Seeds priming with "Pluronic" P-85 grafted single-walled carbon nanotubes results in functional alterations in the photosynthetic apparatus of pea plants

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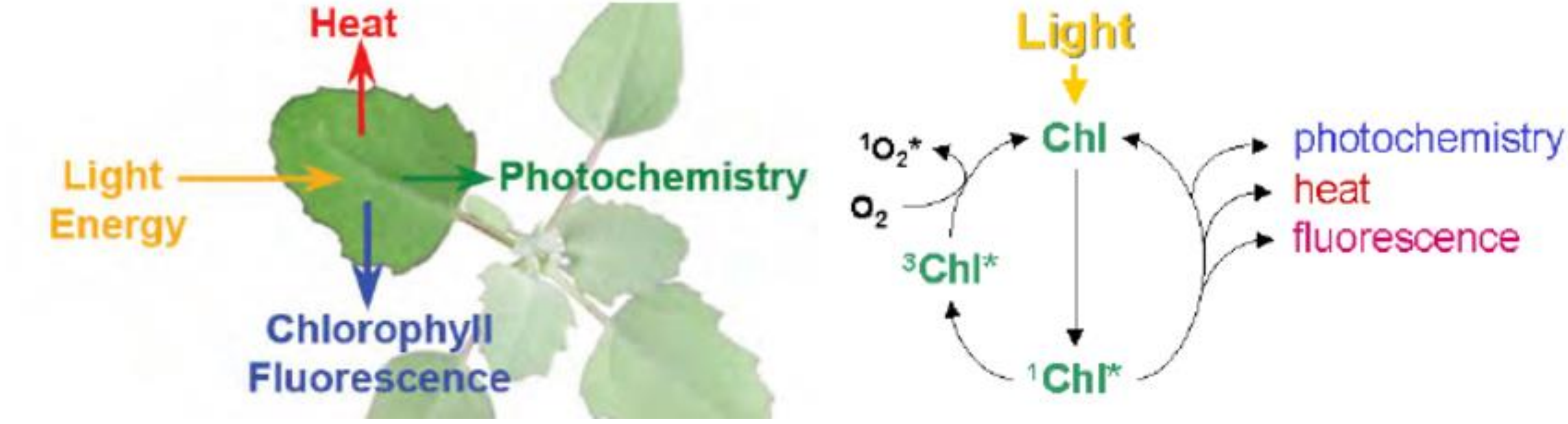
Aim: To explore the effect of seeds priming with "Pluronic" P-85 polymer grafted single walled carbon nanotubes (SWCNT-P85) on the photosynthetic operation of pea plants with emphasis on photosystem II functionality



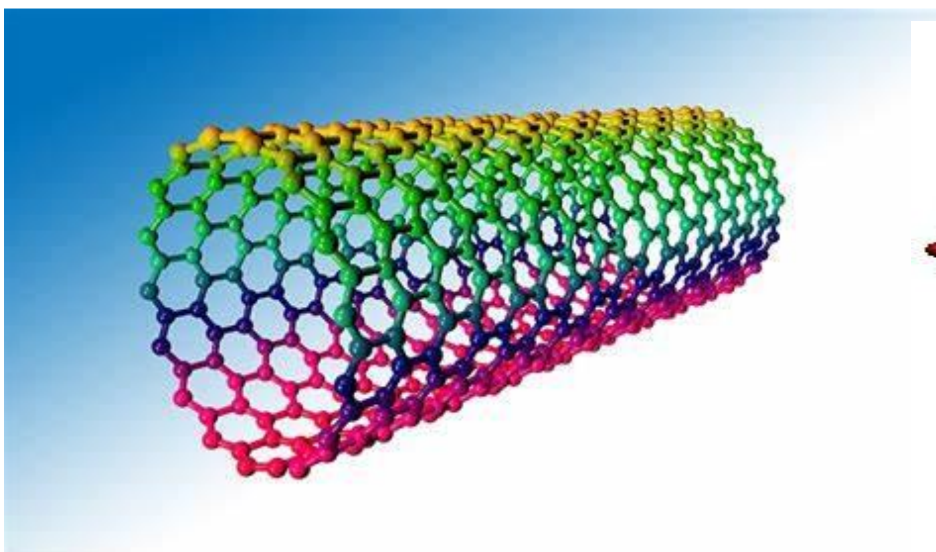
Scheme of distribution of solar energy

♦ SWCNTs are nano-objects of high research and industrial interest in the last years;

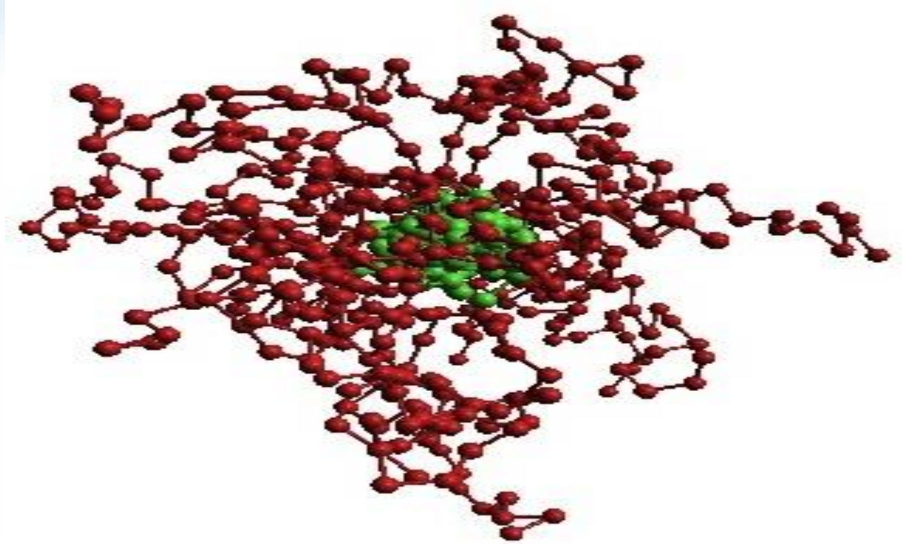
♦ They have a small size and the capability to trespass plant tissues and cell membranes;

