abundans* NA1 (D), *Nostoc paludosum* NA2 (N), and *Tildenella Torsiva* NA3 (T) on the macronutrients content in bean seeds under field conditions. Data are presented as the means of two seasons (2022 and 2023). Means with different letters (a-d) are significantly different according to duncan's post hoc test at $p < 0.05$

Treatment	Concentration	N (%)	P (%)	K (%)	Protein (%)
D	20%	2.0 ^{bc}	0.2 ^{bc}	1.3 ^{ab}	13.1 ^{bc}
	10%	1.9 ^{cd}	0.2 ^{bc}	1.2 ^{bc}	12.3 ^{cd}
	5%	1.8 ^d	0.2 ^c	1.1 ^d	11.5 ^d
N	20%	2.1 ^b	0.3 ^b	1.1 ^{cd}	13.7 ^b
	10%	1.9 ^{cd}	0.2 ^{bc}	1.2 ^{bc}	12.3 ^{cd}
	5%	1.9 ^d	0.2 ^{bc}	1.2 ^{bc}	11.9 ^d
T	20%	2.4 ^a	0.3 ^b	1.4 ^a	15.5 ^a
	10%	2.1 ^b	0.2 ^{bc}	1.2 ^{bc}	13.6 ^b
	5%	2.2 ^b	2.7 ^a	1.4 ^a	14.0 ^b
Control		1.9 ^{cd}	0.2 ^{bc}	1.2 ^c	12.1 ^{cd}
LSD 0.05		0.1	0.0	0.07	0.7

plant weight (109.6 g) and root biomass (4.5 g), its contribution to final seed yield (1798.2 kg/feddan) was lower than that of D + T and D + N + T combinations. The D + N treatment exhibited the least overall effect, with yields ranging from 1297.5 to 1825.5 kg/feddan; however, these values were still significantly ($p \leq 0.05$) higher than the control (85.2 kg/feddan). Generally, the results show that the strongest stimulation of growth, reproductive potential, and yield potential is provided by mixed algal inoculation, especially the three-species treatment at high concentration.

Effect of microalgal inoculants as individual and combined on bean seeds quality

Individual microalga inoculum effectiveness on common bean seed quality was demonstrated in the (Table 9). Both the type and concentration of algal inoculant had a significant ($p \leq 0.05$) impact on the macronutrient composition of bean seeds. *T. torsiva* NA3 (T) produced the significant ($p \leq 0.05$) highest nutrient enrichment at 20% application, with the highest nitrogen (2.4%), potassium (1.4%), and protein content (15.5%) values noted. This strain is particularly effective at promoting nutrient absorption, as seen by similar improvements observed at 5% concentration, when potassium and nitrogen levels were still greater than the control. 20% *N. paludosum* NA2 (N) enhanced seed nitrogen (2.1%) and protein (13.7%), whilst *D. abundans* NA1 (D) treatments showed a more moderate response, with advantages consistently lower than those obtained with *T. torsiva* NA3 (T) but still above the control. For all inoculants, high concentrations (20%) frequently significantly ($p \leq 0.05$) outperformed 10% and 5%, although sometimes even the lowest doses shown clear advantages over the control, reveal an obvious concentration-dependent trend. It is also worth noting that the effectiveness of algal inoculant depends not only on the species applied but also on the algal density, which in turn influences nutrient translocation and the biochemical composition of the seeds.

The combined algal inoculations significantly ($p \leq 0.05$) enhanced the macronutrient composition of bean seeds, when compared to the untreated control, as referred in (Table 10). The highest enhancement across all parameters was obtained by the triple mixture (D + N + T) at a 20% concentration, reaching maximum protein content

Table 10 Effect of the combined microalgal inoculants; *Desmodesmus abundans* NA1 (D), *Nostoc paludosum* NA2 (N), and *Tildeniella Torsiva* NA3 (T) on the macronutrients content in bean seeds. Data are presented as the means of two seasons (2022 and 2023). Means with different letters (a-h) are significantly different according to duncan's post hoc test at $p < 0.05$

