

IRON OXIDE NANOPARTICLES AS A TOOL FOR ENHANCING RAPESEED VIABILITY UNDER EXCESS WATER CONDITIONS

V. Kolbjonoks*, A. Petjukevics, M. Krasovska, N. Skute

Daugavpils University,
13 Vienības Str., Daugavpils, LV-5401, LATVIA
*e-mail: vadims.kolbjonos@du.lv

Flooding is a critical abiotic stressor that adversely impacts crop growth, including rapeseed (*Brassica napus* L.). Iron oxide (Fe_3O_4) nanoparticles (NPs) are emerging as a promising approach for mitigating such environmental stressors. This study aimed to assess the potential of soil-applied Fe_3O_4 NPs to alleviate the adverse physiological effects of flooding on rapeseed. Under controlled climate chamber conditions, plants were subjected to flooding following a pre-treatment application of Fe_3O_4 NPs at concentrations of 0, 50, and 100 mg/L. Results demonstrated that the 50 mg/L treatment significantly mitigated flooding stress, evidenced by increased total chlorophyll content, improved root development, and enhanced scavenging of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2). These antioxidant mechanisms reduced oxidative stress, thereby preserving cellular integrity and photosynthetic function. However, the beneficial effects were strictly dose-dependent; the 100 mg/L concentration exhibited diminished efficacy and induced phytotoxicity compared to the optimal 50 mg/L dosage. These findings demonstrate that optimised, low-concentration Fe_3O_4 NP applications can effectively enhance rapeseed resilience to flooding. Future field studies are required to validate these outcomes and elucidate the underlying mechanisms for practical agricultural implementation.

Keywords: Carotenoids, chlorophyll, crops, Fv/Fm, iron oxide nanoparticles, rapeseed, ROS.

1. INTRODUCTION

Abiotic stresses negatively impact plant growth and development, severely affecting crop yields and leading to significant economic losses. With global climate change, both the magnitude and frequency of severe weather events, such as extreme temperatures and altered precipitation patterns, are predicted to increase [1]. Waterlogging, or root flooding, is a major agricultural challenge worldwide, particularly in low-lying rain-fed areas. Because water is an extremely poor medium for gas diffusion, hampered gas exchange within flooded organs restricts two vital plant processes: respiration and photosynthesis [2]. Rapeseed (*Brassica napus* L.) is an oilseed plant cultivated globally; its meal contains approximately 40 % protein. It is a strategic crop used for animal feed, cooking oil production, and, increasingly, as a source for biofuel.

The photosynthetic apparatus within the chloroplast is a primary and highly susceptible target of environmental stress. Changes in the functionality of the photosynthetic apparatus, photosynthetic pigment content, and their respective ratios are critical indicators of a plant's physiological state under stress [3]-[5]. As a consequence of waterlogging, plant cells experience pronounced internal fluctuations in O_2 and CO_2 , elevated ethylene levels, and an accumulation of reactive oxygen species (ROS). During the onset of flooding-induced oxygen deprivation, ROS are generated primarily due to the inhibition of the mitochondrial electron transport chain [6]. The primary sources of ROS in plant cells include chloroplasts, mitochondria,

and peroxisomes [6]. To mitigate the cytotoxic effects of ROS, plants deploy various non-enzymatic antioxidants (e.g., carotenoids, ascorbate) and enzymatic defense systems (e.g., superoxide dismutase, catalase). While these adaptive mechanisms improve plant resilience, they demand significant energy and metabolite expenditure to restore cellular homeostasis [6]-[8].

In recent years, the application of nanomaterials in agriculture has gained considerable attention for its potential to enhance crop growth, stress resilience, and nutritional value. Iron is a vital micronutrient for plants, playing a critical role in chlorophyll synthesis, respiration, and redox reactions [9]. As a structural component of chloroplasts, it is indispensable for plant growth and development. Iron deficiency typically induces chlorosis, a condition characterized by impaired chlorophyll synthesis, reduced photosynthetic capacity, and disrupted respiratory function. Apical (young) leaves are particularly vulnerable, exhibiting yellowing and eventual desiccation. Consequently, iron-based nanomaterials may offer a more bioavailable and efficient source of this crucial element compared to bulk ionic forms.

Therefore, the goal of this study was to synthesize iron oxide (Fe_3O_4) nanoparticles and evaluate their efficacy in mitigating flooding stress in rapeseed (*Brassica napus* L.). To achieve this goal, we analyzed key stress indicators and the functional state of the photosynthetic apparatus, specifically focusing on photosystem II efficiency and photosynthetic pigment content.

2. MATERIALS AND METHODS

2.1. Materials

The following chemicals were obtained from (Merck, Germany):

- Iron(III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, CAS: 10025-77-1);
- Iron(II) chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, CAS: 13478-10-9);
- Ammonium hydroxide solution (NH_4OH , 32%, CAS: 1336-21-6);
- Cobalt(II) nitrate hexahydrate ($\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, CAS: 10026-22-9);
- Urea (NH_2CONH_2 , CAS: 57-13-6);
- Sodium chloride (NaCl , CAS: 7647-14-5);
- Potassium nitrate (KNO_3 , CAS: 7757-79-1);
- Glucose ($\text{C}_6\text{H}_{12}\text{O}_6$, CAS: 50-99-7);
- Citric acid ($\text{HOC}(\text{COOH})(\text{CH}_2\text{COOH})_2$, CAS: 77-92-9);
- Ascorbic acid ($\text{C}_6\text{H}_8\text{O}_6$, CAS: 50-81-7);
- Hydrogen peroxide solution (H_2O_2 , 30%, CAS: 7722-84-1).