♦ SWCNTs are regarded as plausible nanocarriers of beneficial substances that might enhance plant growth in different environmental conditions;

♦ SWCNTs themselves might affect and even enhance photosynthetic ffunctionality and plant development in general.



Single-walled Carbon Nanotubes (SWCNT)



Pluronic,, P-85 ‘’nanoparticles formed by stabilized micelles (PNP)

6 hours treatment with NPS solutions → Drying to constant weight → 2 hours treatment with d H2O → 4 days germination → 14-day-old pea plants growing hydroponically

- ***Treatments***: Seed priming with different concentrations of SWCNT-P85, P85 and PNP.
- ***Materials:***

- *Pisum Sativum* seeds (n=50) are used for each treatment
- SWCNT-P85 solutions in the range: 0.4–300 mg/L
- P85 solutions in the range : 0.04–30 g/l
- PNP solutions in the range: 0.008-0.4 g/l

- ***Methods***:

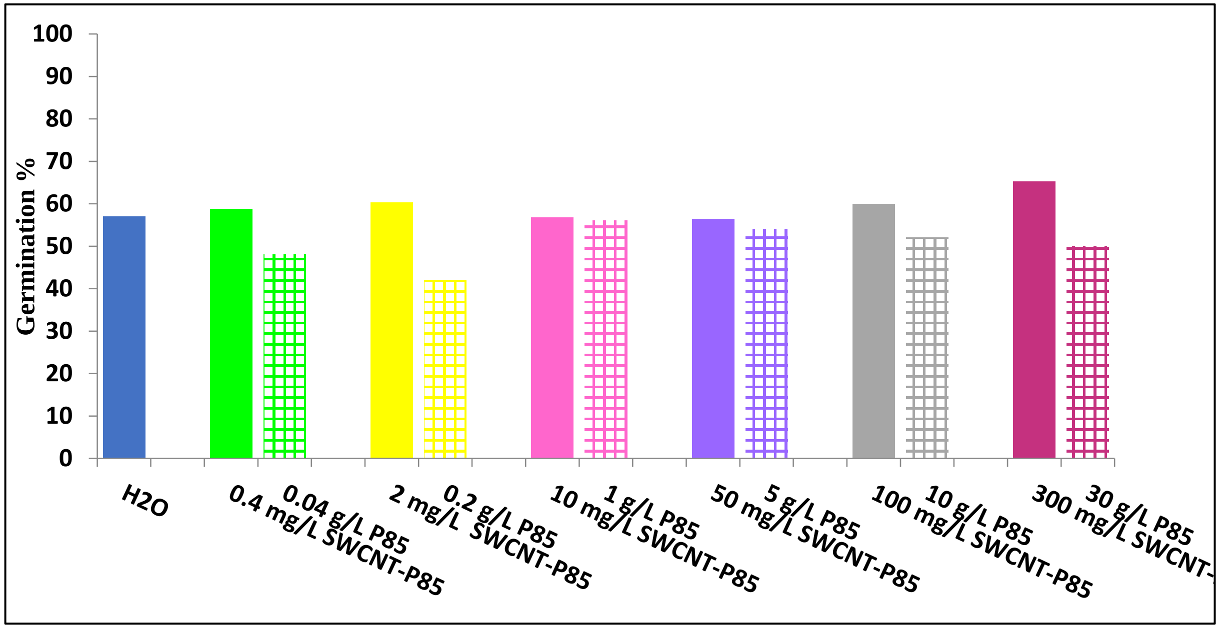
- Germination parameters were determined according Ranal MA (849-855, 2009)
- PAM Imaging was used (MAXI version; Walz, Germany) to determine specific chlorophyll fluorescence parameters of 14-day-old plants.
- Chlorophyll content and leaf temperature were measured with DUALEX leaf clip sensor.

- **Statistical analysis - Student's t-test**

♦ - significant difference compared to the control (H_2O)

* - significant difference compared to the corresponding concentration of P85 in SWCNT-P85 solution.

