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RESEARCH PAPER

Mitigation of arsenic-induced stress in Turnip (*Brassica rapa* L.) plants through foliar application of *Chlorella vulgaris*

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Key Message: Foliar application of *Chlorella vulgaris*, particularly at 10% concentration, significantly alleviated arsenic-induced toxicity in turnip (*Brassica rapa* L.). It reduced arsenic accumulation and oxidative stress while enhancing antioxidant defense, photosynthetic efficiency, chlorophyll content, and plant growth.

Abstract

Plants are constantly exposed to various biotic and abiotic stress factors that adversely affect their growth, development, and overall productivity. Abiotic stress includes a variety of environmental stresses such as drought, salinity, severe temperatures, and chemical toxicity, with heavy metal stress considered as one of the most harmful abiotic stress. Arsenic (As) is one of the most poisonous heavy metals to crops. Excessive As levels in plants can cause biochemical and physiological abnormalities. They cause oxidative stress by accumulating ROS (reactive oxygen species), disrupting normal plant processes. *Chlorella vulgaris*, a green microalga, detoxifies heavy metal ions in soil and preserves the environment. This study examines how microalgae affect biochemical and physiological alterations in two turnip cultivars (Golden Ball and Purple Top). Plants were exposed to different arsenic concentrations (25 mM and 50 mM). As treatment reduced roots and shoots biomass, pigments produced by photosynthesis, and photosystem-II performance, but increased As accumulation in leaves, ROS (reactive oxygen species), and lipid peroxidation. Positive responses were seen at different microalgae concentrations, although the highest level of effectiveness was seen at 10% microalgae concentration. Arsenic stress caused damage to plants across different concentrations, with the highest level of damage was observed at 50 mM arsenic. Hence, arsenic stress increased As accumulation in the leaves of both turnip varieties. However, foliar application of *Chlorella vulgaris*, particularly at 10%, significantly reduced As accumulation and alleviated arsenic-induced oxidative stress, as evidenced by decreased reactive oxygen species (ROS) and malondialdehyde (MDA) levels. Therefore, it can be concluded that foliar spray of microalgae effectively regulates anti-oxidative pathways under heavy metal stress and serve as a possible defensive regulator to increase plant biomass and tolerance. © 2026 The Author(s)

Keywords: Arsenic toxicity, *Brassica rapa*, *Chlorella vulgaris,* Photosystem I & II, Reactive oxygen species, ROS scavenging system

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Introduction

Environmental pollution continues to pose a major global challenge, threatening ecosystems and human well-being (Kumar et al., 2025). Heavy metals pose a significant environmental threat due to their toxic effects, long-term persistence, and ability to accumulate within living organisms. Once released into the atmosphere, these contaminants may be transported over considerable distances before being deposited on land and water surfaces, thereby contributing to environmental contamination (Mustapha et al., 2026). Plants can absorb heavy metals through various physiological and transport mechanisms, which may disrupt their metabolic processes and adversely affect growth and development (Mohamed et al., 2025). When vegetables are cultivated in contaminated soils, heavy metals may accumulate in their edible tissues and subsequently enter the food chain. Continued exposure to these contaminants can pose significant risks to human health, including kidney damage, respiratory complications, neurological disorders, and certain types of cancer (Mititelu et al., 2025). The increasing prevalence of heavy metal contamination has therefore intensified the need for effective strategies to mitigate its environmental impacts (Kaur et al., 2021).

There are two major types of stress factors: biotic and abiotic stresses. Biotic stress arises from the effects of living organisms on plants and their biological interactions, including fungi, bacteria, oomycetes, worms, and herbivorous animals, which may adversely affect plant health under particular conditions (Kamran et al., 2025). In contrast, abiotic stress is caused by unfavorable environmental factors that are non-

living in nature, such as excessive radiation, salinity, flooding, drought, temperature extremes, and heavy metal exposure. These stresses can impair plant physiological functions, reduce productivity, and threaten the sustainability of agricultural production worldwide (Kamran et al., 2025). Industrial activities, particularly mining and the processing of chemicals and metals, are important sources of soil contamination because they can release metal-containing wastes into the surrounding environment (Kalisz et al., 2022). Through the movement of contaminants among soil, water, and air, heavy metals can spread across different environmental compartments and interfere with plant metabolism, physiological functioning, and overall development (Ghosh et al., 2022).

Algae comprise a diverse group of photosynthetic organisms characterized by the presence of chlorophyll a in many major groups, although they do not constitute a single evolutionarily related lineage. They contribute substantially to global photosynthetic productivity and exhibit considerable variation in size and structural complexity, ranging from unicellular organisms to large seaweeds that may attain lengths of approximately 60 m (Alvarez et al., 2021). Based on their size and growth form, algae are commonly classified into two broad categories: microalgae and macroalgae. In agricultural systems, microalgae are increasingly recognized as valuable biological resources because their nutrient-rich biomass and bioactive compounds can support crop growth, improve plant productivity, contribute to biological pest management, and enhance soil quality (Solomon et al., 2023; Gurau et al., 2025; Sun et al., 2026). *Chlorella vulgaris* is a unicellular green microalga with considerable potential for wastewater treatment. Its adaptability to different cultivation conditions and growth media makes it a promising candidate for applications in wastewater management (Abuhasheesh et al., 2025; Melo et al., 2026). Its use may be particularly beneficial in treating industrial effluents, including wastewater generated by textile manufacturing (Coronado-Reyes et al., 2022). Beyond its environmental applications, this microalga has also been found to promote agricultural crops, including wheat, maize, rice, and turnip (Chroho et al., 2026). Microalgal treatments have been associated with improvements in root and shoot development and total plant biomass. They may also influence chlorophyll and lipid contents while reducing the accumulation of reactive oxygen species (ROS), proline, and hydrogen peroxide under stress conditions (Gharib et al., 2024). Arsenic exposure can substantially disrupt the physiological and biochemical functioning of turnip cultivars, resulting in chlorophyll degradation, restricted plant growth, impaired photosystem II efficiency, and increased ROS accumulation and lipid peroxidation (Liu et al., 2024; Ahmed et al., 2025). However, the application of microalgae may help alleviate these adverse effects. In particular, foliar application of a 10% microalgal preparation has been reported to improve plant growth, recover chlorophyll levels, enhance the efficiency of photosystems I and II, and decrease ROS and malondialdehyde (MDA) accumulation under arsenic stress. These beneficial responses may be associated with reduced arsenic uptake and enhanced antioxidant defense mechanisms (Gharib et al., 2024). Collectively, these findings suggest that microalgal applications could provide a promising approach for improving plant tolerance to arsenic contamination and supporting crop performance in metal-contaminated environments.

Both living and non-living biomass can participate in biosorption, although the mechanisms involved may differ depending on the biological material and environmental conditions (Rajput & Yanning, 2015). In algae, metal removal primarily occurs through two mechanisms: passive binding to cell surfaces and the exchange of metal ions with cations, such as Ca^{2+}, Mg^{2+}, Na^{+}, and K^{+}, associated with the cell wall. These processes are facilitated by various surface functional groups capable of interacting with dissolved metal ions (Gu & Lan, 2021). Biosorption has consequently received considerable attention as a potentially economical approach to pollutant removal, particularly because non-living biomass can be used without the need to maintain cellular growth and metabolic activity (Yaashikaa et al., 2021). The cell walls and outer surfaces of algae contain proteins, lipids, and polysaccharides that provide binding sites for contaminants. Interactions between these biomolecules and pollutants can promote their attachment to algal biomass and facilitate their separation from wastewater, thereby reducing contaminant concentrations (Sayanthan et al., 2024; Patel & Patel, 2026). Functional groups present in microalgal cell walls play a particularly important role in this process by providing sites for the adsorption and binding of pollutants (He & Chen, 2014).

In addition to surface-based removal, certain contaminants can cross the cell membrane and accumulate within the cytoplasm or intracellular organelles. This process, known as bioaccumulation, involves the uptake and retention of substances within living algal cells (Chen et al., 2023; Sarma et al., 2024). Unlike passive surface binding, intracellular accumulation may require metabolic energy when substances are transported through active cellular mechanisms. Following uptake, microalgae may also contribute to contaminant detoxification through biochemical transformation, sequestration within cellular compartments, or complex formation with intracellular molecules. These processes can reduce the toxicity or biological availability of contaminants and may support the recovery of useful biomass or other value-added products (Sun et al., 2022). Algae possess diverse defense mechanisms that enable them to tolerate and mitigate heavy metal toxicity. These mechanisms include antioxidant production, ion exchange, chelation, complexation, gene regulation, and heavy metal immobilization (Faruque et al., 2024). Through these processes, algae reduce the harmful effects of heavy metals on cellular proteins, membranes, and enzyme activity (Machado et al., 2024; Yang et al., 2025; Parveen & Selvasembian, 2026). Furthermore, they contribute to heavy metal removal and detoxification through biosorption, biotransformation, and other bioremediation processes (Xiao et al., 2023; Farokhpour et al., 2026; Gu et al., 2026). Prokaryotic and eukaryotic algae, including cyanobacteria and Chlorella

species, respectively, employ different transport systems for arsenic uptake (Tang & Foo, 2024; Chen et al., 2026).

Turnip (*Brassica rapa* L.) is an agriculturally important crop cultivated across diverse environments worldwide, with considerable variation in the morphology of its edible organs (Zhang et al., 2014). Its diverse morphological characteristics also make it a useful model for investigating the genetic and molecular mechanisms underlying plant traits (Alemu et al., 2024). Plant-derived foods are important components of a balanced diet because they supply essential nutrients and biologically active compounds associated with various health benefits (Zhu & Yin, 2025). In recent years, increasing research attention has focused on plant secondary metabolites, commonly known as phytochemicals, because of their potential roles in promoting human health. Turnip contains several phytochemicals that may contribute to its nutritional and therapeutic value (Javed et al., 2019; Paul et al., 2019). Nutritionally, turnip provides dietary fibre, vitamins, carbohydrates, and other essential nutrients. Previous studies have reported its protein, starch, and soluble carbohydrate contents, although the reported concentrations may vary with cultivar, plant part, growing conditions, and analytical method (Khan et al., 2015). Both the fleshy roots and leafy portions can be used for human consumption, while the crop may also serve as livestock feed. According to Paul et al. (2019), 100 g of turnip bulbs contains approximately 34 kcal of energy, dietary fibre (2.2 g), carbohydrates (7.84 g), protein (1.10 g), and fat (0.12 g), with no cholesterol. In addition to their nutritional contribution, leafy brassica vegetables contain phenolic compounds with antioxidant activity. These compounds have attracted interest because of their potential roles in reducing oxidative stress and supporting the prevention of certain chronic diseases, including cancers affecting the gastrointestinal tract, pancreas, lungs, urinary bladder, and breast (Jan et al., 2018). Keeping in view these facts, the present research study was conducted to examine the effect of arsenic stress on the morphological and biochemical characteristics of *Brassica rapa* and also to determine the effectiveness of *C. vulgaris* application in lowering the toxicity of As in turnip.

Materials and Methods

Biological material and growth conditions

Seeds of two turnip (*Brassica rapa* L.) varieties, Top Purple and Golden Ball, were purchased from a local market in Multan, Pakistan. Sodium arsenate (Na_3AsO_4), used as the source of arsenic in the experiment, was obtained from Bahauddin Zakariya University (BZU), Multan, Pakistan. The algal strain *Chlorella vulgaris* ZAA1 was obtained from the Department of Biotechnology, BZU, Multan, Pakistan. Before sowing, the turnip seeds were surface-sterilized by immersion in 70% ethanol for 1 min, followed by treatment with a 4% bleach solution for 20 min. The seeds were washed repeatedly with distilled water to eliminate any remaining traces of the sterilizing solution. Five seeds were sown in each pot containing autoclaved sand supplemented with nutrients, with adequate spacing between seeds to minimize overcrowding during germination. The pots were placed in the growth room of the Institute of Molecular Biology and Biotechnology, BZU, Multan, Pakistan. The plants were grown under controlled conditions, with the temperature maintained at 20 ± 1 °C and relative humidity at 70–80%. They were exposed to fluorescent lighting for 14 hours daily at a light intensity of 250 ± 25 µmol m^{-2} s^{-1}. Seed germination generally occurred within 1–2 weeks after sowing. When the seedlings developed three to four leaves, they were separated and transferred individually into pots containing sand. Following this thinning procedure, the plants were supplied with Hoagland nutrient solution to provide essential mineral nutrients for growth and development (Hoagland & Arnon, 1950).

Preparation of growing medium

The sand used as the growing medium was sterilized by autoclaving for 20 min and subsequently allowed to cool to room temperature. After cooling, the sterilized sand was transferred into plastic pots measuring 6–8 cm in diameter. This preparation procedure was intended to reduce microbial contamination and provide a suitable medium for turnip growth, with adequate drainage and appropriate growing conditions.