All reagents had a minimum purity of 99.8 %. Additional materials included:

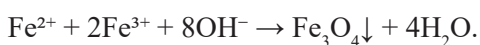
- Iron wires (2 mm thickness, 99.9 % purity) (Sigma-Aldrich, US);
- Ag/AgCl wires (A-M Systems, US);
- Carbon rods: 5 mm diameter (Sigma-Aldrich, US).

2.2. Synthesis and Characterisation of Fe_3O_4 Nanoparticles

Fe_3O_4 nanoparticles were synthesized via the co-precipitation method, which is well-suited for producing small, plant-compatible nanoparticles. A solution of 0.2334 g $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 0.0858 g $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ in 100 mL distilled water was prepared. Then, 0.54 mL of 25% NH_4OH was added dropwise under continuous stirring. This reaction yielded a black precipitate weighing 72 mg. To stabilize the nanoparticles, an aqueous citric acid solution was added. The Fe_3O_4 precipitate was magnetically separated from the solution, washed several times with distilled water, and removed when the washing water became clear.

The overall hydrothermal synthesis

reaction can be summarised as:



The morphology and size of the Fe_3O_4 nanoparticles were analyzed using field emission scanning electron microscopy (FESEM) MAIA 3 (Tescan, Czech Republic). The chemical composition was examined with an EDS attachment (Inca, Oxford Instruments). SEM images revealed spherical nanoparticles averaging 60 nm in diameter, often appearing as agglomerates. Energy-dispersive spectroscopy (EDS) confirmed that the powder was free of impurities. The nanoparticles dissolved easily in water when treated ultrasonically.

2.3. Chemical Composition Determination

Chemical composition was assessed by energy-dispersive spectroscopy under the following conditions:

- Accelerating voltage: 30 keV;
- Chamber pressure: 9×10^{-3} Pa;
- Working distance: 7 mm;
- Electron beam spot size: 90 nm;
- Absorption current: 510 pA;
- Magnification: 7 kx.

2.4. Plant Material, Growth Conditions, and Stress Treatment

Rapeseed (*Brassica napus* L.) seeds of the cultivar Agrs Clearfield (InV 140 CL), provided by BASF Corporation (Germany), were germinated in a universal peat-based substrate. The plants were grown in a Versatile Environmental Climate Test Chamber (Sanyo, Japan) under controlled conditions: a 16/8-hour light/dark photoperiod, light intensity of $30 \mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of $20 \pm 1^\circ\text{C}$, and relative humidity of approximately 70 %. After one week of growth,

the seedlings were divided into experimental groups. Fe_3O_4 nanoparticles, dissolved in water via ultrasonication, were applied daily by watering the plants with solutions at concentrations of 50 mg/L and 100 mg/L. The control group received only water. To induce flooding stress, the plant containers were placed in trays filled with water to a level of 2 cm above the substrate surface for the duration of the experiment.

2.5. Determination of Photosynthetic Pigments by Spectrophotometry

Chlorophyll (Chl *a*, Chl *b*) and carotenoid concentrations were measured at room temperature (20°C). Fresh plant material (200 mg) was ground with 5 mL of 80 % acetone in a chilled mortar. The volume was then brought to 10 mL with acetone, and the mixture was incubated

in the dark for 24 hours. After extraction, the mixture was centrifuged at 8,000 rpm, and the supernatant was transferred to clean tubes. Absorbance was measured in a quartz cuvette (10 mm path length) at 663, 646, and 470 nm using a deNovix DS-7, US spectrophotometer [10], [8].

2.6. Measurement of Chl *a* Fluorescence

For all fluorescence measurements carried out a handheld pulse modulated fluorometer OS-30p (Opti-Sciences, US) equipped with a leaf-clip holder for plant leaves; all measurements were made at room temperature (20°C). By simultaneously monitoring the reactions of PSII and PSI and recording the induction of changes in delayed fluorescence, it was possible to simultaneously record fluorescence and assesses the pigment's redox state of the PSI reaction-center. An array of red LEDs at 660 nm was used as the saturation light source. Fluorescence measurements and the evaluation of the redox state by the change

in absorbance were carried out at 700–750 nm wavelengths. The beam saturation intensity was exposed at 6000 μM . The test lasts between 0.1 and 1.5 seconds, the detection mechanism is pulse-modulated, and the automatic light calibration is set to 3500 μM . The software uses a rolling eight-point 25 ms average to calculate F_0 and F_m , even though the default saturation pulse duration was set at 2.0 seconds. For plant dark adaptation phase: samples were left in the dark for 30 minutes, 60 minutes, 90 minutes, 120 minutes, and 24 hours, and fluorescence index measurement was accomplished on each stage [11]–[13].