Seed germination



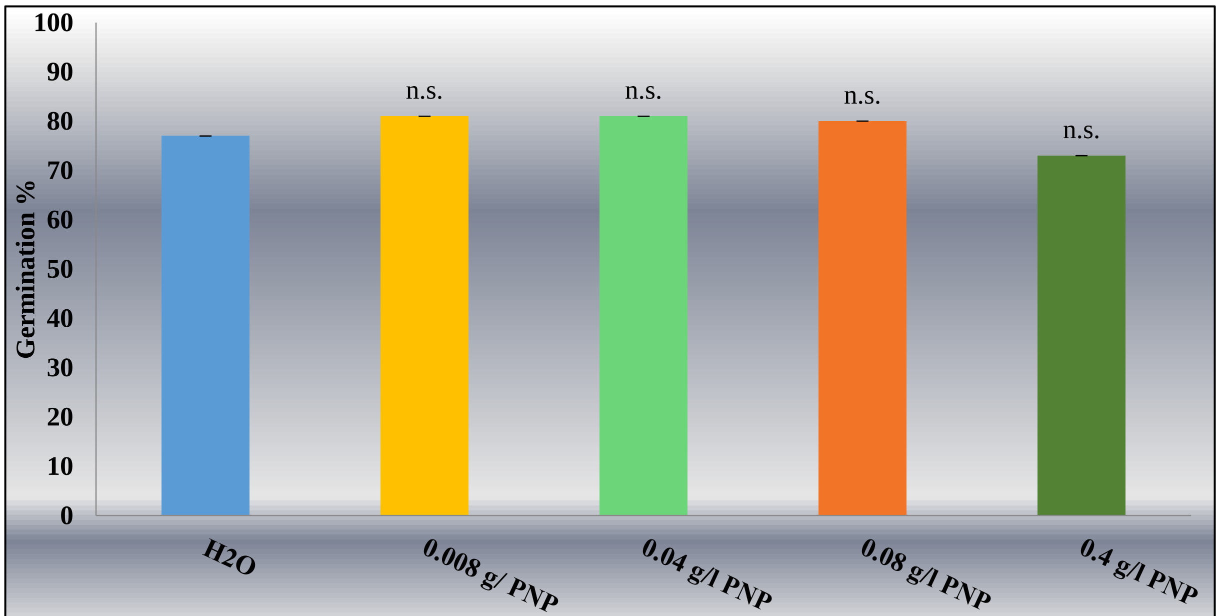
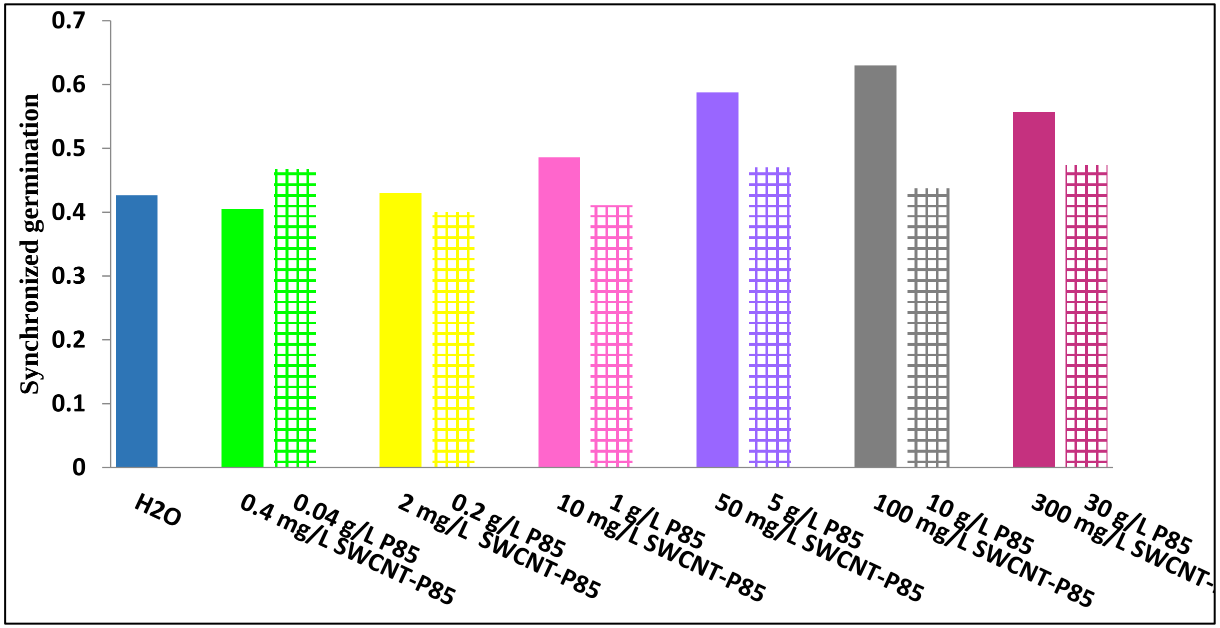


Fig. 1. Germination (%)*-expressing the number of germinated seeds in the last day of tested period compared to the total number of seeds.*

SWCNT-P85 treatment does not effect germination process significantly, while **P85** application causes negative influence on this parameter. The highest concentration of **SWCNT-P85** leads to increased number of germinated seeds.

The germination process is not affected by any dose of **PNP** with exception of slightly negative effect at the highest concentration.