Microalgal and arsenic treatments

Dried biomass of *Chlorella vulgaris* was used to prepare the microalgal treatments. The dried algal biomass was suspended in distilled water to prepare two concentrations (5% and 10%). The required quantities of dried biomass were mixed thoroughly with distilled water to obtain homogeneous suspensions. Tween-20 was added at a concentration of 0.1% (v/v) as a surfactant to facilitate foliar application. The water-control plants received distilled water containing the same concentration of Tween-20 (0.1%, v/v) to ensure uniform treatment conditions. Five-week-old plants of both turnip varieties were selected according to their homozygosity and transferred to separate trays for the respective treatments. The prepared microalgal suspensions were applied to the plants by foliar spraying at a volume of 15 mL per pot. Foliar application of *C. vulgaris* extract was performed weekly throughout the treatment period. Arsenic stress was imposed 48 h after the initial microalgal application. Sodium arsenate (Na_3AsO_4) solutions were prepared in Hoagland nutrient solution to achieve the target arsenic concentrations of 25 and 50 mM. Arsenic exposure was initiated at 5 mM, and the concentration was gradually increased until the designated treatment level was reached. Each tray received 10 mL of the arsenic solution. Before each subsequent application, the trays and pots were washed to remove accumulated arsenic residues. Arsenic treatments were administered at 24-h intervals.

Photosynthetic activity measurement using DUAL-PAM-100

Photosynthetic performance was assessed using a DUAL-PAM-100 fluorimeter to evaluate chlorophyll fluorescence and photosystem activity (Bonjoch & Tamayo, 2001). Leaf samples were positioned appropriately within the instrument's measuring area to record fluorescence signals. Measurements were used to assess the functional efficiency of photosystem I (PSI) and photosystem II (PSII) under the experimental treatment conditions. The instrument detects fluorescence signals within the relevant wavelength range, extending up to 830 nm, and allows photosynthetic responses to be evaluated using different measurement protocols. Before measurements, the leaves were dark-adapted for 30 min to determine dark-adapted fluorescence parameters. The effective quantum yields of PSI and PSII were represented by (Y(I)) and (Y(II)), respectively. These fluorescence parameters provide useful indicators of photosystem performance and the effects of environmental stress on photosynthetic processes. The DUAL-PAM-100 fluorimeter therefore enabled the evaluation of treatment-related changes in photosynthetic efficiency in turnip plants.

Fresh and dry weight

Following completion of the experimental treatments, the plants were gently uprooted from their pots and rinsed thoroughly with tap water to remove any sand particles attached to their surfaces. Excess surface moisture was removed using tissue paper, and the fresh weights of the roots and leaves were recorded using a weighing balance. Following fresh weight measurement, the plant samples were separated into shoot and root portions using scissors, placed in labeled envelopes, and dried in an oven maintained at 65°C for 15 days. After the drying period, the samples were removed from the oven and weighed to determine their dry weights.

Estimation of photosynthetic pigments (mg/g F.W)

Chlorophyll and carotenoid contents were determined following the method described by Arnon (1949). Briefly, 0.1 g of fresh leaf tissue was homogenized in 2.5 mL of 80% acetone to extract the photosynthetic pigments. The homogenate was kept for 24 h to facilitate pigment extraction and subsequently filtered through filter paper to remove plant debris. The optical density of the resulting filtrate was measured using a spectrophotometer at wavelengths of 470, 645 and 663 nm. The concentrations of photosynthetic pigments were then calculated from the recorded absorbance values using the appropriate equations:

$$\text{Chl a} = [(12.7 \times \text{OD}_{663}) - (2.69 \times \text{OD}_{645})] \times \frac{\text{V}}{\text{W}} \times \frac{1}{1000}$$

$$\text{Chl b} = [(22.91 \times \text{OD}_{645}) - (4.68 \times \text{OD}_{663})] \times \frac{\text{V}}{\text{W}} \times \frac{1}{1000}$$

$$\text{Carotenoids} = 4.07 \times (\text{OD}_{450}) - (0.0435 \times \text{Chlorophyll a} + 0.367 \times \text{Chlorophyll b})$$

Estimation of proline content

Proline accumulation is an important physiological response in plants exposed to environmental stress. Proline concentration in fresh leaf tissue was measured using the procedure outlined by Bates et al. (1973). Briefly, 0.1 g of fresh leaf material was homogenized in 4 mL of 3% sulfosalicylic acid, followed by filtration through Whatman filter paper. A 3 mL aliquot of the resulting filtrate was combined with equal volumes of glacial acetic acid and ninhydrin reagent. The mixture was heated in a water bath at 100 °C for 1 h and subsequently cooled rapidly in an ice bath. After cooling, 3 mL of toluene was added, and the mixture was shaken vigorously for 15–20 s to promote phase separation. The upper toluene phase, containing the colored reaction product, was collected carefully, and its absorbance was recorded at 520 nm using a spectrophotometer. Proline concentrations were determined using a standard curve prepared from solutions of known proline concentrations.

ROS estimation

Reactive oxygen species (ROS) act as important secondary messengers in plants, regulating defense-related signaling pathways and participating in responses to environmental stress. They are also continuously generated as by-products of normal cellular oxygen metabolism. Reactive oxygen species (ROS) levels were assessed using the protocol of Velikova et al. (2000).

Malondialdehyde (MDA) estimation (nmol/g FW)

Malondialdehyde (MDA) content, a widely used marker of lipid peroxidation and stress-induced damage to cellular membranes, was quantified following the procedure of Cakmak and Horst (1991), as discussed by Das et al. (2022). MDA content was estimated from the difference between the absorbance readings at 532 and 600 nm, using a molar extinction coefficient of 156 $\text{mmol}^{-1}\ \text{cm}^{-1}$:

$$\text{MDA}\left(\frac{\text{nmol}}{\text{g}}\ \text{FW}\right) = 6.45 \times (\text{OD}_{532}\text{nm} - \text{OD}_{600}\ \text{nm}) - 0.56 \times \text{OD}_{450}\ \text{nm}$$

Enzymatic antioxidants

Antioxidant enzymes were extracted according to the method of Ananieva et al. (2004).

Catalase (CAT) estimation

Catalase (CAT) activity was assayed using the method of Chance & Maehly (1955). Catalase plays a crucial role in cellular antioxidant defense by converting hydrogen peroxide (H_2O_2) into water and oxygen, thereby reducing oxidative stress. The reaction mixture was prepared using 1 mM potassium phosphate buffer (pH 7.0) as the buffering medium, enzyme extract, and H_2O_2 at the specified concentrations. After mixing the components to initiate the reaction, absorbance was recorded at 240 nm using a spectrophotometer at 20-second intervals over a total duration of 1 min. CAT activity was assessed by measuring the decline in absorbance, reflecting the enzymatic decomposition of H_2O_2.

Peroxidase (POD) Estimation

Peroxidase (POD) activity was determined through the guaiacol oxidation assay based on Chance & Maehly (1955), following the procedure referenced by Das et al. (2022).

Ascorbate peroxidase (APX) estimation

Ascorbate peroxidase (APX) activity was determined according to the method of Hossain & Asada (1984). Ascorbate peroxidase (APX) activity was evaluated using a reaction solution comprising ascorbic acid, hydrogen peroxide (H_2O_2), potassium phosphate buffer, and 0.1 mL of enzyme extract. The solution was maintained at pH 7.7, and absorbance changes were recorded at 290 nm with a spectrophotometer to determine APX activity.

Protein quantification

The total protein concentration in the enzyme extract was determined using the Bradford assay, following the method described by Bradford (1976).

Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was assessed according to the method of Giannopolitis and Ries (1977), based on the inhibition of nitroblue tetrazolium (NBT) photoreduction.

Statistical analysis

The experimental data were analyzed using CoStat software, version 6.3 (Cohort Software, Berkeley, California, USA). Factorial analysis of variance (ANOVA) was performed to evaluate the individual and combined effects of cultivar, arsenic (As) levels, and *Chlorella vulgaris* treatments on the studied growth, physiological, biochemical, and photosynthetic parameters of turnip plants. Treatment means were compared using the least significant difference (LSD) test, following the approach described by Bukhat et al. (2020).

Results

Biomass analyses

The fresh and dry weight of shoot and roots were significantly reduced due to arsenic (As) stress; Moreover, the more reduction was observed at 50 mM As. However, the foliar treatment of microalgae (both 5 and 10%), mitigated the negative effect of As-stress and significantly improved the fresh and dry weight of shoot and root of both turnip varieties (Fig. 1). However, a maximum improvement in all these growth parameters was observed at 10% microalgae (*C. vulgaris*). Notably, the highest recovery was observed in "Top Purple" variety in comparison to "Golden Ball" variety (Fig. 1 and 2).

Shoot and root length

To examine the effects of arsenic (As) stress on the development of turnip seedlings, shoot and root lengths were measured (Fig. 3). Both 25 and 50 mM As treatments significantly reduced shoot and root lengths in both turnip varieties compared with their respective controls, with greater reductions generally observed at 50 mM As. In Golden Ball, shoot length decreased by 20.0% and 39.3% under 25 and 50 mM As treatments, respectively, whereas the corresponding reductions in Top Purple were 20.3% and 25.4%. Similarly, root length decreased by 14.8% and 29.0% in Golden Ball and 21.9% and 25.5% in Top Purple under 25 and 50 mM As treatments, respectively. Application of *Chlorella vulgaris* extract at 5% and 10% significantly alleviated As-induced growth inhibition in both varieties. The 10% microalgal treatment produced the greatest improvement in shoot and root lengths. Compared with the corresponding As-only treatments, 10% *C. vulgaris* application increased shoot length by 20.5% and 22.8% in Golden Ball under 25 and 50 mM As stress, respectively. In Top Purple, the corresponding increases were 35.3% and 26.4% (Fig. 3).

Photochemical quantum yield of PSI Y(I) and PSII Y(II)

The photochemical quantum yields of Photosystem I (PSI) and Photosystem II (PSII) were significantly reduced under arsenic (As) stress in both turnip varieties (Fig. 4). The inhibitory effect was more pronounced at 50 mM As. In Golden Ball, Y(I) decreased by 34.7% and 61.8% under 25 and 50 mM As treatments, respectively, compared with the control. Similarly, Y(I) decreased by 33.3% and 58.5% in Top Purple under the corresponding treatments. For Y(II), reductions of 18.5% and 36.9% were observed in Golden Ball, whereas Top Purple exhibited reductions of 30.7% and 25.3% under 25 and 50 mM

As treatments, respectively. Application of *Chlorella vulgaris* extract at 5% and 10% significantly improved the photochemical quantum yields of both photosystems under As stress, with the greatest improvement generally observed following 10% microalgal application. Compared with the corresponding As-only treatments, 10% *C. vulgaris* application increased Y(I) by 51.1% and 61.8% in Golden Ball under 25 and 50 mM As stress, respectively. In Top Purple, the corresponding increases were 57.9% and 63.4%. Similarly, Y(II) increased by 22.7% and 26.3% in Golden Ball and 50.4% and 28.2% in Top Purple under 25 and 50 mM As treatments, respectively (Fig. 4).

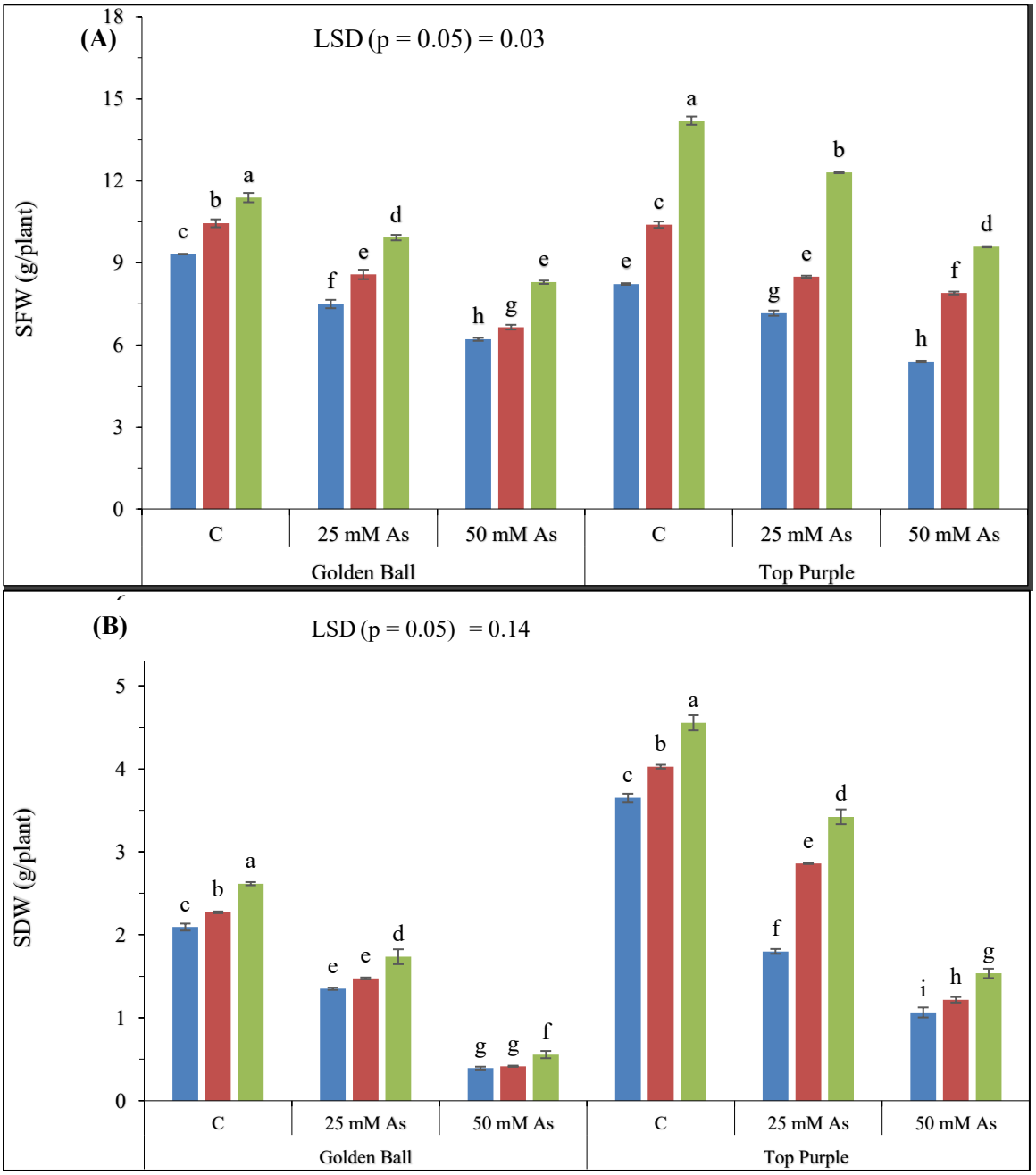


Fig. 1 Effect of microalgae (*C. vulgaris*) application on biomass analyses (A) Shoot fresh weight (B) Shoot dry weight in *Brassica rapa* subjected to As-stress; Values exhibited the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