Treatment	Concentration	N (%)	P (%)	K (%)	Protein (%)
D+N+T	20%	2.5 ^a	0.3 ^a	1.7 ^a	16.0 ^a
	10%	2.2 ^{cd}	0.3 ^{bc}	1.6 ^{abc}	14.1 ^{cd}
	5%	2.0 ^e	0.3 ^{bc}	1.6 ^{abc}	13.0 ^e
N+T	20%	2.0 ^e	0.2 ^e	1.5 ^{cd}	13.0 ^e
	10%	2.1 ^e	0.3 ^{cd}	1.4 ^{de}	13.1 ^e
	5%	1.9 ^f	0.2 ^f	1.1 ^h	12.0 ^f
D+T	20%	2.4 ^{ab}	0.2 ^e	1.6 ^{bc}	15.3 ^{ab}
	10%	2.3 ^{bc}	0.3 ^b	1.4 ^{de}	14.5 ^{bc}
	5%	2.1 ^{de}	0.2 ^e	1.3 ^{ef}	13.6 ^{de}
D+N	20%	2.5 ^a	0.3 ^{bcd}	1.7 ^{ab}	15.7 ^a
	10%	2.4 ^{ab}	0.3 ^a	1.2 ^{fg}	15.2 ^{ab}
	5%	2.1 ^{de}	0.3 ^d	1.5 ^{cd}	13.6 ^{de}
Control		1.9 ^f	0.2 ^f	1.2 ^{gh}	12.1 ^f
LSD 0.05		0.11	0.008	0.10	0.70

(16.0%), phosphorus (0.3%), potassium (1.7%), and nitrogen (2.5%).

The mixture of these two strains was highlighted by the significant ($p \leq 0.05$) good performance of D+N at 20% and 10% among the dual combinations, especially in nitrogen (2.5 and 2.4%) and protein content (15.7 and 15.2%). With nutritional levels just marginally higher than the control, particularly at lower concentrations, the N+T treatment demonstrated very modest benefits, while the D+T combination at 20% produced significant ($p \leq 0.05$) increases in potassium (1.6%) and protein (15.3%).

Overall, the data highlight that the best approaches for improving the nutritional value of bean seeds involve multi-strain treatments, especially the triple mixture and the D+N combination, through synergistic enhancement of nutrient uptake and biosynthesis.

Discussion

Our research explored the dual role of microalgal inoculants as biocontrol agents and biofertilizers simultaneously in the cultivation of common bean plants. *Rhizoctonia solani* was isolated based on damping-off symptoms observed in common bean plants previously collected from the governorates of Giza, El-Sharkia, Fayoum and Beni-suef, with variable virulence. These findings are consistent with those of Helmy and Abu-Hussien (2024) and Ghoneem et al. (2023), who documented *R. solani* as the predominant damping-off pathogen of *Phaseolus vulgaris* in Giza and El-Sharkia.

Pathogenicity tests showed that the Beni-Suef isolate exhibited the highest virulence, as it caused the most

severe damping-off symptoms. Consequently, this isolate was chosen to be studied further, identifying Beni-Suef as a hotspot for seedling damping-off.

Antagonistic tests demonstrated that all investigated microalgal strains suppressed *R. solani* mycelial growth to different extents. It is important to note that, *Tildeniella torsiva* NA3 exhibited the highest antifungal activity, even though it produced moderate levels of flavonoids and had lower antioxidant capacity than *Desmodesmus abundans* NA1. This means that the properties of antifungal activity is not solely reliant on phenolic content but also influenced by the constitution and synergistic interactions of secondary metabolites, such as peptides, alkaloids, fatty acids, hydrolytic enzymes, and volatile organic compounds (VOCs) (Asimakidis et al. 2022). The current observation can be compared to previous studies that showed that algal antifungal mechanisms are multifactorial in nature and various types of metabolites play a combined role in inhibiting pathogen growth (López-Arellanes et al. 2025). The novelty of the present findings lies in the absence of previous studies on the biocontrol potential of *Tildeniella* spp., recommending further investigation into its metabolite profile.