Synchronized germination



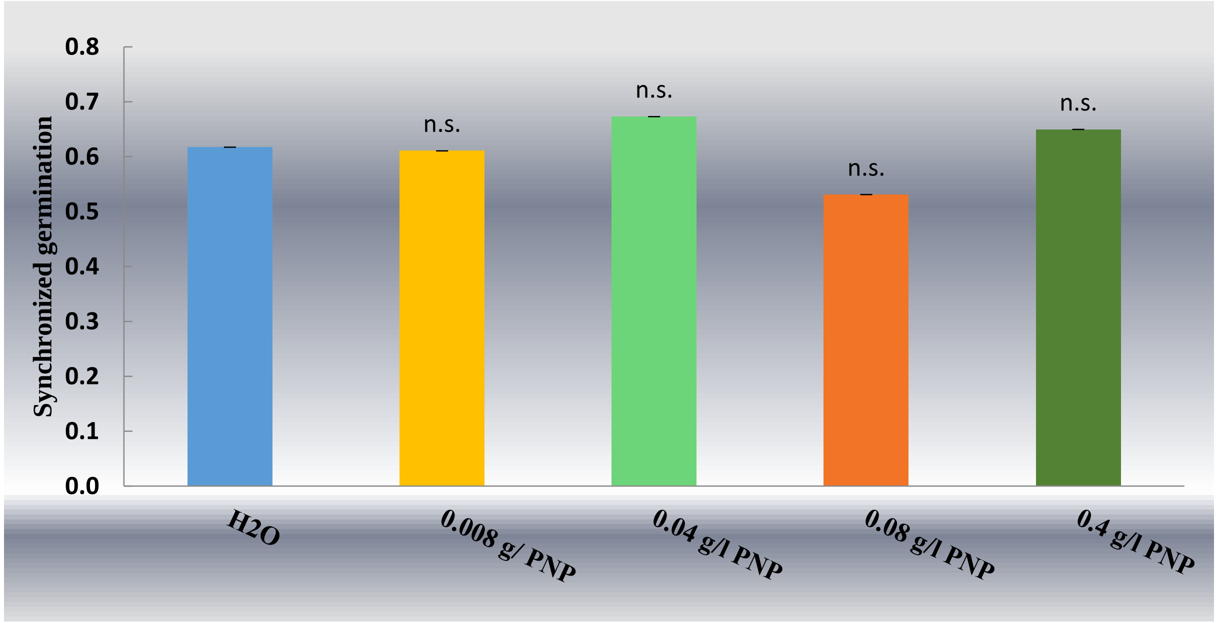


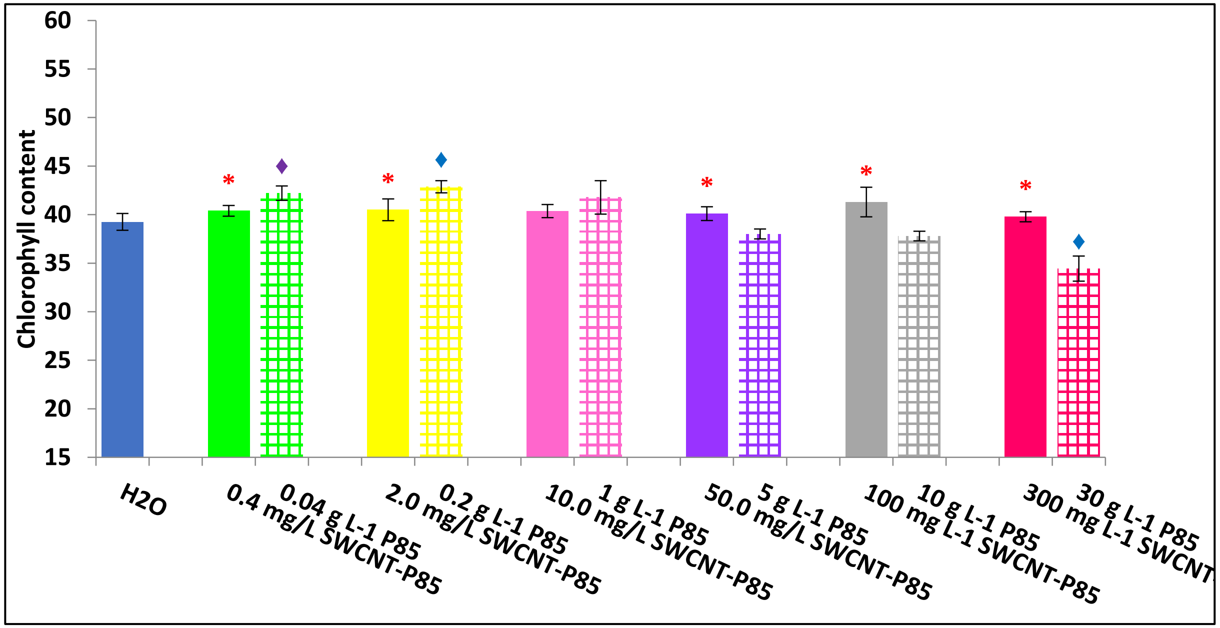
Fig. 2. Synchronized germination.

Treatment with **SWCNT-P85** solutions causes beneficial effect on germination synchronization

P85 application does not alter significantly this parameter.

Seeds soaked in **PNP** solutions germinate relatively similar to the control with slightly negative but insignificant effect in 0.08 g/l treatment.