Electron transport rate of PSI and PSII

Electron transport rate in the photosystem I and II was significantly reduced due to the both concentrations (25 mM and 50 mM) of arsenic. Moreover, more reduction was observed at 50 mM As (Fig. 5). These results showed that the toxic effects of As were significantly overcome by microalgae application. However, ETR I and II were positively affected with both concentrations of microalgae in all control and As-stress plants. The results indicated that applying

Chlorella vulgaris at 5% and 10% under arsenic stress substantially enhanced electron transport rates in both Photosystem I (PSI) and Photosystem II (PSII) across the two turnip varieties (Fig. 5). However, more improvement was observed in "Top Purple".

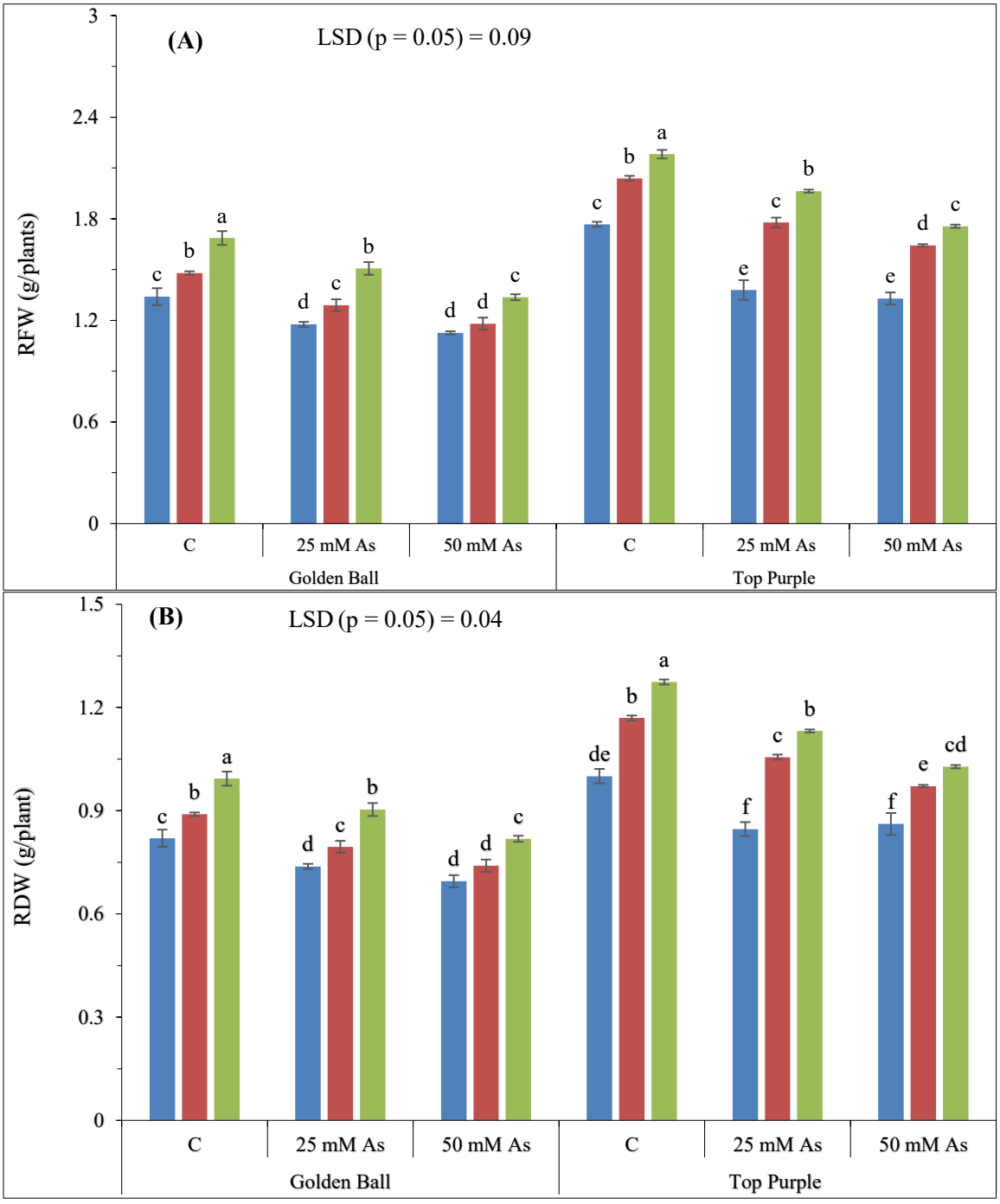


Fig. 2 Effect of microalgae (*C. vulgaris*) application on biomass analyses (A) Root fresh weight (B) Root dry weight in *Brassica rapa* under arsenic stress; Values proven that the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

YND & YNA (Non-photochemical energy dissipation and quantum yield of PSI)

Arsenic exposure at 25 and 50 mM altered the energy-dissipation parameters of Photosystem I (PSI), including YND and YNA. Both parameters declined markedly under the 50 mM arsenic treatment (Fig. 6). Applying microalgae at 5% and 10% improved the measured responses, with the strongest effect observed at 10%. However, the Golden Ball

cultivar responded less strongly to microalgal treatment than Top Purple (Fig. 6).

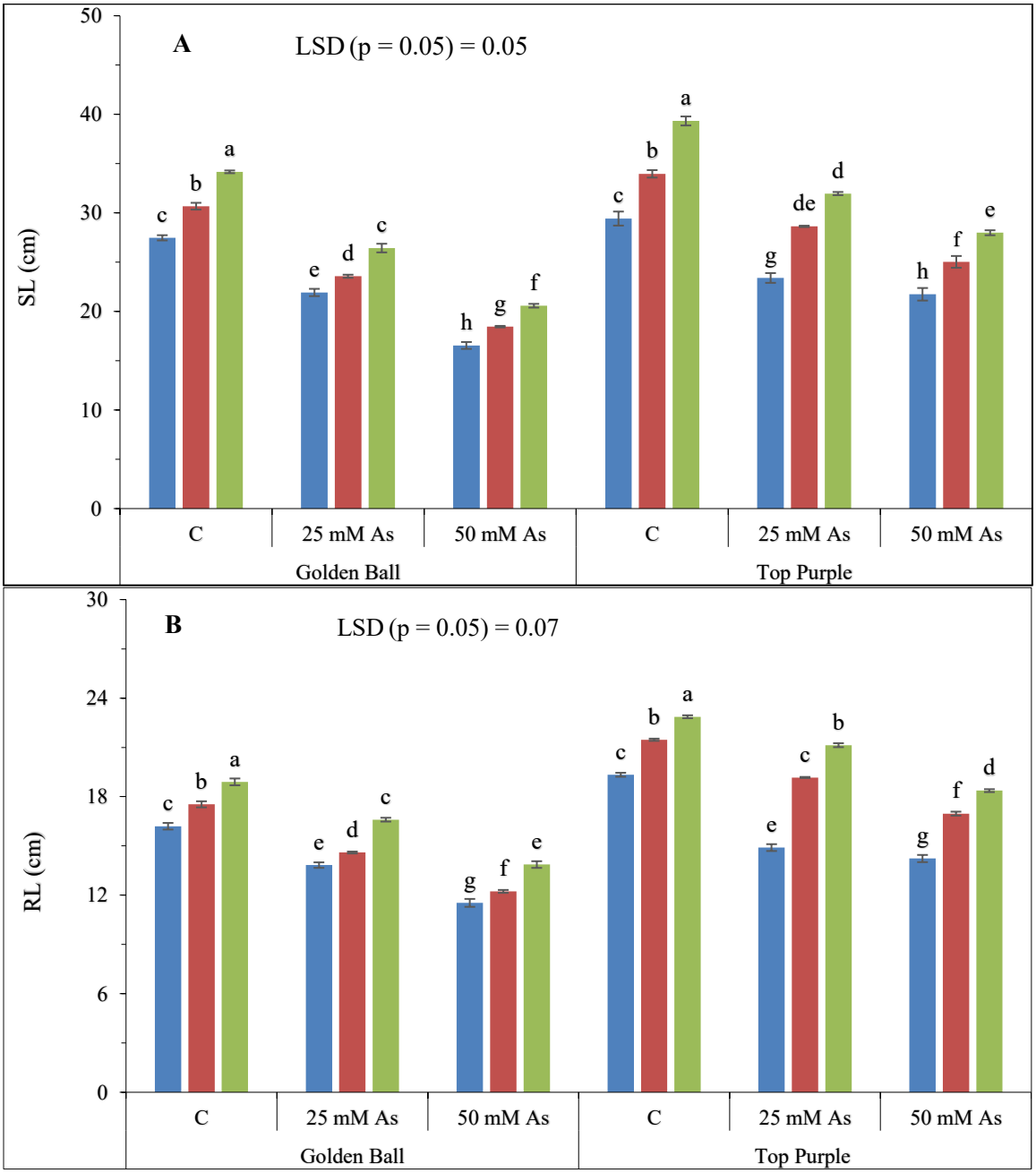


Fig. 3 Effect of microalgae (*C. vulgaris*) application on root and shoot analyses (A) Shoot length (B) Root length in *Brassica rapa* under As stress; Values demonstrated the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

NPQ and Y(NPQ) Quantum yield that regulated energy dissipation

Plants under arsenic stress exhibited energy dissipation and non-photochemical quantum yield at different concentrations (25 mM and 50 mM). More energy dissipation and no-photochemical quantum yield was markedly increased at 50 mM As (Fig. 7). Foliar spray of microalgae at different concentrations 5% and 10%, exhibited higher recovery rate that decreased the negative effects of arsenic stress. The most significant effects were observed at the 10% microalgal treatment level. Among the two cultivars, "Top Purple" exhibited a stronger response than "Golden Ball," indicating greater responsiveness to microalgal treatment under the tested conditions (Fig. 7).