Achieved insights into the remarkable antioxidant potential of *D. abundans* NA1, which might be attributed to the fact that it has a tremendous potential of producing phenolic compounds (Shin et al. 2025). This is due to the fact that the phenolic compounds are effective antioxidants because they supply electrons through their hydroxyl groups, thus eliminating the reactive oxygen species and the free radicals (Aryal et al. 2019). These results are in agreement with previous studies that verified the ability of microalgae to produce flavonoids and phenolic compounds, showing their antioxidant potential as a stress-adaptive response. Moreover, the production of bioactive substances with antimicrobial properties enables microalgae to survive and compete by preventing pathogens from colonizing their environment (Helal et al. 2023). In contrast, no phenolic compounds were detected in *N. paludosum* NA2 and *T. torsiva* NA3, which can be attributed to the lack of nitrogen in their growth media (Akgül and Kösel 2023). Nevertheless, they synthesized notable levels of flavonoid levels with moderate antioxidant activity, consistent with previous finding on *N. paludosum* extracts showing antioxidant effects (Guerreiro et al. 2020). Accordingly, these results highlight the crucial role of nutrient availability in regulating the biosynthesis of bioactive metabolites.

Greenhouse experiments displayed that all microalgal inoculants were effective in damping-off control especially, *D. abundans* NA1 which exhibited the highest efficiency. Drenching of soil was more effective than seed soaking, which could possibly be attributed to improved root colonization, the sustained activity of bioactive

metabolites, and direct exposure to soil-borne pathogens (Delai et al. 2024). Moreover, microalgae improve the ability of plants to defend them by activating hydrolytic enzymes, increasing lignin deposition and promoting beneficial microbial interactions of microbes in the rhizosphere (Righini et al. 2025). Collectively, these effects, together with enhanced microbial activity and nutrient availability, leads to the overall health and vigor of plants (Solomon et al. 2025). Similarly, El-Sheekh et al. (2022) and Sara et al. (2022) highlighted the biocontrol capability of *Nostoc* species, in agreement with our findings, demonstrating their ability to suppress soil-borne pathogens in tomato and fennel plants, respectively. On the same note, Eckstien et al. (2024), presented the antifungal properties of microalgae, specifically, *Desmodesmus* sp. against soil-borne pathogens, which signifies their potential usefulness as biocontrol agents.

The results of greenhouse were confirmed in a field experiment conducted in two seasons, indicating that a single and a combination of microalgal inoculations produced significant positive effects on plant growth, survival, and total yield. The triple combination (D+N+T) and multi-strain inoculation with a strong synergistic effect have been observed to reduce the pre- and post-emergence damping-off and enhanced seedling development. This synergistic interaction may be explained by the complementary mechanisms which include the production of more antifungal metabolites, growth-hormones (auxins, cytokinins, gibberellins) and improving nutrient mobilization (Mohamed et al. 2025). This is consistent with reports of multi-strain consortia in rice and maize where combined microalgal inoculants were better than single strains in improving nutrient uptake and stress response (Liu et al. 2023).

All microalgal applications significantly increased photosynthetic pigment contents, with *D. abundans* NA1 and *N. paludosum* NA2 showing the most noticeable effects at higher concentrations. The elevated concentrations of chlorophyll a, chlorophyll b, and carotenoid levels may result from improved nutrient uptake and growth hormone-mediated chloroplast development, which delays leaf aging and enhances photosynthetic efficiency (Abdelwahab et al. 2024). Consequently, these physiological improvements directly led to increased plant biomass, improved reproductive performance, and higher yield. In harmony with our finding, Ghazal et al. (2025), detected a remarkable enhancement in chlorophyll content and growth characters of *Lupinus luteus* plants.

The usage of microalgal inoculants also significantly increased grain quality. In particular, triple-microbial inoculants at 20% recorded the highest levels of protein, nitrogen, phosphorus, and potassium, whilst the dual combinations (D+N) also indicated notable enhancements. Therefore, these improvements can be attributed

to the combined effects of nitrogen fixation, growth hormone production, and enhanced nutrient bioavailability, emphasizing the practical capability of multi-strain inoculants in enhancing both yield and seed quality. These results are in parallel with previous findings by González-González and de-Bashan (2023), who demonstrated that algal-bacterial consortia improved seed nutrient composition by promoting mineral mobilization and nitrogen fixation.