Chlorophyll content



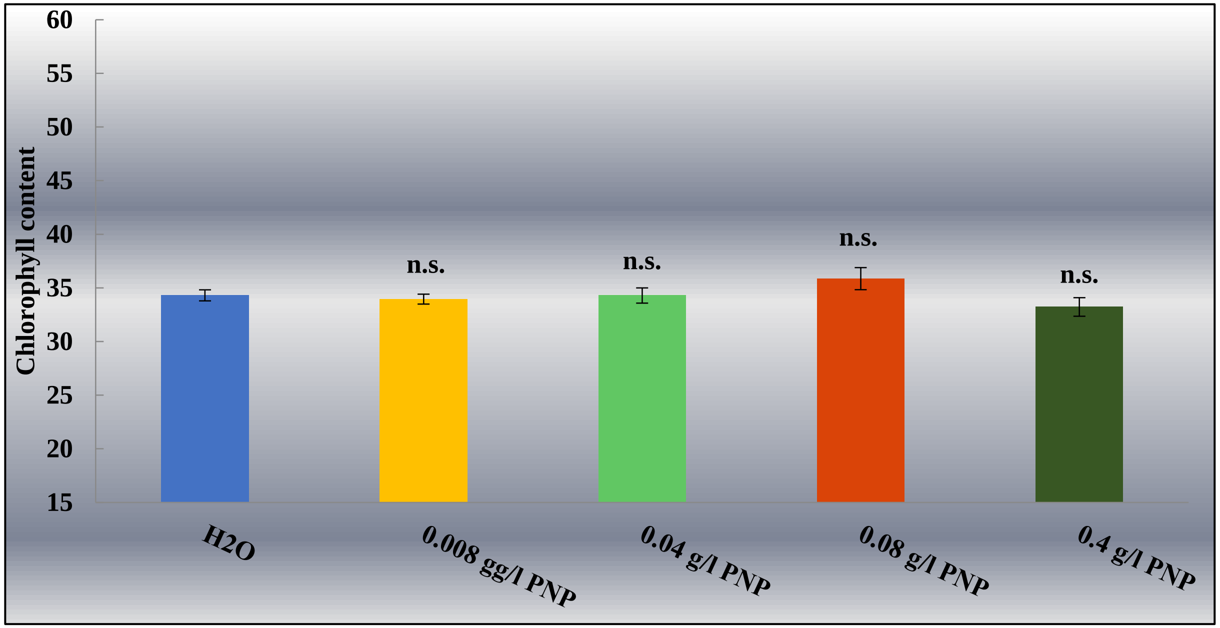
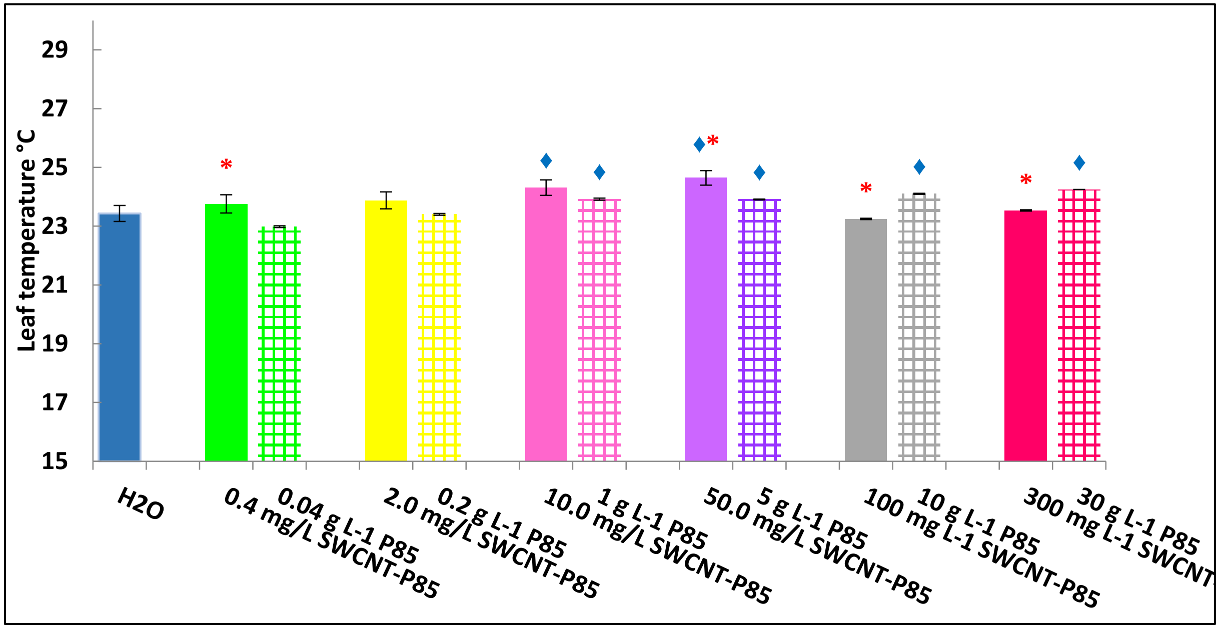


Fig. 3. Chlorophyll content.

Only lower doses of **SWCNT-P85** and **P85** have a positive effect on chlorophyll content, with optimal values at 2 mg/l **SWCNT-P85** and 0.2 g/l **P85**.
There is no effect of **PNP** treatment on chlorophyll concentration.