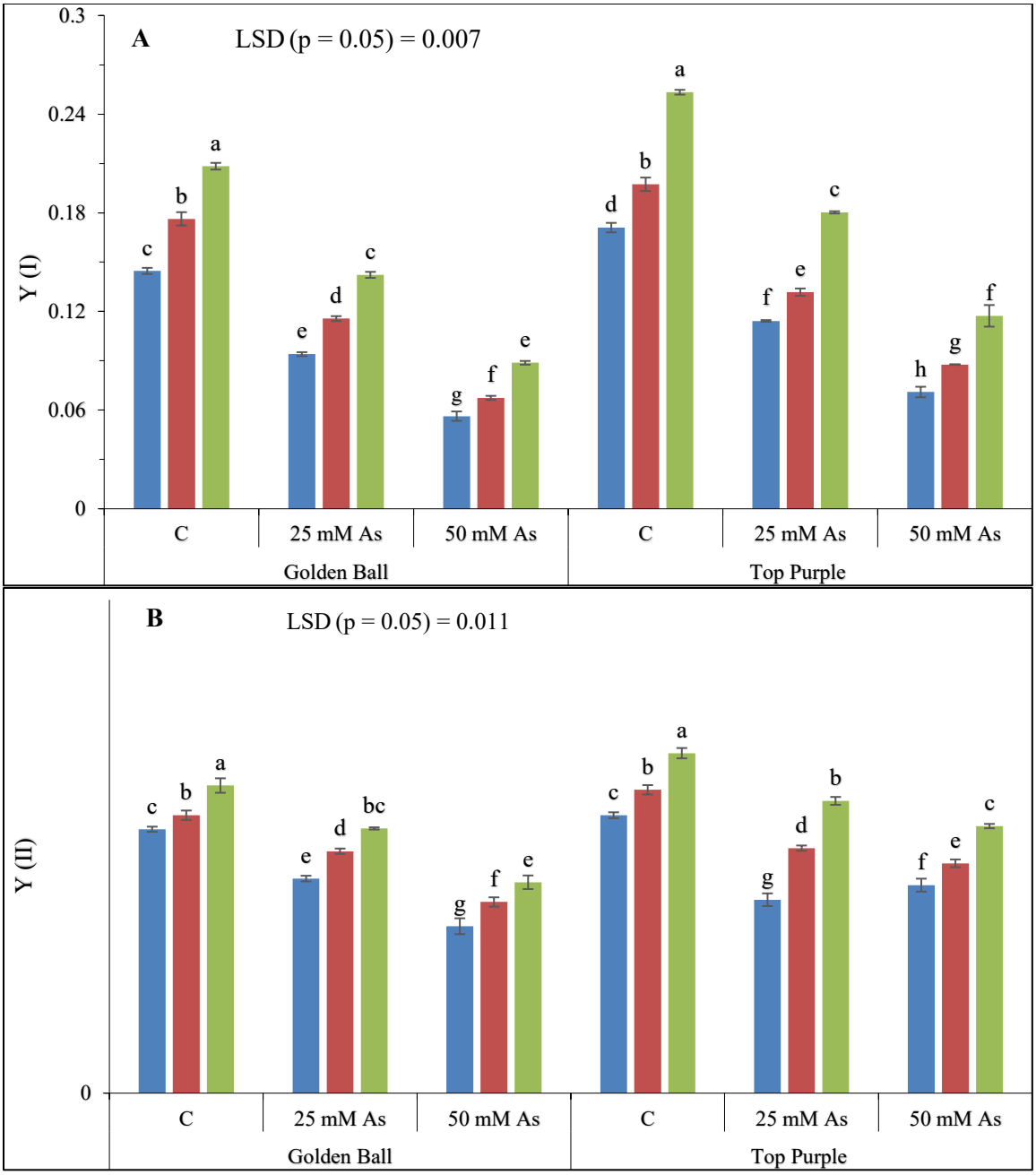


Fig. 4 Effect of microalgae (*C. vulgaris*) application on yield (A) Yield I (B) Yield II in *Brassica rapa* subjected to As-stress; Values exhibited the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

Non-photoprotective energy dissipation in PSI

Arsenic exposure increased non-regulated energy dissipation in Photosystem II (PSII), with a more pronounced increase in Y(NO) observed under the 50 mM arsenic treatment (Fig. 8). Co-application of *Chlorella vulgaris* at concentrations of 5% and 10% reduced Y(NO) in both turnip varieties, indicating improved regulation of energy dissipation under arsenic-induced stress. Among the treatments, the 10% microalgal concentration produced the greatest reduction in Y(NO). Top Purple exhibited a more pronounced response to microalgal application than Golden Ball (Fig. 8).

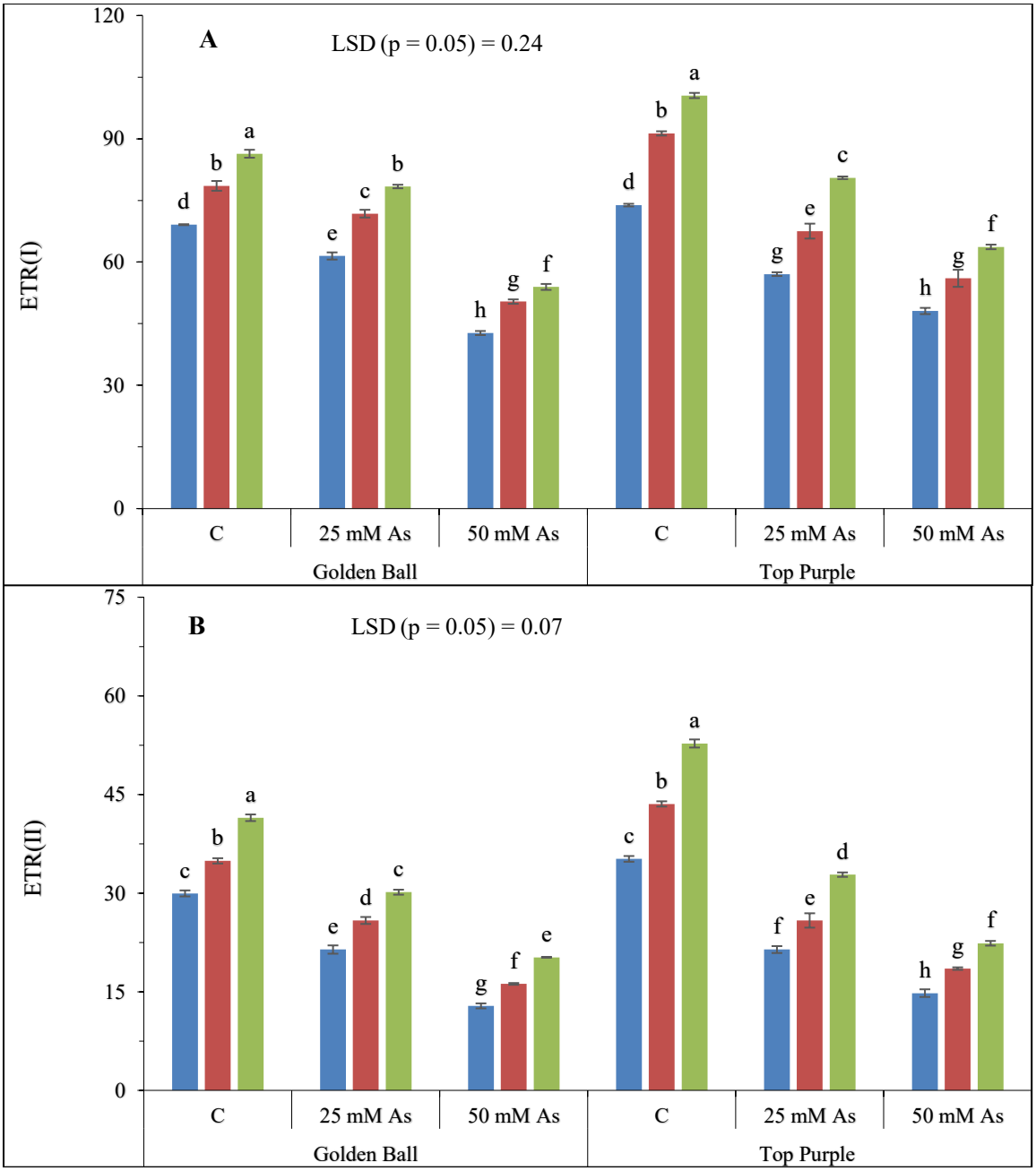


Fig. 5 Effect of microalgae (*C. vulgaris*) application on electron transport rate (A) ETR I (B) ETR II in *Brassica rapa* under As-stress; Values exhibited the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

Photosynthetic pigments measurement

In this study, we found that arsenic stress with two concentrations (25 mM and 50 mM) negatively affect the photosynthetic pigments. Our results showed that the level of chlorophyll *a, b* contents, total chlorophyll and carotenoid content were significantly decreased in all arsenic-treated turnip plants as compared to untreated plant. The more reduction was seen at higher concentration (50 mM) of arsenic (Fig. 9). However, application of microalgae considerably ameliorates the negative effects of As-stress on photosynthetic pigments. Microalgae with both concentrations considerably increased all these photosynthetic pigments in both varieties. Moreover, our results indicated that 10% microalgae caused more enhancements in these chlorophyll pigments in "Top Purple" variety than "Golden Ball" variety (Fig. 10).

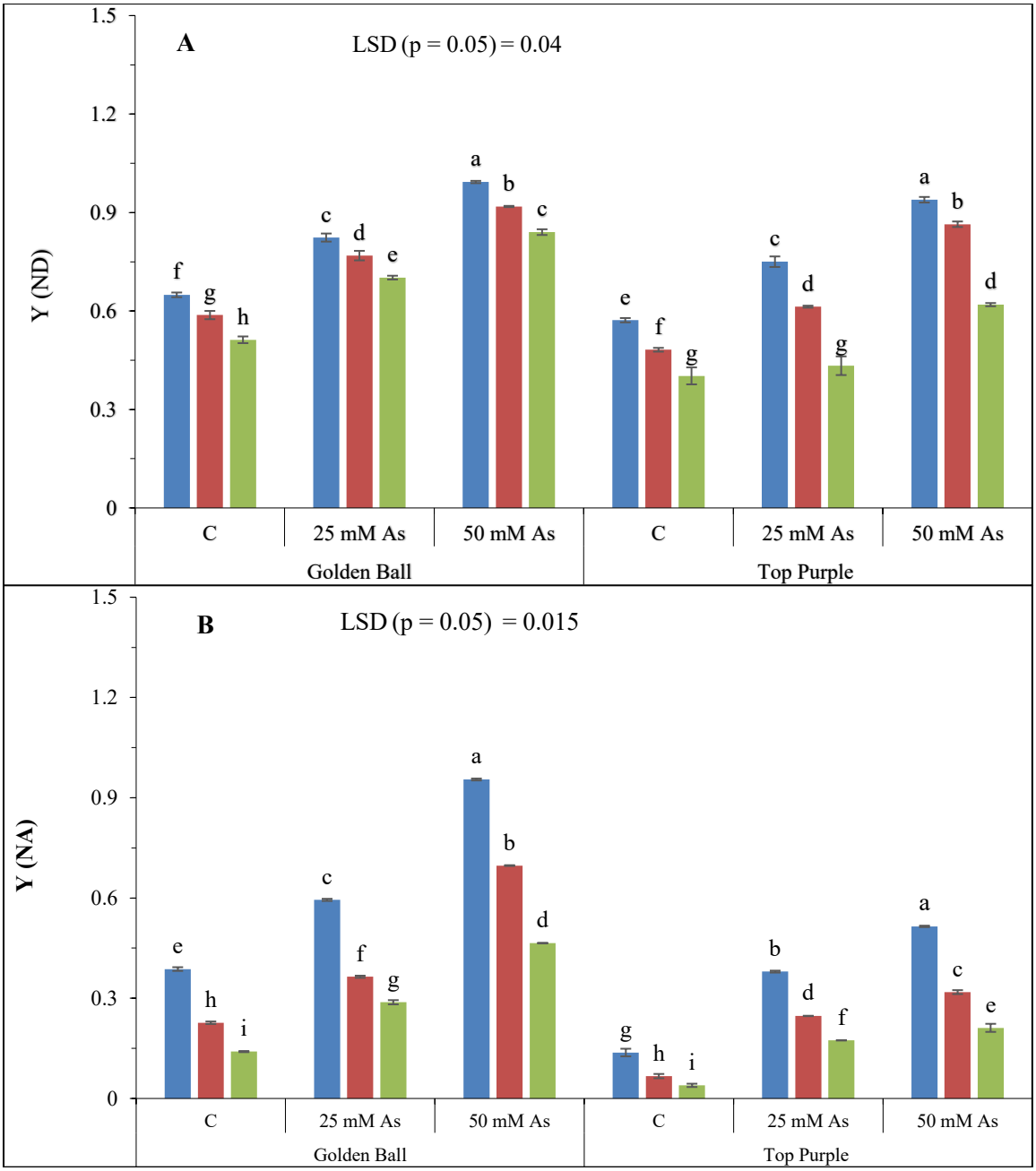


Fig. 6 Effect of microalgae (*C. vulgaris*) application on energy dissipation and quantum yield (A) Y (ND) (B) Y (NA) in *Brassica rapa* subjected to As-stress; Values showed the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

ROS and MDA analyses

H_2O_2 and MDA content were analyzed to investigate the effects of arsenic stress on the regulation of As-stress on turnip plants. Results showed that As-treatment with both 25 and 50 mM concentration induced a considerable increase in H_2O_2 and MDA level, as compare to un-treated turnip plants. Meanwhile, microalgae (*C. vulgaris*) application with both concentrations induced a noticeable decrease in MDA and H_2O_2 concentration. Our results depicted in Fig. 11 showed that 10% microalgae concentration induced more reduction in both H_2O_2 and MDA content as compare to 5% microalgae concentration. Moreover, 10% microalgae induced more reduction in these above mentioned parameters in "Top Purple" compared to "Golden Ball" turnip variety (Fig. 11).