2.7. Preparation of Co_3O_4 Nanostructured Electrodes for H_2O_2 Detection

Wire-like Co_3O_4 nanostructures were synthesized and used in a sensor for detecting hydrogen peroxide, as detailed in prior publications [9]. Electrodes were pre-

pared through hydrothermal synthesis and subsequent thermal decomposition. Iron wires were cleaned in 0.1 M HCl, rinsed, and cut into 6 cm segments. The wires

were then immersed in a solution of 0.1 M $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ and urea in 80 mL of distilled water, which was stirred until dissolved. The wires and solution were heated at 95 °C for 5 hours, forming nanostructured

$\text{Co}(\text{OH})_2$ coatings. These were rinsed, dried at 90 °C for 3 hours, and annealed at 450 °C for 1 hour to convert $\text{Co}(\text{OH})_2$ into Co_3O_4 nanostructures.

2.8. Electrochemical Measurements:

A custom-designed electrochemical cell was employed, consisting of:

- a nanostructured $\text{Fe}/\text{Co}_3\text{O}_4$ working electrode;
- a carbon counter electrode;
- an Ag/AgCl reference electrode;

- a 0.1 M NaOH electrolyte solution (pH 13).

Cyclic voltammetry was performed with a potential range from -1.3 V to +0.5 V, starting at 0 V, and a scan rate of 100 mV/s.

2.9. H_2O_2 Detection Methodology and Quantification₂

The detection mechanism for H_2O_2 relies on the catalytic activity of nanostructured Co_3O_4 electrodes. The nanostructures serve as catalysts, promoting the decomposition of H_2O_2 into CoOOH and H_2O . The Co_3O_4 surface offers numerous active sites where H_2O_2 oxidation occurs, forming CoOOH . This catalytic reaction is evidenced by a marked increase in the amplitude of oxidation and reduction peaks on cyclic voltammetry (CV) graphs upon introducing H_2O_2 into the solution. These reversible reactions and the underlying detection mechanism can be summarized as follows [9].

To quantify H_2O_2 in rapeseed samples, a NaOH-based leaf extract was prepared.

To prepare the extract, 200 mg of fresh leaf tissue was homogenized in 5 mL of 0.1 M NaOH solution. The homogenate was then centrifuged at 10,000 rpm for 15 minutes, and the supernatant was collected for analysis. Since the actual H_2O_2 concentration in stressed rapeseed samples was unknown, known amounts of H_2O_2 were manually added to the extract during measurements. The recorded data were used to construct a calibration curve based on the 0.1 M NaOH electrolyte. H_2O_2 concentration in the sample was calculated by subtracting the added H_2O_2 from the total concentration detected. For each measurement, 70 mL of analyte solution was used.

3. RESULTS AND DISCUSSION

Iron oxide (Fe_3O_4) nanoparticles were synthesized as a black amorphous powder. Energy-dispersive X-ray spectroscopy (EDS) confirmed the chemical purity of the nano-powder, indicating the presence of only iron and oxygen in a stoichiomet-

ric ratio consistent with Fe_3O_4 . Particle size, determined via secondary electron microscopy (SEM), ranged from 10 to 60 nm, confirming the nanoscale nature of the synthesized samples.

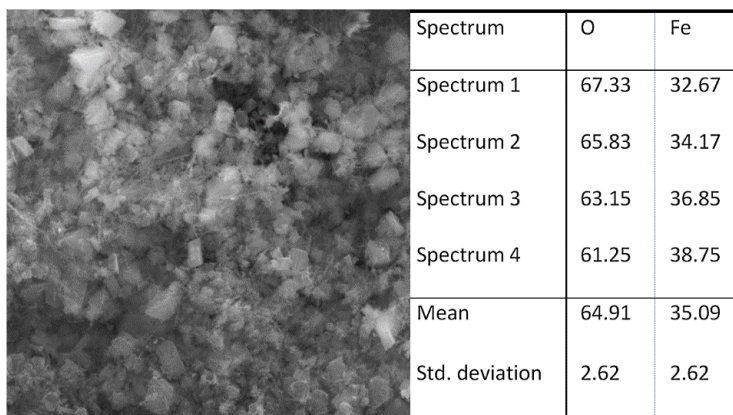


Fig. 1. SEM image of Fe_3O_4 nanoparticle powder (left), chemical composition of nanoparticles (right).

Waterlogging or root-zone flooding is one of the most significant climate-related challenges adversely impacting global crop growth and yield, particularly in low-lying, rainfed agricultural regions. This stress arises primarily from the poor gas diffusion properties of water, which hinder gas exchange in submerged plant organs and consequently disrupt two critical physiological processes: respiration and photosynthesis. During chlorophyll *a* fluorescence measurement, weakly modulated light is applied at intensity sufficient to induce antenna fluorescence without triggering photosynthetic activity.

To evaluate the physiological impact of flooding and NP treatment, chlorophyll *a* fluorescence was measured. A weakly modulated light was applied to induce antenna fluorescence without triggering photosynthetic electron transport, allowing for the accurate determination of the minimum fluorescence yield after dark adaptation, where photosystem II (PSII) remains fully oxidized. Under these conditions, related processes such as the xanthophyll cycle, thylakoid lumen pH gradient, and state transitions are effectively suppressed [14], [2].