Leaf temperature



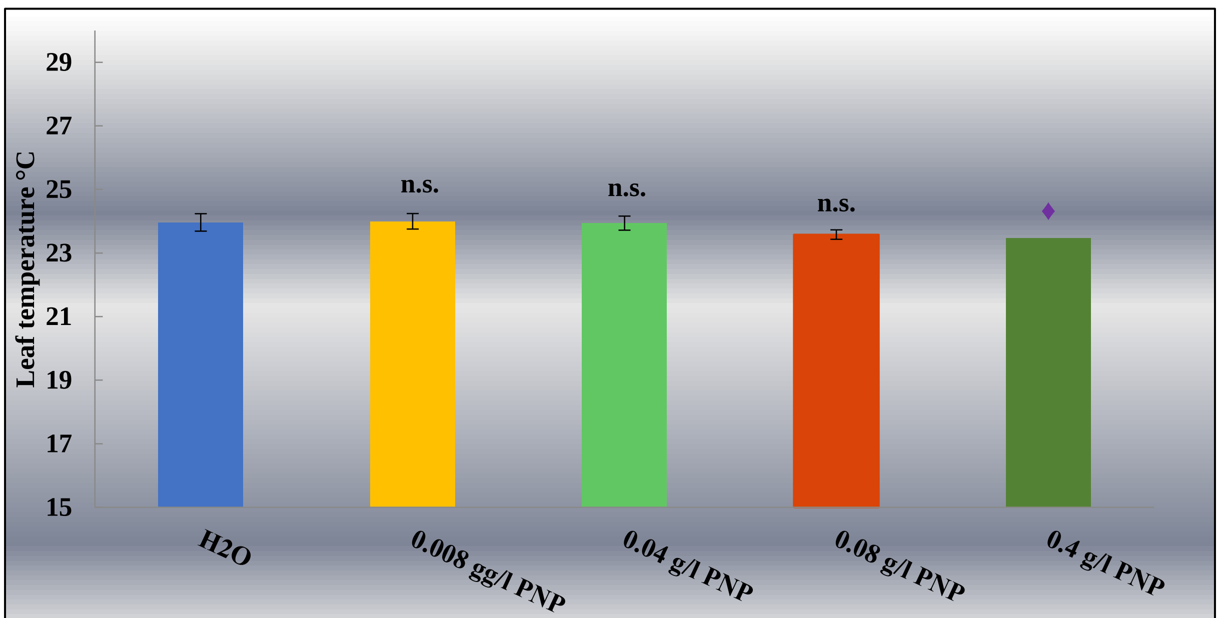
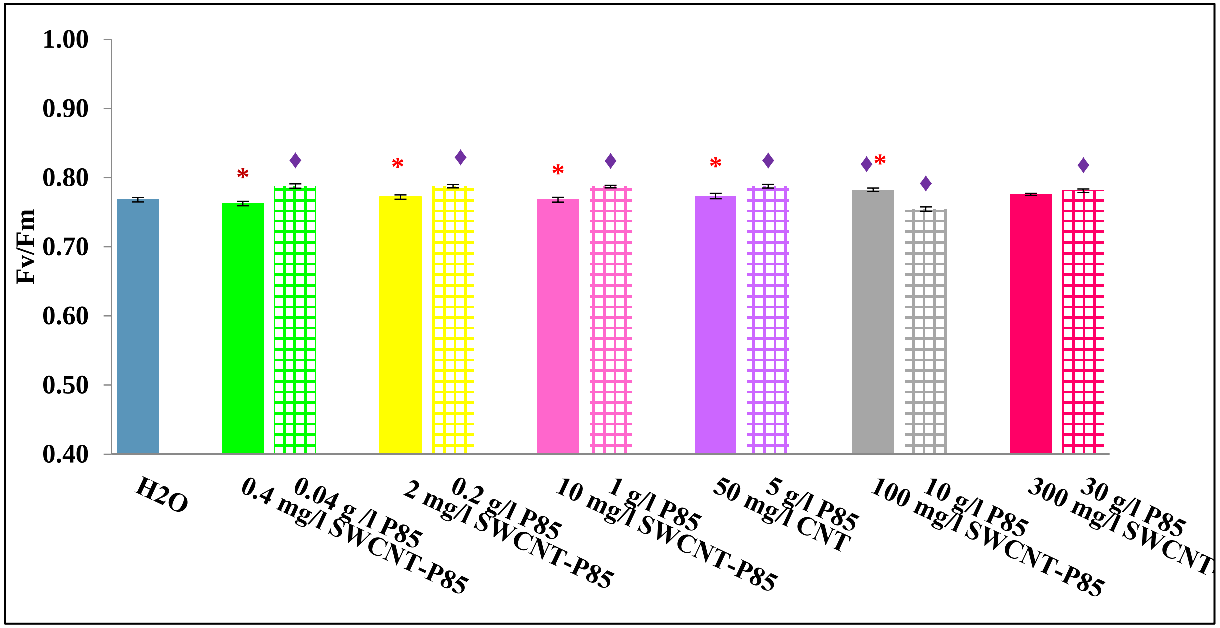


Fig. 4. Leaf temperature (oC). Treatments with concentration of and higher than 1 g/L **P85** exhibit higher leaf temperature. **SWCNT-P85** treatment, on the other hand, leads to increased leaf temperature only for 10 and 50 mg/L concentrations.

On the contrary, **PNP** application does not lead to significant changes in leaf temperature and only the highest dose (0.4 g/l) leads to reduction of this parameter.