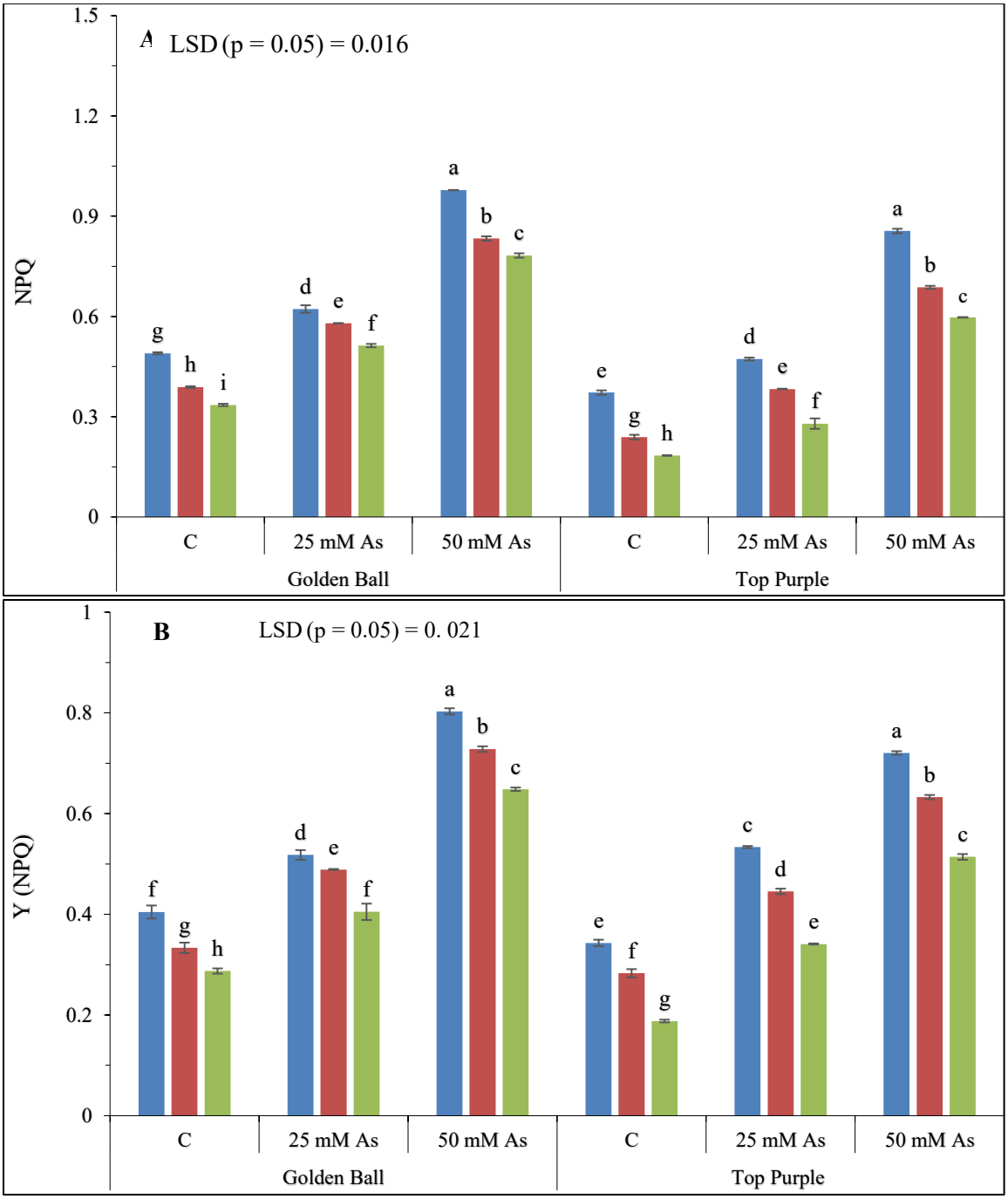


Fig. 7 Effect of microalgae (*C. vulgaris*) application on (A) NPQ (B) Y (NPQ) in *Brassica rapa* under arsenic stress; Values demonstrated the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

Antioxidant enzymes activity

The results demonstrated that arsenic (As) exposure significantly altered the activities of antioxidant enzymes, including peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and superoxide dismutase (SOD), in turnip shoots (Figs. 12 and 13). Foliar application of *Chlorella vulgaris* extract modulated antioxidant enzyme activities under As stress, with responses varying according to the extract concentration and cultivar. Compared with the corresponding water-control treatments under As stress, 5% and 10% microalgae supplementation increased POD activity by 6–35% and 16–56%, respectively. Similarly, CAT activity increased by 17–30% with 5% extract and 27–43% with 10% extract. APX activity also increased by 11–27% and 28–51% following the application of 5% and 10% microalgae extract, respectively. In contrast, SOD activity decreased by 12–16% under 5% extract and 24–33% under

10% extract compared with the respective water-control treatments (Figs. 12 and 13).

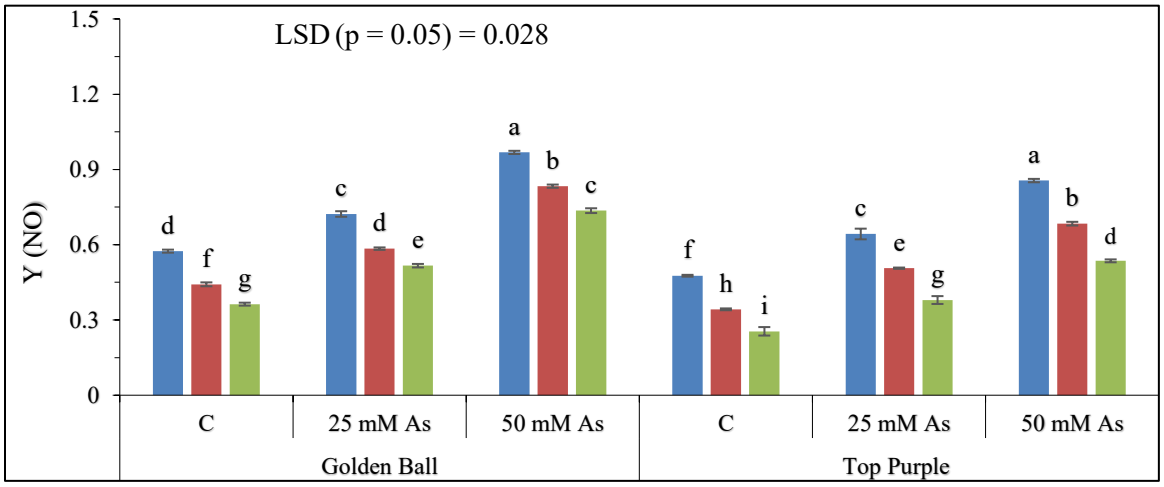


Fig. 8 Microalgae and their interaction with Y (NO) in turnip (*Brassica rapa*) under arsenic stress; Values showed the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

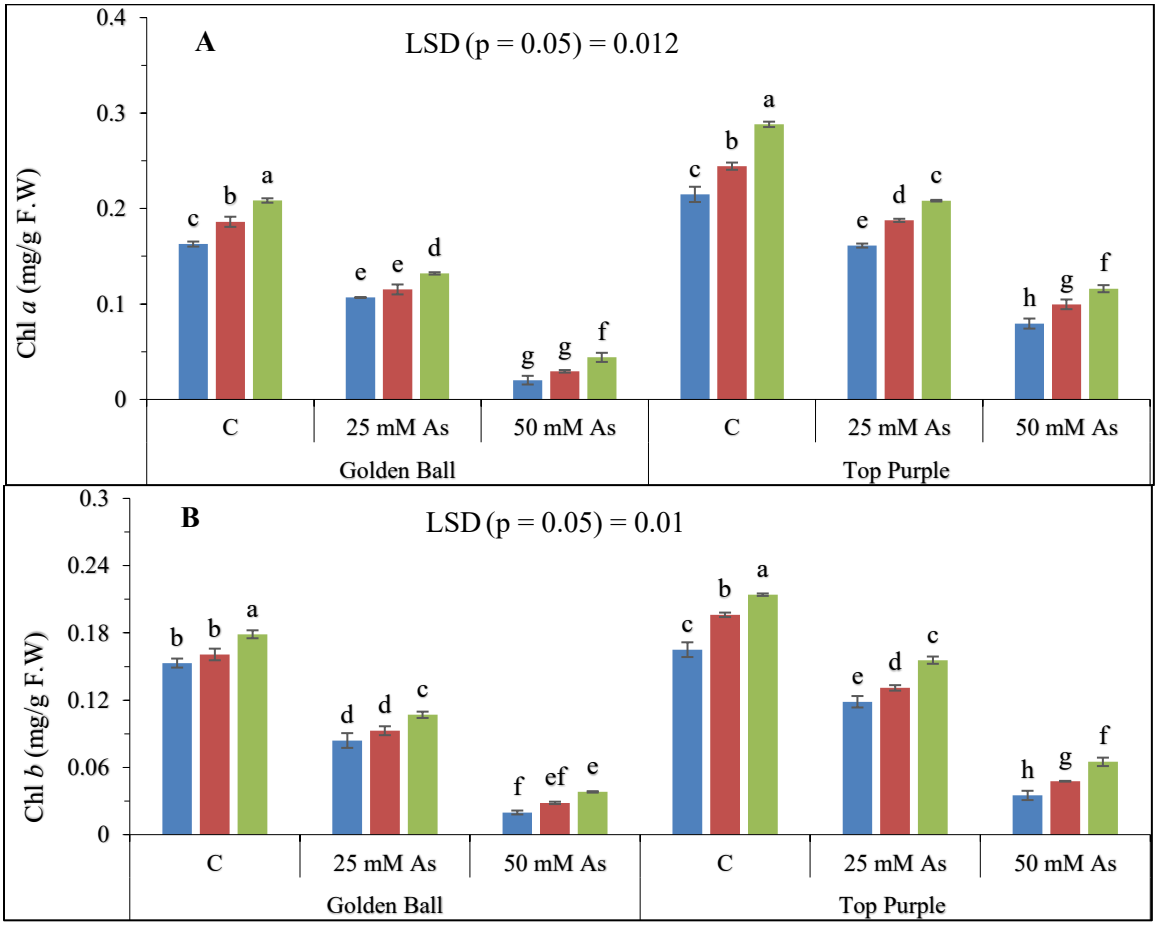


Fig. 9 Effect of microalgae (*C. vulgaris*) application on photosynthetic pigments (A) Chl a (B) Chl b in *Brassica rapa* under As stress; Values showed the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

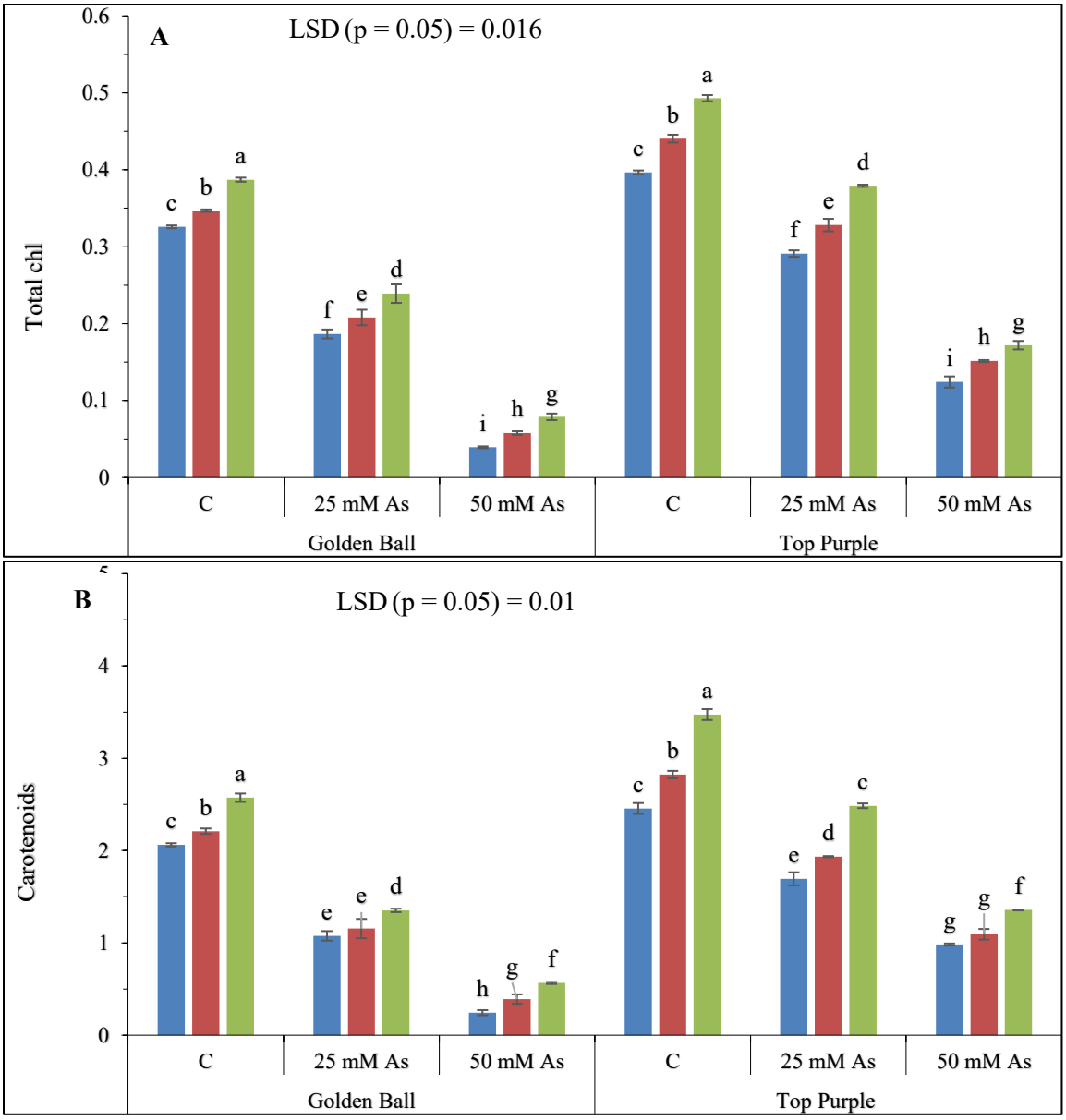


Fig. 10 Effect of microalgae (*C. vulgaris*) application on photosynthetic pigments (A) Total Chl (B) Carotenoids in *Brassica rapa* subjected to As-stress; Values exhibited the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