In addition to all of above, the improvement in the growth characteristics of all treated bean plants, this may be attributed to the positive effect of algal inoculants on root nodule growth and function. Notably, earlier studies have shown that certain microalgae, such as *Nostoc* species, produce extracellular polysaccharides (EPS), which act as natural adhesives, binding soil particles together to improve porosity, moisture retention, and aeration. Collectively, this result in enhancement microbial activity, particularly *rhizobium* sp. and root development. Also, EPS can form hydrogel-like structures that protect *Rhizobium* from desiccation's effects (Gonzalez-Gonzalez and de-Bashan 2023). Microalgae provide a carbon source that promotes nodulation and provide energy for *Rhizobium* activity and metabolism (Ramakrishnan et al. 2023). Also, microalgae may produce growth hormones such as (auxins, cytokinins and gibberellins) which are essential for the development of nodules and root system. For instance, auxins promote the formation of cortical cell divisions and root hair, increasing the surface area of the root and providing *Rhizobium* with additional sites for infection (Nagata and Suzuki 2014). Meanwhile, cytokinins control the number of nodules and activate crucial nodulation genes including ERN1 and NIN. These hormones signals precisely regulate the formation of symbiotic partnerships through calcium signaling pathways (Buhian and Bensmihen 2018). Furthermore, cytokinins influence germination, shoot/root growth, and leaf senescence, auxins regulate cell division and enlargement, and gibberellins mainly encourage the elongation and growth of cells (Do et al. 2021).

Notably, *Nostoc paludosum* NA2 was the promising strain assessed to improve pod formation and greatly increase the number of seeds per pod, may be attributed to its nitrogen-fixing capacity. This observation is in line with Herridge et al. (2008) who demonstrated that biological nitrogen fixation plays a crucial part in enhancing grain legume output by encouraging seed filling and pod development.

Overall, the present study confirms that microalgal inoculants, particularly multi-species formulations, provide an effective and synergistic approach for controlling damping-off, enhancing photosynthetic performance, and improving yield and seed quality in common bean. This observation has confirmed earlier by Ghazal et al.

(2025) who highlighted the important role of microalgae in controlling soil borne pathogens and improving the crop productivity at the same time.

Crucially, the large-scale production and field applicability of microalgal inoculants have become increasingly achievable in recent years. Recent advanced technologies in large-scale cultivation techniques, such as open raceway ponds and photobioreactors, have supported cost-effective production of algal biomass suitable for agricultural practice (Skifa et al. 2025). Microalgae have already been efficiently applied as biofertilizers and biofungicides across various crops, including tomato, rice, maize, and legumes, contributing to enhanced soil fertility, effective disease control, and yield improvement (Jurado-Flores et al. 2025). Moreover, These inoculants could be available in a variable forms such as, dried powders, encapsulated beads, or liquid suspensions, which can be easily applied through seed coating, soil drenching, or fertigation (Ibáñez et al. 2023). One of the major challenges in applying biofertilizers is ensuring their compatibility with other microbial fertilizers. In this context, microalgal inoculants offer a distinct advantage, as they are compatible with other biofertilizer agents, including rhizobacteria, yeast and mycorrhizal fungi, displaying no antagonistic effects and even promoting synergistic interactions that enhance nutrient cycling and overall plant health (Su et al. 2024). Collectively, these advances support the feasibility of integrating microalgae-based bioinoculants into existing sustainable agricultural frameworks, paving the way for their commercial adoption in field-scale crop production (Kapoor et al. 2021).

Conclusion

This study shed out on the significant biocontrol and biofertilizing capacity of soil microalgal inoculants, *D. abundans* NA1, *N. paludosum* NA2, and *T. torsiva* NA3, whether applied singly or in combination. At a 20% concentration, the triple inoculant completely controlled post-emergence damping-off and greatly increased plant survival and yield. The synthesis of phenolic compounds, flavonoids, antioxidant metabolites, and phytohormones was responsible for the strong antifungal and growth-promoting effects. Microalgal inoculants improved nitrogen uptake and seed quality, as well as photosynthetic pigments and nodulation, leading to yields that exceeded 3 tons/feddan under field conditions. These results emphasized the innovative potential of the triple microalgae inoculant as environmentally beneficial bio-stimulant and biocontrol agents in the development of sustainable legumes.

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Author contributions

NA designed the experimental approach, carried out the field crop study, contributed to full plant and soil analyses, interpreted the results, wrote and drafted the manuscript. AG carried out the field crop study. SA designed the experimental approach, contributed to full plant and soil analyses, data analysis and writing of the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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Data availability

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

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Competing interests

The authors declare no competing interests.

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