Under flooding-induced oxygen deprivation, the inhibition of the mitochondrial electron transport chain leads to the exces-

sive generation of reactive oxygen species (ROS), including the superoxide radical (O_2^-), which is subsequently converted to hydrogen peroxide (H_2O_2) via dismutation. This oxidative stress disrupts key cellular processes, including chlorophyll synthesis and photosynthesis.

Our data confirm a substantial increase in H_2O_2 production under flooding conditions; however, pre-treatment with iron oxide nanoparticles (IONPs) significantly alleviated this oxidative stress. The data presented in Fig. 2 illustrate the effect of IONPs on H_2O_2 accumulation in rapeseed tissues under both control and flooded conditions. In the control group, IONP application reduced endogenous H_2O_2 concentrations by 33 % and 13 % at dosages of 50 mg/L and 100 mg/L, respectively. Under flood-stressed conditions, the mitigation was even more pronounced, with reductions of 35 % and 30 % for the 50 mg/L and 100 mg/L concentrations, respectively (Fig. 2).

These results demonstrate that IONPs consistently suppress H_2O_2 accumulation, likely by augmenting intrinsic antioxidant defense mechanisms, thereby stabilizing physiological functions under hypoxic stress [8]. Notably, the 50 mg/L concentration exhibited superior efficacy compared to the 100 mg/L treatment. This concentra-

tion-dependent response suggests that while optimal (lower) doses stimulate intrinsic defense mechanisms and offer significant protective benefits, excessive concentrations may induce phytotoxic effects. Consequently, precise dosage calibration is critical for maximizing the therapeutic benefits of nanoparticle-based interventions while mitigating potential risks to plant health.

This underscores the importance of dosage optimization in nanoparticle-based interventions. The agricultural and biotechnological implications of these findings are significant, as IONPs may serve as a novel strategy for improving plant resilience and productivity. Their application has the potential to reduce oxidative stress, enhance plant health, and contribute to sustainable crop management practices. Furthermore, their use may facilitate new biotechnological approaches to plant protection. The efficacy of IONPs is influenced by several factors, including their concentration, size, morphology, surface properties, and the physiological state of the plant.

While moderate concentrations tend to promote growth and stress tolerance, excessive levels may produce phytotoxic effects. Therefore, careful calibration of nanoparticle properties and application protocols is essential to maximize benefits while mitigating risks. In summary, the application of iron oxide nanoparticles appears to be a promising approach for reducing hydrogen peroxide accumulation, enhancing plant stress tolerance, and promoting sustainable agricultural development. Under flooding-induced oxygen deprivation, the inhibition of mitochondrial electron transport leads to the generation of reactive oxygen species (ROS), including superoxide (O_2^-), which is subsequently converted to H_2O_2 by dismutation. This oxidative stress disrupts key cellular processes such as photosynthesis and chlorophyll synthesis. Our data confirm that H_2O_2 production increases under these conditions; however, treatment with IONPs significantly alleviates oxidative stress, in agreement with previous findings [15]–[17].

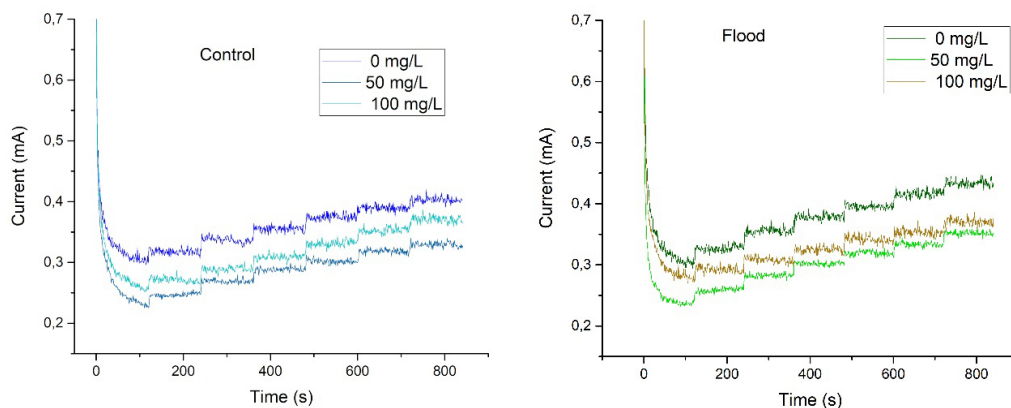


Fig. 2. Dynamics of hydrogen peroxide (H_2O_2) concentration in leaves of *Brassica napus* L. plants exposed to varying concentrations of Fe_3O_4 nanoparticles under abiotic stress conditions: control group (left side) and flooding stress (right side), measured at the third week of treatment.