Measurement of proline and total soluble protein

Arsenic (As) stress significantly increased proline and total soluble protein contents in both turnip varieties (Golden Ball and Top Purple) compared with their respective water controls (WC) (Fig. 14). In Golden Ball, proline content increased by 74% and 130% under 25 and 50 mM As treatments, respectively, compared with the control. Similarly, in Top Purple, these increases were 25% and 71%, respectively. Foliar application of *Chlorella vulgaris* further enhanced proline accumulation, with the 10% extract generally producing higher values than the 5% extract. For example, under 50 mM As stress, the 10% extract increased proline content by 14% in Golden Ball and 15% in Top Purple compared with the 5% extract. A similar trend was observed for total soluble protein content (Fig. 14B). Under 25 and 50 mM As treatments, protein content increased by 56% and 122%, respectively, in Golden Ball, while the corresponding increases in Top Purple were 39% and 65% compared with their respective controls. Furthermore, under 25 mM As stress, the application of 10% *C. vulgaris* extract increased total soluble protein content by approximately 9% in Golden Ball and 27% in Top Purple compared with the 5% extract.

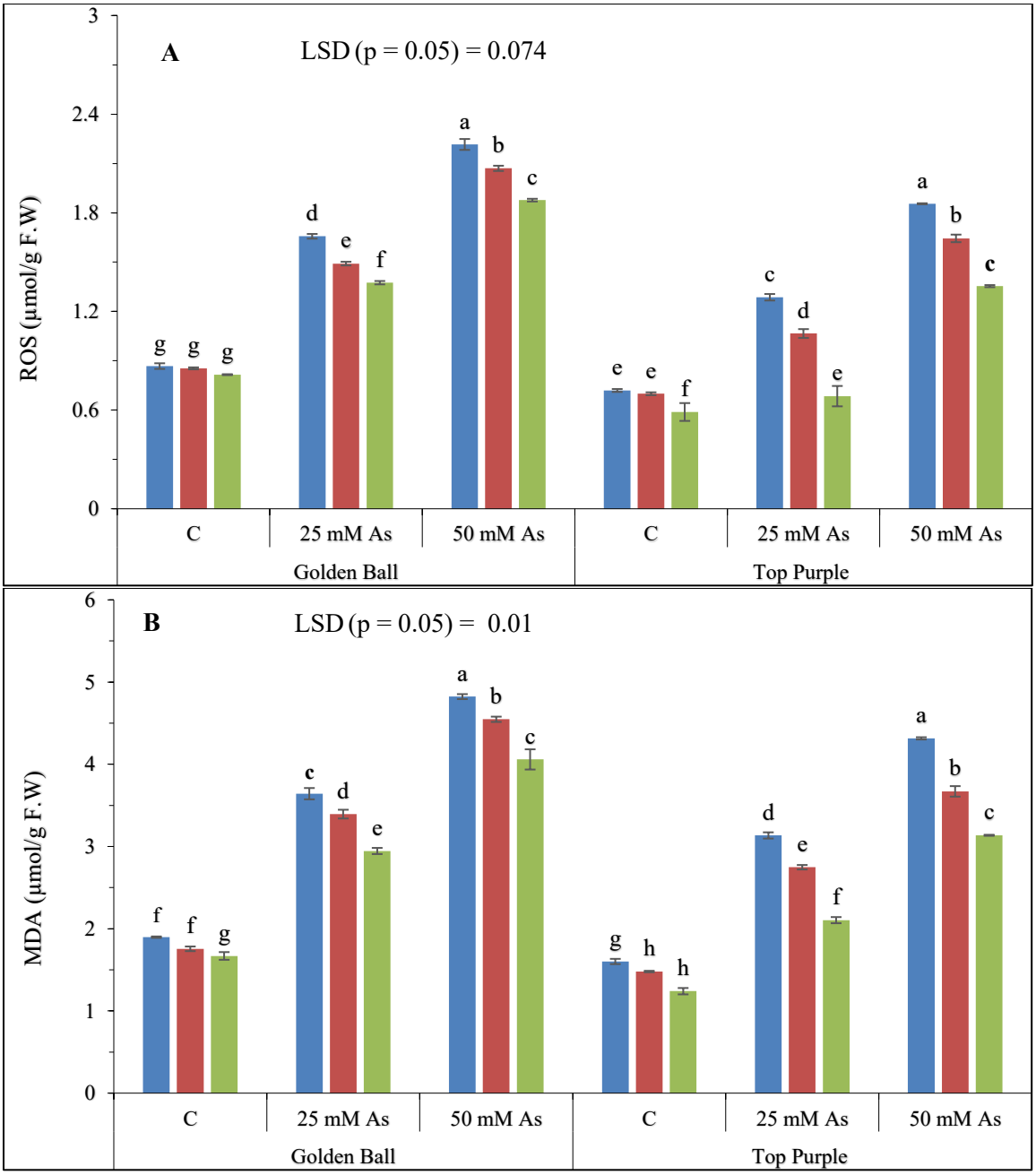


Fig. 11 Effect of microalgae (*C. vulgaris*) application on (A) ROS (B) MDA in *Brassica rapa* subjected to As-stress; Values showed the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

Factorial analysis of variance (ANOVA)

The factorial analysis of variance (ANOVA) revealed significant effects of *Chlorella vulgaris* treatments (Algae), arsenic (As) levels, and cultivar (varieties) on most of the investigated growth, biochemical, antioxidant, and photosynthetic parameters (Table 1). Among the main effects, arsenic levels exhibited considerable effects on plant growth and physiological responses, particularly shoot fresh weight, root length, shoot length, shoot dry weight, and proline content. Similarly, cultivar effects were significant across the studied parameters, indicating differences in the responses of Golden Ball and Top Purple cultivars to arsenic stress. The interaction between algae and arsenic (Algae × As) was significant for most parameters, demonstrating that the effectiveness of *C. vulgaris* treatments varied with the level of arsenic stress. Likewise, the Algae × Varieties interaction indicated cultivar-dependent responses to microalgal application, particularly for growth attributes, chlorophyll contents, antioxidant activities, and photosynthetic parameters. The As × Varieties interaction was also significant for several parameters, suggesting that the two cultivars differed in their responses to increasing arsenic concentrations.

Importantly, the three-way interaction (Algae × As × Varieties) was significant for several physiological and photosynthetic parameters, including shoot fresh weight, root length, chlorophyll a, carotenoids, NPQ, and ETR (I) and ETR (II). These findings indicate that the response to C. vulgaris application under arsenic stress was dependent on both arsenic concentration and cultivar. However, the three-way interaction was non-significant for several other parameters, including root fresh weight, shoot length, root dry weight, chlorophyll b, total chlorophyll, malondialdehyde, proline, and Y(I), Y(II), and Y(NO). The results demonstrate that cultivar, arsenic concentration, and microalgal application, both individually and in combination, influenced the growth, biochemical, antioxidant, and photosynthetic responses of turnip plants.

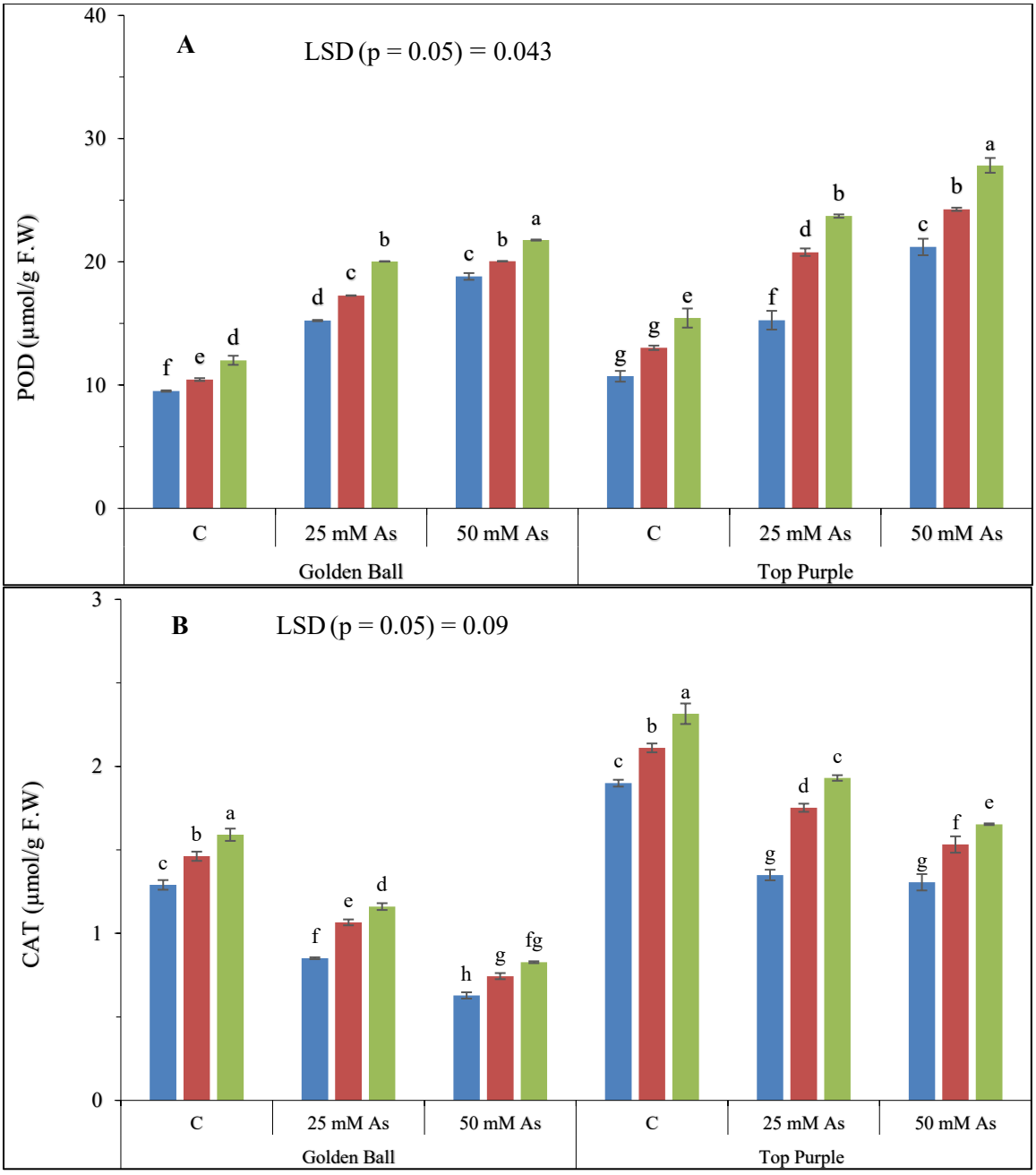


Fig. 12 Effect of microalgae (*C. vulgaris*) application on antioxidant enzymes activity (A) POD (B) CAT in *Brassica rapa* subjected to As-stress; Values demonstrated the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

Table 1 Results of factorial ANOVA showing the main and interaction effects of cultivar, arsenic levels, and *Chlorella vulgaris* treatments on the studied parameters