Photosystem II functionality



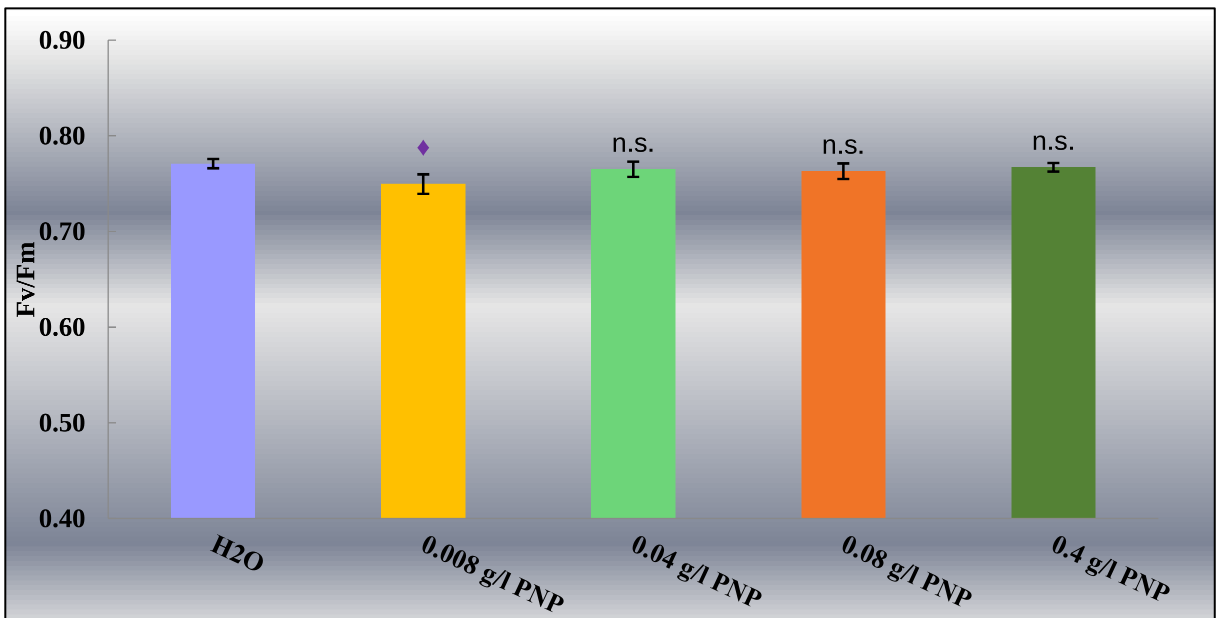
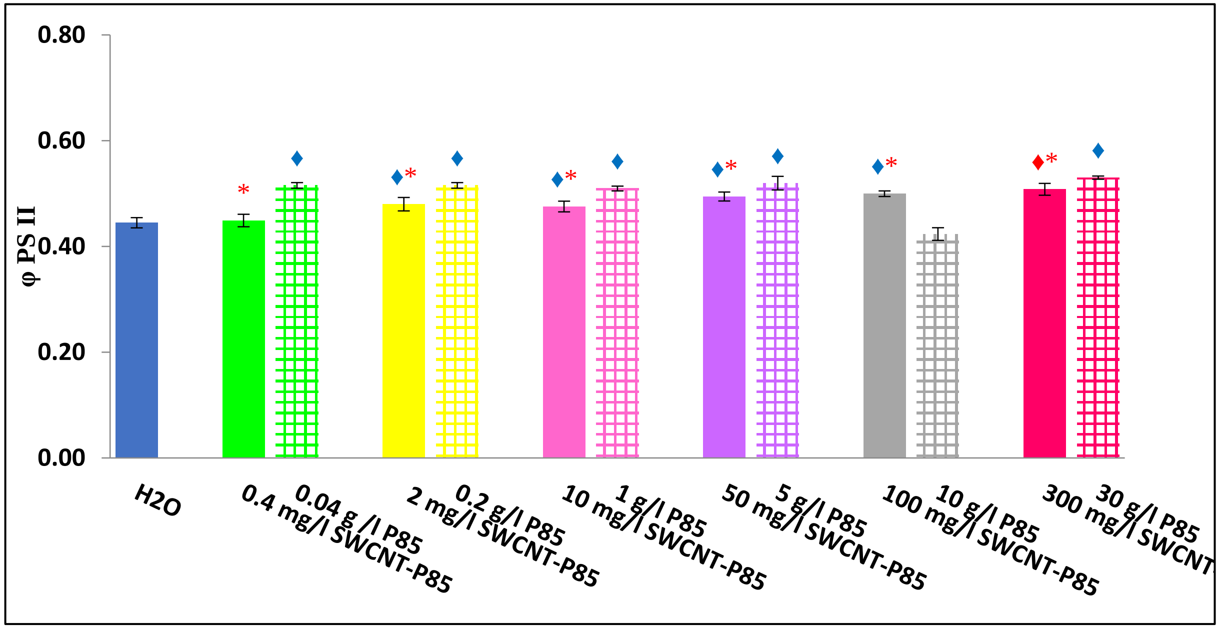


Fig.5. Fv/Fm - maximum efficiency of photosystem II in dark-adapted plants

SWCNT-P85 and **P85** alone exert different effects that strongly depend on the applied concentration. Optimal influence is observed for 100 mg/l **SWCNT-P85** treatment.
PNP do not effect Fv/Fm with exception of the lowest concentration.