Measurements of Chl a Fluorescence Parameter. Alterations in the structural organization of the photosynthetic apparatus in

rapeseed (*Brassica napus* L.) leaves under flooding stress were closely associated with a marked suppression of photosystem II

(PSII) photochemical activity. As a baseline indicator of maximum quantum efficiency, the v/F_m ratio was evaluated. Under flooding conditions, the control group (without nanoparticle treatment) exhibited a significant 5.6% reduction in the F_v/F_m ratio, confirming the onset of photoinhibition and stress-induced impairment of photosynthetic electron transport (Fig. 3). The observed decrease in F_v/F_m under flooding stress reflects a decline in PSII photochemical efficiency and suggests an inhibition of primary photochemistry [18]. This reduction was primarily driven by a significant drop in maximal fluorescence (F_m) coupled with a concurrent increase in minimal fluorescence (F_0), both of which are hallmark indicators of stress-induced damage to the PSII reaction centers. Following this, the leaves were exposed to a high-intensity saturating light pulse, sufficient in strength and duration to completely reduce or close all PSII reaction centers, thereby enabling precise determination of the maximum fluorescence yield (F_m) and calculation of the F_v/F_m ratio [18], [19]. The observed decrease in the F_v/F_m ratio under flooding stress reflects a decline in PSII photochemical efficiency and suggests inhibition of primary photochemistry [18]. Tracking mini-

mal (F_0) and maximal (F_m) fluorescence over a three-week observation period provided further insights into the redox state of chlorophyll a and PSII functionality [10]. In non-flooded plants, treatment with Fe_3O_4 NPs at 50 mg/L and 100 mg/L induced an increase in F_0 . Notably, the 50 mg/L concentration exerted a more pronounced effect on F_0 values (increasing from 232 to 262 in week one, 263 to 304 in week two, and 259 to 325 in week three) compared to the 100 mg/L treatment. A baseline gradual increase in F_0 was also observed in untreated, non-stressed control plants (from 232 to 259). This elevation in F_0 indicates a shift in the PSII redox equilibrium, typically associated with the closure of PSII reaction centers or the partial dissociation of the outer light-harvesting antenna complex from the PSII core [20], [21].

Under flooding conditions, F_0 levels increased even in the absence of nanoparticle treatment, reaching 297 in the first week. However, the application of Fe_3O_4 nanoparticles at 50 mg/L during flooding stress reversed this increase, reducing F_0 to values comparable to the initial control level (from 297 to 230, versus control at 232 in week one). This mitigating effect was less evident at the higher concentration of 100 mg/L.

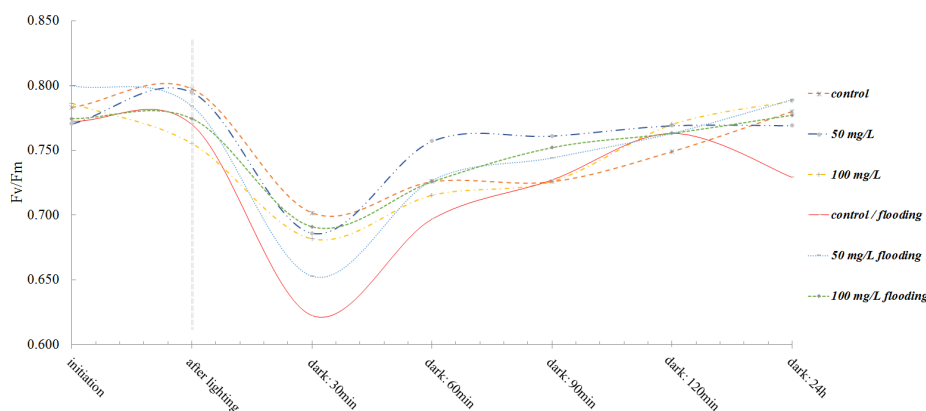


Fig.3. Chlorophyll a F_v/F_m parameter changes in *Brassica napus* L. under flooding and different Fe_3O_4 nanoparticles concentrations.

Similarly, an increase in maximal fluorescence (F_m) was observed in control group (non-flooded) plants treated with nanoparticles (from 1210 to 1280, 1207 to 1265, and 1180 to 1249 across the respective weeks). Under flooding conditions, F_m also rose in untreated plants (from 1210 to 1320 in the first week). The subsequent decrease in F_m observed in flooded plants may be attributed to inhibition of the oxygen-evolving complex [22], [23] and structural disintegration of the photosynthetic machinery. These disruptions reduce PSII photochemical efficiency, enhance energy dissipation as fluorescence, increase non-radiative energy losses, and promote a shift in excitation energy flow toward PSI [20].

The application of iron oxide (Fe_3O_4) nanoparticles influenced the F_v/F_m ratio differently depending on their concentration and the environmental conditions. Under non-flooded conditions, treatment with 50 mg/L of nanoparticles caused a slight decrease in the F_v/F_m ratio, whereas 100 mg/L led to a mild enhancement in photochemical efficiency, reflected by an increase in F_v/F_m (Fig. 3). Under flooding condi-

tions, plants treated with 100 mg/L of Fe_3O_4 nanoparticles exhibited a slight increase in the F_v/F_m ratio relative to the initial stage of stress exposure, indicating improved resilience of the photosynthetic apparatus. In contrast, the 50 mg/L treatment helped mitigate the decline in F_v/F_m by 1.4% compared to untreated flooded controls, suggesting a partial protective effect (Fig. 3).