Sources of variation	**df**	**Root fresh weight**	**Shoot fresh weight**	**Root length**	**Shoot length**
Algae	2	0.671***	60.855***	59.056***	199.387***
As	2	0.582***	49.825***	106.234***	530.544***
Varieties	1	2.3***	4.805***	182.969***	288.773***
Algae × As	4	0.007*	0.482***	0.897***	3.871***
Algae × Varieties	2	0.064***	9.711***	6.448***	11.637***
As × Varieties	2	0.023***	0.012 ns	0.39**	10.841***
Algae × As × Varieties	4	0.004 ns	0.997***	0.905***	0.762 ns
Error	36	0.09	0.03	0.071	0.051
Sources of variation	**df**	**Shoot dry weight**	**Root dry weight**	**Chlorophyll *a***	**Chlorophyll *b***
Algae	2	2.071***	0.185***	0.007***	0.004***
As	2	24.635***	0.138***	0.109***	0.087***
Varieties	1	20.988***	0.614***	0.055***	0.012***
Algae × As	4	0.196***	0.003*	0.0003***	0.00008 ns
Algae × Varieties	2	0.478***	0.017***	0.0006***	0.00031**
As × Varieties	2	0.998***	0.003*	0.00005 ns	0.0005***
Algae × As × Varieties	4	0.117***	0.0006 ns	0.0001 ns	0.00005 ns
Error	36	0.007	0.04	0.012	0.01
Sources of variation	**df**	**Total Chlorophyll**	**Carotenoids**	**Malondialdehyde**	**Proline**
Algae	2	0.019***	1.404***	2.284***	178.182***
As	2	0.393***	15.059***	27.911***	1044.323***
Varieties	1	0.137***	7.411***	4.687***	393.232***
Algae × As	4	0.0005***	0.069***	0.204***	7.1002***
Algae × Varieties	2	0.0008***	0.151***	0.097***	11.672***
As × Varieties	2	0.001***	0.048**	0.233***	5.416***
Algae × As × Varieties	4	0.0001 ns	0.027**	0.016 ns	0.952 ns
Error	36	0.016	0.01	0.01	0.051
Sources of variation	**df**	**APX**	**SOD**	**Protein**	**POD**
Algae	2	186.0002***	58.641***	5.34***	112.859***
As	2	637.754***	256.45***	26.972***	508.541***
Varieties	1	497.423***	113.45***	34.722***	112.306***
Algae × As	4	3.959***	1.327***	0.257***	3.772***
Algae × Varieties	2	10.366***	2.448***	0.461***	12.019***
As × Varieties	2	2.286***	15.755***	0.334***	4.95***
Algae × As × Varieties	4	0.952*	0.087 ns	0.099***	0.659 ns
Error	36	0.025	0.014	0.01	0.043
Sources of variation	**df**	**CAT**	**ROS**	**NPQ**	**ETR (I)**
Algae	2	0.591***	0.459***	0.154***	1553.213***
As	2	2.03***	5.243***	0.973***	4284.34***
Varieties	1	6.466***	1.843***	0.351***	351.586***
Algae × As	4	0.014**	0.058***	0.004***	32.993***
Algae × Varieties	2	0.037***	0.043***	0.0041***	52.09***
As × Varieties	2	0.017**	0.129***	0.003***	194.009***
Algae × As × Varieties	4	0.003 ns	0.006*	0.0003*	8.156*
Error	36	0.09	0.074	0.016	0.24
Sources of variation	**df**	**ETR (II)**	**Y(I)**	**Y (II)**	**Y (NO)**
Algae	2	531.489***	0.015***	0.01***	0.267***
As	2	2204.564***	0.055***	0.021***	0.588***
Varieties	1	184.464***	0.009***	0.005***	0.188***
Algae × As	4	22.127***	0.0004***	0.0002**	0.001**
Algae × Varieties	2	12.268***	0.00046***	0.0006***	0.003***
As × Varieties	2	66.201***	0.0001*	0.001***	0.004***
Algae × As × Varieties	4	5.168***	0.00003 ns	0.00009 ns	0.0007 ns
Error	36	0.07	0.007	0.011	0.028
Sources of variation	**df**	**Y (NPQ)**	**Y (ND)**	**Y(NA)**	
Algae	2	0.111***	0.188***	0.349***	

As	2	0.616***	0.486***	0.585***
Varieties	1	0.063***	0.209***	0.686***
Algae × As	4	0.0008**	0.004***	0.019***
Algae × Varieties	2	0.004***	0.021***	0.024***
As × Varieties	2	0.006***	0.006***	0.059***
Algae × As × Varieties	4	0.0005*	0.004***	0.0015***
Error	36	0.021	0.04	0.015

ROS = Reactive oxygen species; NPQ = Non-photochemical quantum; ETR (I); Electron transport rate through photosystem I (PSI); ETR (II) = Electron transport rate through photosystem II (PSII);Y(I) = Photochemical quantum yield of Photosystem I (PSI); YII = Photochemical quantum yield of Photosystem II (PSII); Y (NO) = Non-regulated energy dissipation in Photosystem II (PSII); Y (NPQ) = Quantum yield of regulated non-photochemical energy dissipation in Photosystem II (PSII); Y (ND) = Quantum yield of non-photochemical energy dissipation due to donor-side limitation in Photosystem I (PSI); Y(NA) = Quantum yield of non-photochemical energy dissipation due to acceptor-side limitation in Photosystem I (PSI).

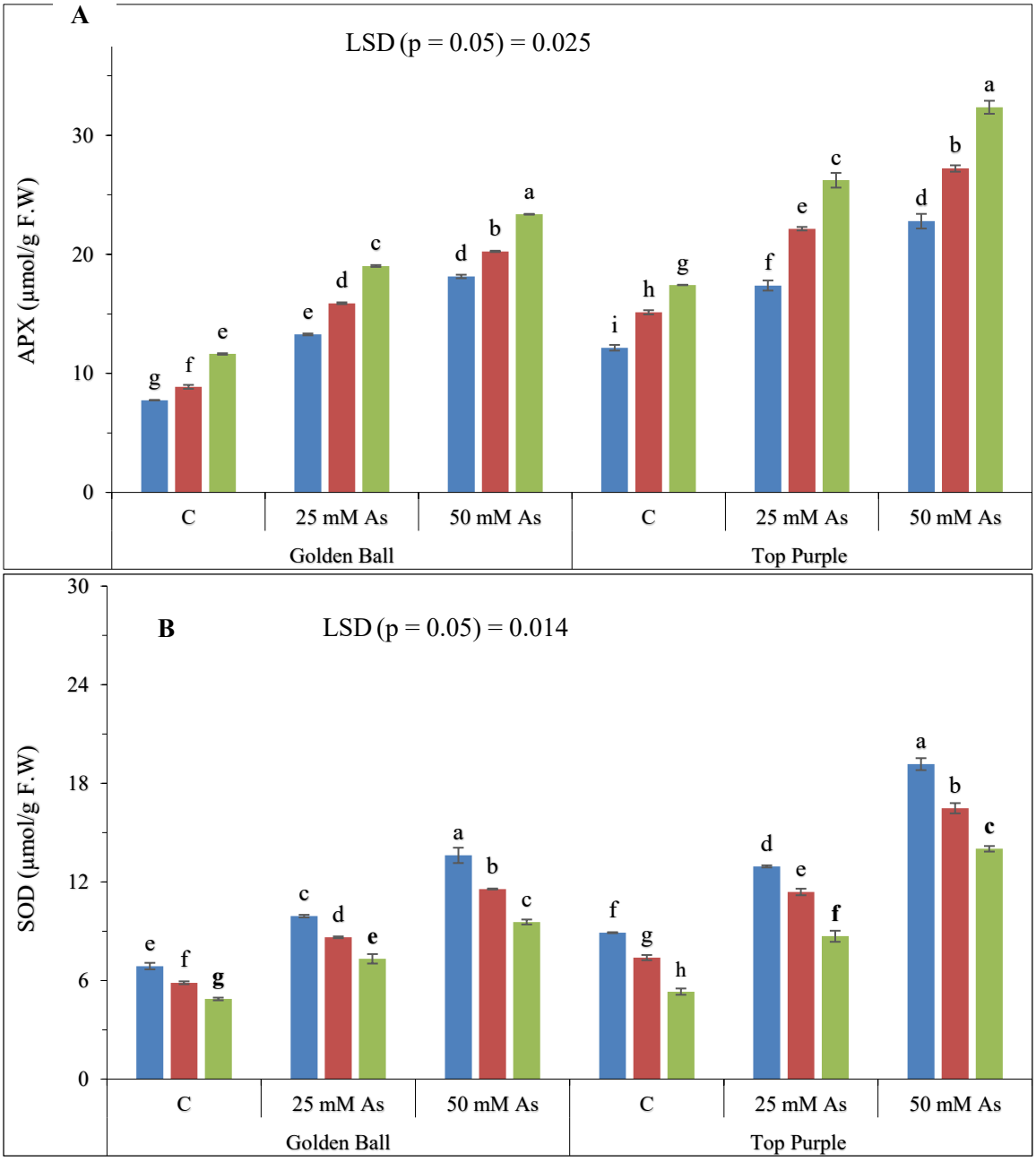


Fig. 13 Effect of microalgae (*C. vulgaris*) application on antioxidant enzymes activity (A) APX (B) SOD in *Brassica rapa* under As stress; Values presented the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC

(control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

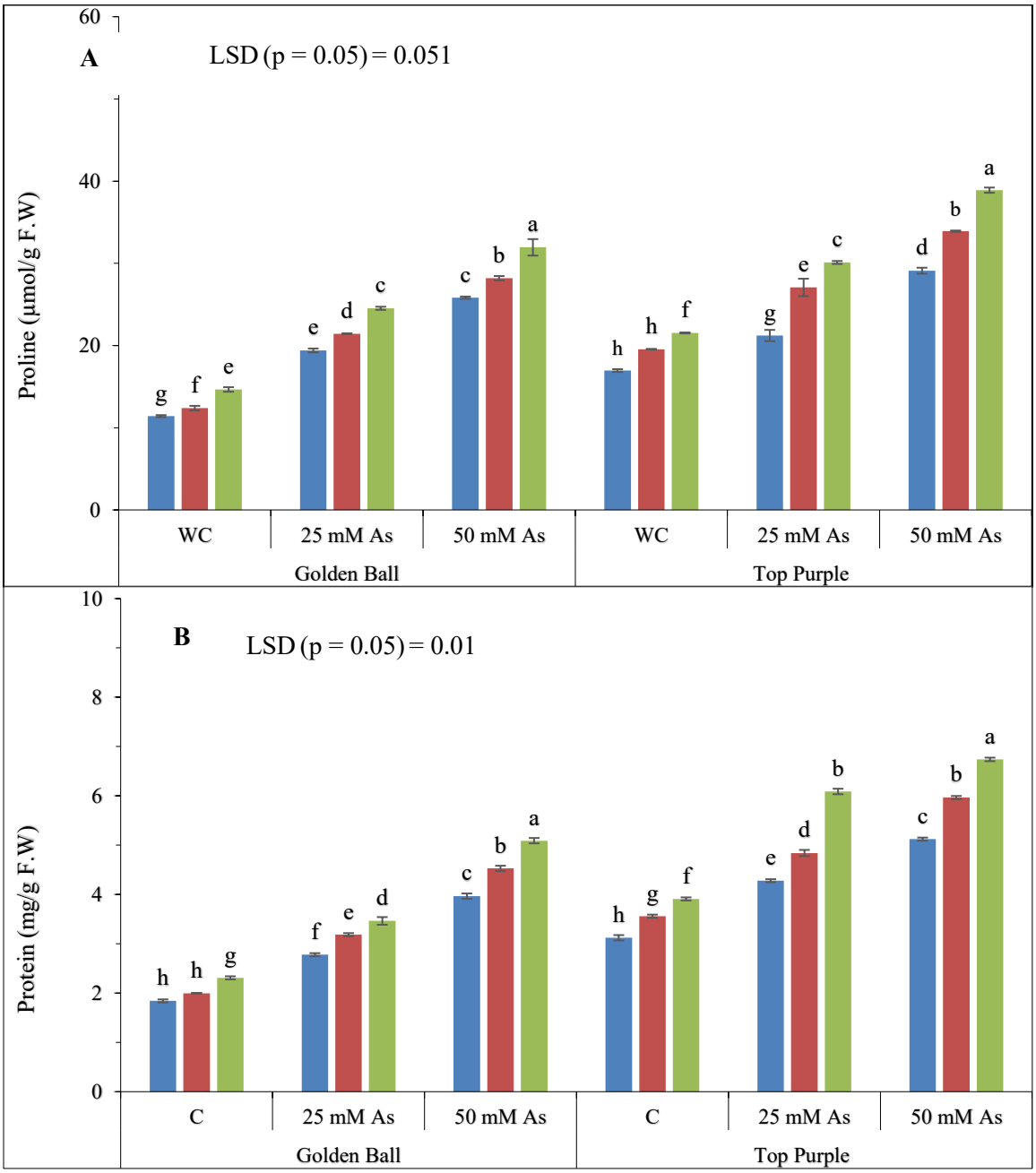


Fig. 14 Effect of microalgae (*C. vulgaris*) application on Proline and total soluble protein in *Brassica rapa* subjected to As-stress; Values demonstrate the means of three replicates ± SD; C = Control; As = Arsenic; Blue bars indicate WC (control); Red bars indicate 5% *C. vulgaris* extract, while light green bars indicate 10% *C. vulgaris* extract