These results suggest that flooding leads to both structural and functional impairments of the photosynthetic machinery, including damage to PSII reaction centres [5]. However, the application of iron nanoparticles particularly at higher concentrations may contribute to the stabilization of PSII function and enhance the tolerance of rapeseed plants to flooding stress by maintaining higher levels of photochemical efficiency.

Photosynthesis, being central to plant growth and development, is closely associated with chlorophyll content [24]. However, when the absorption of light energy exceeds the photochemical capacity, excess energy may lead to the generation of reactive oxygen species (ROS), such as superoxide anions and hydroxyl radicals [25], [26].

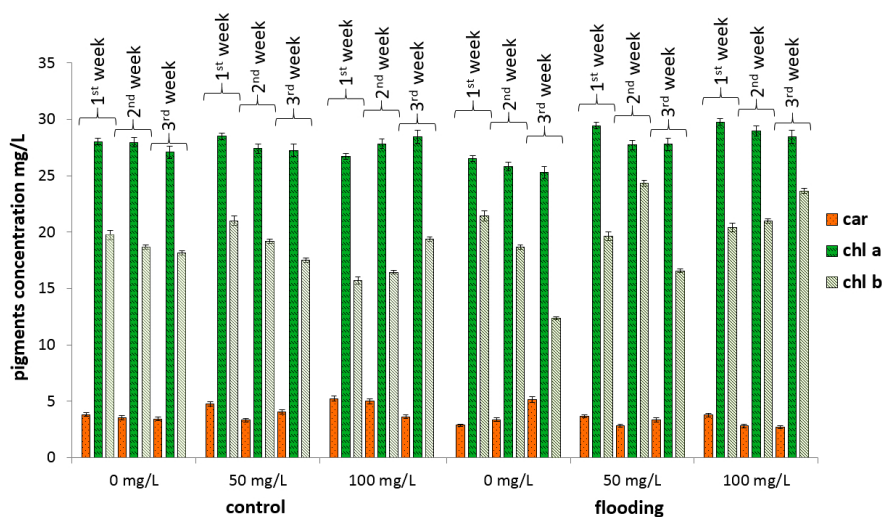


Fig. 4. Measurements of pigments: Chl a, Chl b, and Car dynamics in leaves of *Brassica napus* L. under the influence of different concentrations of Fe_3O_4 nanoparticles and abiotic stress factor: flooding, during the 1st week.

The dynamics of major photosynthetic pigments – chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*), and carotenoids (Car) – in *Brassica napus* L. leaves were monitored over a three-week period under varying concentrations of Fe₃O₄ nanoparticles (0, 50, and 100 mg/L) across two environmental regimes: optimal (control) and flooding stress (Fig. 4).

Under control conditions, pigment concentrations remained relatively stable or exhibited a gradual increase, indicative of healthy photosynthetic performance. Notably, within this non-stressed group, treatment with 100 mg/L Fe₃O₄ yielded the highest levels of Chl *a* and Chl *b* by week three, suggesting that iron oxide supplementation effectively enhances chlorophyll biosynthesis and supports the structural development of the photosynthetic apparatus.

Conversely, under flooding stress, untreated plants exhibited a sharp decline in total chlorophyll content due to stress-induced degradation. However, pre-treatment with 50 mg/L Fe₃O₄ NPs significantly attenuated this loss, maintaining Chl *a* and Chl *b* levels at higher point. A characteristic decrease in total pigment concentrations (Chl *a*, Chl *b*, and Car) was observed during natural plant development. Interestingly, the increase in carotenoids, particularly at 50 mg/L, may reflect the activation of non-photochemical quenching mechanisms for photoprotection. In contrast, flooding stress caused a significant decline in all pigment contents most notably Chl *a* in untreated plants, indicating damage to the photosynthetic system likely due to reduced chlorophyll synthesis, accelerated degradation, and

photoinhibition under hypoxic conditions. However, Fe₃O₄ nanoparticle treatments mitigated these adverse effects, and at 100 mg/L, pigment levels remained relatively stable throughout the stress period. Chl *a* increased by ~27.5%, Chl *b* by ~17.5%, and carotenoids by ~11% compared to week one under optimal conditions. Under flooding, untreated plants showed pigment losses of ~37.5% for Chl *a*, ~30% for Chl *b*, and ~25% for Car. However, at 50 mg/L Fe₃O₄, the decrease in Chl *a* was limited to ~11% and carotenoids to ~6%; at 100 mg/L, Chl *a* even slightly increased by ~5%, and Chl *b* rose by ~25% relative to week one. In contrast, the control group showed a ~6% reductions in Chl *b*, likely due to normal developmental senescence. These findings indicate that Fe₃O₄ nanoparticles, particularly at 100 mg/L, can effectively alleviate flooding-induced pigment loss, presumably by enhancing antioxidant activity, stabilizing chloroplast structure, and maintaining redox balance. Additionally, although carotenoid biosynthesis declined under flooding, the presence of iron nanoparticles appeared to support membrane protection by increasing carotenoid content in stressed leaves, with assumed roles in photoprotection and oxidative stress mitigation. Overall, our results are consistent with previous findings showing that the effects of nanoparticles on plant physiology are concentration-dependent and influenced by environmental conditions. Under flooding, iron nanoparticles supported pigment system recovery, improved photochemical stability, and helped preserve photosynthetic capacity in *Brassica napus* L [2], [27], [28].

4. CONCLUSION

This study demonstrates that iron oxide (Fe₃O₄) nanoparticles can significantly alle-

viate flooding-induced stress in *Brassica napus* L., acting primarily through the sta-

bilization of the photosynthetic apparatus. The application of Fe₃O₄ nanoparticles elicited a dose-dependent response, with lower concentrations providing clear protective and stimulating effects.

The primary mechanism of this protection appears to be the mitigation of oxidative stress. Nanoparticle-treated plants, particularly under flooding, exhibited a marked reduction in hydrogen peroxide (H₂O₂) levels. This enhanced antioxidative capacity helps preserve the integrity of chloroplasts. Consequently, the functional stability of photosystem II was improved, as shown by chlorophyll *a* fluorescence analysis, and the degradation of chlorophyll and carotenoid pigments was lessened. The nanoparticles also supported carotenoid-mediated photo-

protective mechanisms, which are crucial for dissipating excess energy under stress.

While nanoparticles enhanced overall plant development and stress resilience, this beneficial effect was diminished at higher concentrations, highlighting that dosage is a critical factor for avoiding phytotoxicity. Therefore, careful optimization is essential before practical application.

In summary, Fe₃O₄ nanoparticles are a promising tool for enhancing crop resilience in sustainable agriculture. However, future research is crucial to fully elucidate the underlying molecular pathways, assess long-term ecological impacts, and establish safe and effective application protocols for field conditions.

REFERENCES

1. Raza, A., Razzaq, A., Mehmood, S. S., Zou, X., Zhang, X., Lv, Y., & Xu, J. (2019). Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. *Plants*, 8(2), 34. <https://doi.org/10.3390/plants8020034>
2. Sasidharan, R., & Voesenek, L. A. C. J. (2015). Ethylene-mediated acclimations to flooding stress. *Plant Physiology*, 169(1), 3–12. <https://doi.org/10.1104/pp.15.00387>
3. Ashraf, M., & Harris, P. J. C. (2013). Photosynthesis under stressful environments: An overview. *Photosynthetica*, 51(2), 163–190. <https://doi.org/10.1007/s11099-013-0021-6>.
4. Batjuka A., Škute N., & Petjukevičs A. (2016). The influence of antimycin A on pigment composition and functional activity of photosynthetic apparatus of *Triticum aestivum* L. under high temperature. *Photosynthetica* 55(2), 251–263. <https://doi.org/10.1007/s11099-016-0231-9>
5. Cui, L., Li, J., Fan, Y., Xu, S., & Zhang, Z. (2006). High temperature effects on photosynthesis, PSII functionality and antioxidant activity of two *Festuca arundinacea* cultivars with different heat susceptibility. *Botanical Studies*, 47(1), 61–69.
6. Kreslavski, V. D., Los, D. A., Allakhverdiev, S. I., & Kuznetsov, V. V. (2012). Signaling role of reactive oxygen species in plants under stress. *Russian Journal of Plant Physiology*, 59(2), 141–154. <https://doi.org/10.1134/S1021443712020057>
7. Škute, N., Savicka, M., Petjukevičs, A., & Harlamova, N. (2020). Application of the Luminometric Methylatoion Assay for plant ecological researches: The study of global DNA methylation in leaves of *Elodea canadensis* under laboratory conditions and in leaves of fen orchid from wild populations. *Plant Omics*, 13(1), 30–36. <https://doi.org/10.21475/POJ.13.01.20.p2111>
8. Petjukevičs, A., Batjuka, A., & Škute, N. (2015). The impact of different levels of sodium chloride on the quantitative changes of chlorophyll and carotenoids in chloroplasts of *Elodea canadensis* (Michx. 1803). *Biologija* 61(1), 34–41. <https://doi.org/10.6001/biologija.v61i1.3109>