Discussion

Environmental stresses pose major challenges to plant growth and vegetable production, both of which are essential components of the global food supply. Among these stresses, heavy metal contamination is a significant factor limiting crop growth and development, with reported reductions in vegetable productivity ranging from 50% to 70% worldwide (Gull et al., 2019). Arsenic (As) is one of the most toxic metalloids, posing serious risks to both plants and human health. Consistent with findings reported for other crop species, the present study demonstrated that As exposure markedly inhibited the growth and development of turnip plants. The excessive accumulation of arsenic in plant roots can impair physiological processes, restrict overall growth, and reduce crop productivity. Furthermore, arsenic accumulation in edible plant tissues may pose substantial health risks to consumers through the food chain (Van Der Zanden, 2008). In another previous study, arsenic toxicity can disrupt various physiological and biochemical processes in

plants, consequently restricting their growth and productivity (Singh et al., 2015). Soil arsenic concentrations have increased in many regions due to several factors, particularly anthropogenic activities. Arsenic contamination can affect a wide range of plant species, influencing seed germination, vegetative growth, and crop yield. Its uptake by plants, along with its interactions with essential nutrients, may disturb nutrient acquisition and utilization. The movement of water through plants is closely associated with mineral nutrient uptake; however, exposure to heavy metals can interfere with water transport and alter plant water relations. Arsenic distribution within plants may also vary according to species and their tolerance mechanisms, with substantial quantities potentially accumulating in roots and shoots. Excessive arsenic accumulation in roots can impair plant development and reduce productivity, while contamination of edible tissues may threaten human health (Kiran et al., 2022). Previous research has investigated the effects of industrial wastewater irrigation on soil properties and plant performance, including heavy metal accumulation, growth, yield, and biochemical responses in turnip plants. In addition, Carbonell-Barrachina et al. (1999) reported that prolonged arsenic exposure could alter the metabolic processes of turnip plants. Arsenic is generally absorbed through plant roots, although submerged aquatic plants may also take it up through their leaves from the surrounding water (Wolterbeek & van der Meer, 2002). In addition to the potential health concern, plants may not grow normally if there is an excessive amount of As in the soil or water. This can cause yield losses and decrease in biomass. As the amount of Arsenic in water increases, plant height decreases, arsenic-contaminated soils can adversely affect plant morphology and productivity, resulting in reduced yields and decreases in the fresh and dry biomass of both roots and shoots (Garg & Singla, 2011). In the present study, arsenic exposure at 50 mM caused more pronounced damage to photosynthetic pigments than treatment at 25 mM, indicating that the severity of the adverse effects increased with arsenic concentration. These findings are consistent with previous research on rice plants, in which arsenic toxicity was associated with reductions in chlorophyll content, photosynthetic pigments, and overall plant growth (Garg & Singla, 2011).

Among the tested treatments, the 10% concentration produced more pronounced beneficial effects than the 5% concentration. These results are consistent with previous findings indicating that microalgae application can enhance plant growth and photosynthetic pigment accumulation (Mohsenpour et al., 2012). The present study investigated the potential of *C. vulgaris* as a biofertilizer for reducing the adverse effects of arsenic toxicity in turnip plants. The findings demonstrated that microalgal application improved photosynthetic pigments under arsenic stress, with the higher concentration providing greater benefits. Similarly, Ali et al. (2024) reported an increase in photosynthetic pigment levels following microalgal treatment. Chlorophyll depletion can impair plant development by promoting chlorosis and disrupting photosynthetic processes, including the functioning of photosystems I and II (Singh et al., 2020). Arsenic toxicity can further compromise photosynthetic efficiency and damage the photosynthetic apparatus. In the present study, the activity of photosystems I and II was more strongly inhibited at an arsenic concentration of 50 mM than at 25 mM, indicating that higher arsenic exposure intensified the disruption of photosynthetic function. These observations agree with the findings of Huang et al. (2021), who reported adverse effects of arsenic stress on photosystems I and II.

Arsenic toxicity can interfere with electron transport during photosynthesis, thereby altering chlorophyll fluorescence parameters associated with photosystem II (PSII) and photosystem I (PSI). Goussi et al. (2018) reported changes in photosynthetic performance and related fluorescence parameters in Thellungiella salsuginea under arsenic stress. Similarly, Das et al. (2023) observed significant reductions in the quantum yields of PSI and PSII, along with alterations in non-photochemical quenching (NPQ) and related energy dissipation parameters, in turnip plants exposed to heavy metal stress. In the present study, arsenic exposure altered several chlorophyll fluorescence parameters, including Y(ND), NPQ, Y(NPQ), and Y(NA), in both turnip varieties. These changes indicate that arsenic stress affected the regulation of absorbed light energy and the functioning of the photosynthetic electron transport system. The direction and magnitude of these responses varied with the level of metal stress and the plant variety. Chlorophyll is essential for photosynthesis because chlorophyll a and chlorophyll b absorb light energy that supports its conversion into chemical energy (Singh et al., 2015). Carotenoids also play a protective role by reducing photo-oxidative damage under unfavorable environmental conditions. Sharma (2012) highlighted the importance of photosynthetic pigment content as an indicator of photosynthetic efficiency. Environmental stresses can reduce the levels of photosynthetic pigments, thereby limiting the capacity of plants to capture and utilize light energy. A decline in chlorophyll a, chlorophyll b, and total chlorophyll can consequently impair photosynthesis and adversely affect plant growth and development (Gu et al., 2017).

In the present study, arsenic exposure reduced the concentrations of chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll in turnip plants. However, foliar treatment with microalgae improved photosynthetic pigment levels, suggesting a protective role in maintaining photosynthetic performance, metabolic activity, and plant development under arsenic-induced stress. Heavy metal toxicity can trigger oxidative stress through the excessive generation of ROS and the subsequent peroxidation of membrane lipids (de Araújo Silva et al., 2021). In turnip plants, arsenic exposure was associated with increased levels of oxidative stress indicators, including superoxide radicals (O_2^-), hydroxyl radicals (-OH), and hydrogen peroxide (H_2O_2), potentially contributing to damage to the photosynthetic apparatus (Singh et al., 2019). Owing to their high reactivity, ROS can oxidize cellular macromolecules, including lipids, proteins, carbohydrates, and

DNA, thereby compromising cellular integrity and function (Saleem et al., 2023). Excessive ROS production can also promote the peroxidation of membrane fatty acids, resulting in increased malondialdehyde (MDA) accumulation. MDA is widely used as an indicator of lipid peroxidation and oxidative membrane damage in plants (Garg & Manchanda, 2009). Such damage may alter membrane fluidity, impair enzyme activity, increase electrolyte leakage, and disrupt membrane-associated protein channels. Similar responses to heavy metal-induced oxidative stress have been documented in other plant species (Ahmed et al., 2022). The present findings showed that arsenic stress substantially increased ROS accumulation and MDA levels in turnip plants, indicating enhanced oxidative stress and membrane lipid peroxidation. Excessive ROS production, particularly the accumulation of superoxide radicals, can damage cellular membranes and interfere with normal physiological processes. Notably, foliar application of microalgae to arsenic-stressed plants significantly reduced H_2O_2 and MDA concentrations. These results suggest that microalgal treatment may help alleviate oxidative damage to cellular membranes by reducing ROS accumulation and lipid peroxidation. Similar protective effects of microalgal application against oxidative stress and associated membrane damage have also been reported in previous research (Ahmed et al., 2022). By limiting oxidative injury, microalgae may contribute to the maintenance of normal growth and development under arsenic stress.

The synthesis and accumulation of protective compounds, together with the activation of antioxidant enzymes, can strengthen plant defense mechanisms and facilitate recovery from abiotic stress (Suparmaniam et al., 2023). Hamed et al. (2017) also highlighted the importance of proline in plant responses to arsenic toxicity. Similarly, Singh and Prasad (2015) reported increased proline accumulation in wheat under cadmium stress and observed that exogenous microalgal treatment alleviated the adverse effects of cadmium exposure. In the present study, proline levels increased considerably in turnip plants subjected to severe arsenic stress, suggesting that proline accumulation was part of the plants' adaptive response to metal toxicity. However, combined treatment with microalgae may help regulate cellular osmotic balance and maintain intracellular pH, thereby reducing the physiological burden imposed by arsenic stress. These findings suggest that microalgal application may contribute to improved stress tolerance by supporting cellular homeostasis and protective responses in arsenic-exposed turnip plants.

Oxidative stress triggers protective mechanisms in plants, including enhanced activity of antioxidant enzymes such as catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POD). These enzymes contribute to reactive oxygen species (ROS) regulation and help minimize oxidative damage to plant cells (Singh et al., 2015). Khan et al. (2019) reported that cadmium exposure altered the activities of CAT, POD, superoxide dismutase (SOD), and APX in eggplant, mung bean, and cucumber plants. These antioxidant enzymes play essential roles in protecting plant cells against oxidative damage caused by ROS, including superoxide radicals (O_2^-), hydroxyl radicals (-OH), and hydrogen peroxide (H_2O_2) (Sagar et al., 2023). Similarly, increased activities of SOD, CAT, and POD have been reported in *Phaseolus vulgaris* and *Brassica juncea* under stress conditions (Zulfiqar & Ashraf, 2022). The ascorbate–glutathione cycle also contributes to ROS detoxification through a coordinated antioxidant system involving enzymes such as APX and other components that maintain cellular redox balance (Pavlíková et al., 2023). Research on maize has shown that arsenic-induced oxidative stress can stimulate antioxidant defense responses, including increased antioxidant enzyme activity (Bhat et al., 2022). In turnip plants subjected to higher arsenic levels, the activities of peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) exhibited significant alterations, consistent with the findings of the present study. CAT and APX contribute to the detoxification of H_2O_2, while POD participates in peroxide-dependent oxidation reactions. Although increased antioxidant enzyme activity can help protect cellular components, persistent arsenic exposure may generate ROS at rates that exceed the plant's detoxification capacity, potentially damaging proteins and membrane lipids. The alterations in antioxidant enzyme activity therefore indicate how turnip plants respond to arsenic-induced toxicity and the accompanying oxidative stress caused by metal exposure.

The accumulation of specific protective proteins may help plants tolerate adverse environmental conditions and maintain essential metabolic functions. Farooq et al. (2016) reported that soluble protein content initially increased with rising Cd concentrations but subsequently declined, suggesting that prolonged or intensified metal stress can interfere with protein metabolism. However, these responses may not have been sufficient to prevent oxidative damage when ROS accumulation and MDA levels were elevated. The application of microalgae alongside As(V) treatment influenced antioxidant enzyme activities and improved the physiological responses of turnip plants. Changes in CAT, POD, and APX activities following microalgal treatment may reflect adjustments in the plants' antioxidant defense system and their capacity to regulate ROS accumulation and lipid peroxidation. The present study further demonstrated that foliar application of microalgae benefited turnip plants under both arsenic-stressed and non-stressed conditions. Among the tested concentrations, 10% microalgae produced the most favorable responses, followed by the 5% treatment. These findings highlight the potential of microalgae as a biological supplement for improving plant performance under environmental stress. However, the effects of microalgae can vary according to the plant species, treatment concentration, and experimental conditions, as suggested by research on cucumber plants (Wang et al., 2022).

Arsenic accumulation in the shoots of turnip plants increased under arsenic exposure compared with the control treatment. Furthermore, plants exposed to 50 mM arsenic accumulated higher amounts of the metal than those treated with 25 mM, indicating a concentration-dependent increase in

arsenic uptake and accumulation. Similar arsenic accumulation has been documented in several crop species, including wheat, maize, and turnip (Kumar et al., 2019). In the present study, foliar application of microalgae at concentrations of 5% and 10% reduced arsenic accumulation in plant tissues. The 10% microalgal treatment was more effective than the 5% treatment, resulting in lower arsenic accumulation in turnip leaves under arsenic stress. These findings suggest that microalgal application may help restrict arsenic accumulation in aboveground plant tissues. Similarly, a previous investigation in pea plants reported reduced arsenic accumulation following microalgal treatment, supporting the potential use of microalgae to mitigate metal accumulation in plants (Samanta & Roychoudhury, 2021).

Conclusion

The findings of the present study demonstrated that arsenic stress adversely affected the physiological and biochemical characteristics of the tested turnip varieties. Arsenic exposure reduced chlorophyll content, inhibited plant growth, and impaired the efficiency of photosystems I and II. In contrast, the accumulation of reactive oxygen species (ROS) and the extent of membrane lipid peroxidation increased under arsenic stress, indicating enhanced oxidative damage. Overall, these results suggest that arsenic toxicity disrupts photosynthetic activity and cellular homeostasis, thereby limiting the growth and physiological performance of turnip plants. However, the foliar treatment of both 5% and 10% microalgae concentration reduced arsenic-induced toxicity. Higher concentration (10%) of *Chlorella vulgaris* increased growth parameters (root and shoot growth), improved chlorophyll contents, increased efficacy of photosystem I and photosystem II, while reduced ROS and MDA production by reducing Arsenic accumulation, and increased the anti-oxidant activity of CAT, SOD, POD, APX and proline. Therefore, it might be thought that microalgae are a possible solution for decreasing arsenic induced-toxicity.

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