9. Kolbjonoks, V., Petjukevičs, A., Krasovska, M., & Škute, N. (2024). Influence of Fe₃O₄ nanoparticles on oxidative processes and photosynthetic pigments of *Brassica napus* L. under drought. In *2024 IEEE 14th International Conference Nanomaterials: Applications & Properties (NAP)* (pp. 1–6). IEEE. <https://doi.org/10.1109/NAP62956.2024.10739747>
10. Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. *Methods in Enzymology*, *148*, 350–382. [https://doi.org/10.1016/0076-6879\(87\)48036-1](https://doi.org/10.1016/0076-6879(87)48036-1)
11. Antal, T. K., Osipov, V. A., Matorin, D. N., & Rubin, A. B. (2011). Membrane potential is involved in regulation of photosynthetic reactions in the marine diatom *Thalassiosira weissflogii*. *Journal of Photochemistry and Photobiology B: Biology*, *102*(2), 169–173. <https://doi.org/10.1016/j.jphotobiol.2010.11.005>
12. Franklin, L. A., & Badger, M. R. (2001). A comparison of photosynthetic electron transport rates in macroalgae measured by pulse amplitude modulated chlorophyll fluorometry and mass spectrometry. *Journal of Phycology*, *37*(5), 756–767. <https://doi.org/10.1046/j.1529-8817.2001.00156.x>
13. Havaux, M., Ernez, M., & Lannoye, R. (1988). Tolerance of poplar (*Populus* sp.) to environmental stresses: I. Comparative study of poplar clones using the in vivo chlorophyll fluorescence method. *Acta Oecologica/Oecologia Plantarum*, *9*(2), 161–172.
14. Küster, A., Pohl, K., & Altenburger, R. (2007). A fluorescence-based bioassay for aquatic macrophytes and its suitability for effect analysis of non-photosystem II inhibitors. *Environmental Science and Pollution Research*, *14*(6), 377–383. <https://doi.org/10.1065/espr2007.04.410>
15. Giraldo, J. P., Landry, M. P., Faltermeier, S. M., McNicholas, T. P., Iverson, N. M., Boghossian, A. A. & Strano, M. S. (2014). Plant nanobionics approach to augment photosynthesis and biochemical sensing. *Nature Materials*, *13*(4), 400–408. <https://doi.org/10.1038/nmat3890>
16. Rezvani, N., Sorooshzadeh, A., & Farhadi, N. (2012). Effect of nano-silver on growth of saffron in flooding stress. *International Journal of Biological, Life and Agricultural Sciences*, *6*(1), 517–522.
17. Savicka, M., Petjukevics, A., Batjuka, A., & Skute, N. (2018). Impact of moderate heat stress on the biochemical and physiological responses of the invasive waterweed *Elodea canadensis* (Michx. 1803). *Archives of Biological Sciences*, *70* (3), 551–557. <https://doi.org/10.2298/ABS180119016S>
18. Song, Y., Chen, Q., Ci, D., Shao, X., & Zhang, D. (2014). Effects of high temperature on photosynthesis and related gene expression in poplar. *BMC Plant Biology*, *14*, 111. <https://doi.org/10.1186/1471-2229-14-111>
19. Genty, B., Briantais, J.-M., & Baker, N. R. (1989). The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. *Biochimica et Biophysica Acta (BBA) - General Subjects*, *990*(1), 87–92. [https://doi.org/10.1016/S0304-4165\(89\)80016-9](https://doi.org/10.1016/S0304-4165(89)80016-9)
20. Mishra, R. K., & Singhal, G. S. (1992). Function of photosynthetic apparatus of intact wheat leaves under high light and heat stress and its relationship with peroxidation of thylakoid lipids. *Plant Physiology*, *98*(1), 1–6. <https://doi.org/10.1104/pp.98.1.1>
21. Rodríguez, V. M., Soengas, P., Alonso-Villaverde, V., Sotelo, T., Cartea, M. E., & Velasco, P. (2015). Effect of temperature stress on the early vegetative development of *Brassica oleracea* L. *BMC Plant Biology*, *15*, 145. <https://doi.org/10.1186/s12870-015-0535-0>
22. Taran, S., Garip, A. K., & Arslan, H. (2016). Theoretical study of the structures and chemical ordering of CoPd nanoalloys supported on MgO(001). *International Journal of Modern Physics C*, *27*(12), 1650146. <https://doi.org/10.1142/S0129183116501461>
23. Tóth, S. Z., Schansker, G., Kissimon, J., Pálfalvi, L., Garab, G., & Strasser, R. J. (2005). Biophysical studies of photosystem II-related recovery processes after a heat pulse in barley seedlings (*Hordeum vulgare* L.). *Journal of Plant Physiology*, *162*(2), 181–194. <https://doi.org/10.1016/j.jplph.2004.06.010>

24. Vaughan, R. G., Calvin, W. M., & Taranik, J. V. (2003). SEBASS hyperspectral thermal infrared data: Surface emissivity measurement and mineral mapping. *Remote Sensing of Environment*, 85(1), 48–63. [https://doi.org/10.1016/S0034-4257\(02\)00186-4](https://doi.org/10.1016/S0034-4257(02)00186-4)
25. Worrall, E. A., Hamid, A., Mody, K. T., Mitter, N., & Pappu, H. R. (2018). Nanotechnology for plant disease management. *Agronomy*, 8(12), 285. <https://doi.org/10.3390/agronomy8120285>
26. Yin, J.-J., Liu, J., Ehrenshaft, M., Roberts, J. E., Fu, P. P., Mason, R. P., & Zhao, B. (2012). Phototoxicity of nano titanium dioxides in HaCaT keratinocytes—Generation of reactive oxygen species and cell damage. *Toxicology and Applied Pharmacology*, 263(1), 81–88. <https://doi.org/10.1016/j.taap.2012.06.001>
27. Beneragama, C. K., & Goto, K. (2010). Chlorophyll a:b ratio increases under low-light in “shade-tolerant” *Euglena gracilis*. *Tropical Agricultural Research*, 22(1), 12–25. <https://doi.org/10.4038/tar.v22i1.2666>
28. Petjukevics, A., & Skute, N. (2022). Chlorophyll fluorescence changes, as plant early state indicator under different water salinity regimes on the invasive macrophyte *Elodea canadensis* (Michx., 1803). *One Ecosystem*, 7, e82389. <https://doi.org/10.3897/oneeco.7.e82389>