Photosystem II functionality



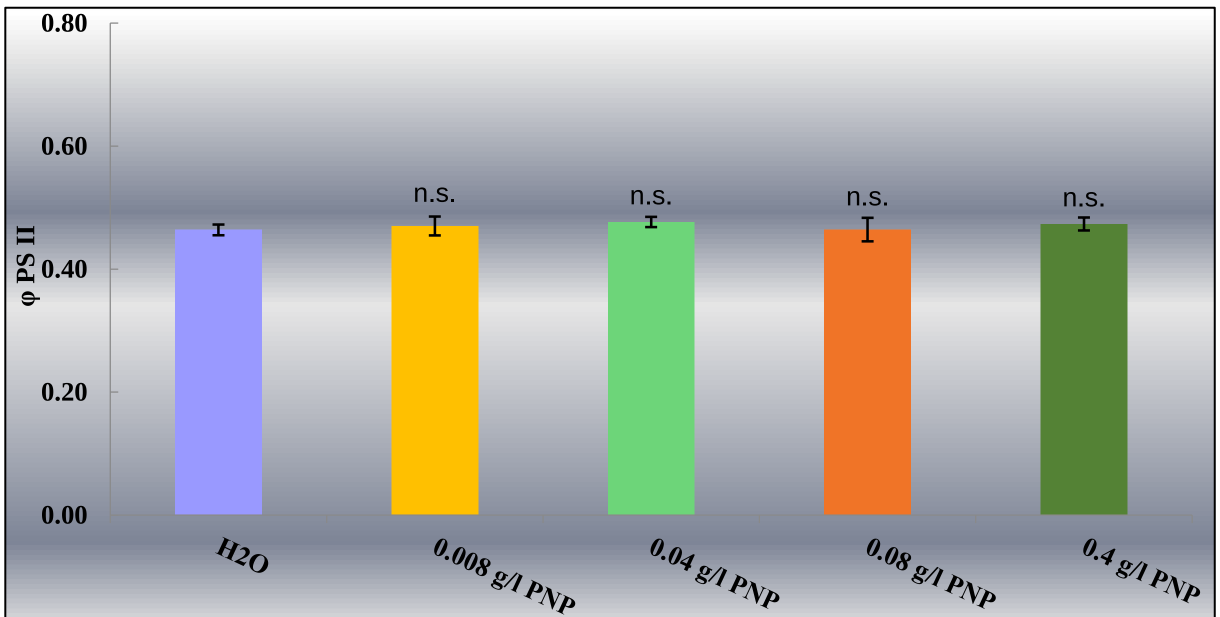
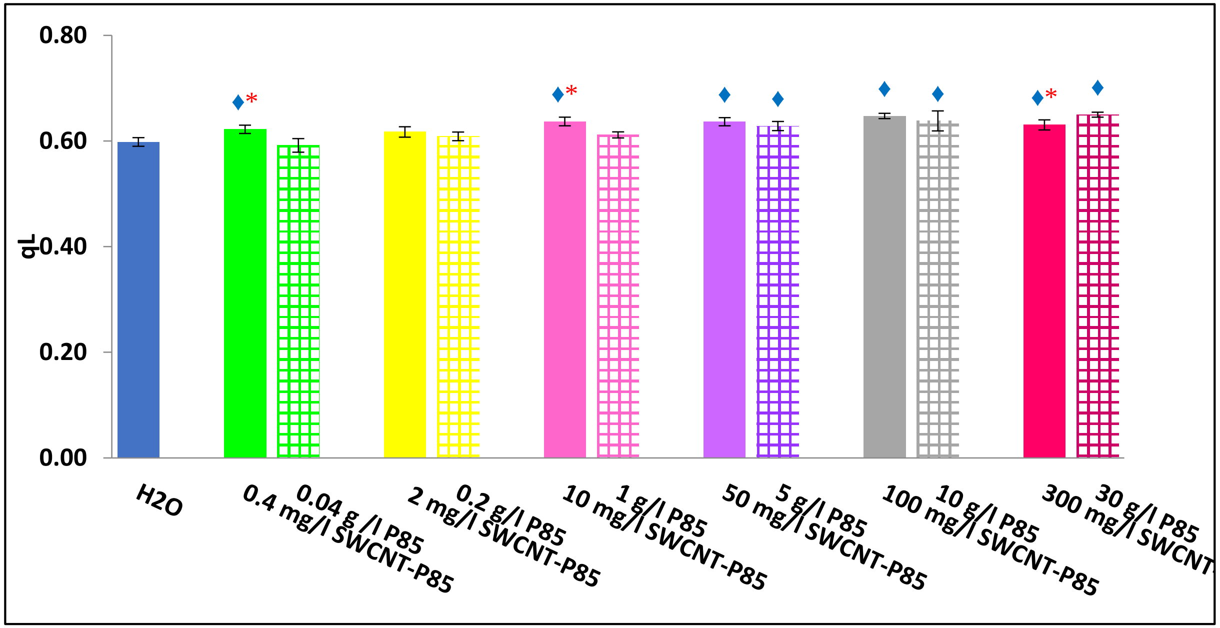


Fig.6. ΦPSII - actual quantum yield of photosystem II*, measure of the rate of linear electron transport driving photosynthesis and photorespiration when leaves are illuminated and photosynthesis is activated*

There is not clear effect of the **PNP** treatments at all tested concentrations, while for **SWCNT-P85** treatment (in the range 0.4 – 300 mg/L) this parameter increases linearly and proportionally to **SWCNT-P85** and **P85** concentrations. The strongest effect on ΦPSII is observed at 300 mg/L **SWCNT-P85.**

Photosystem II functionality



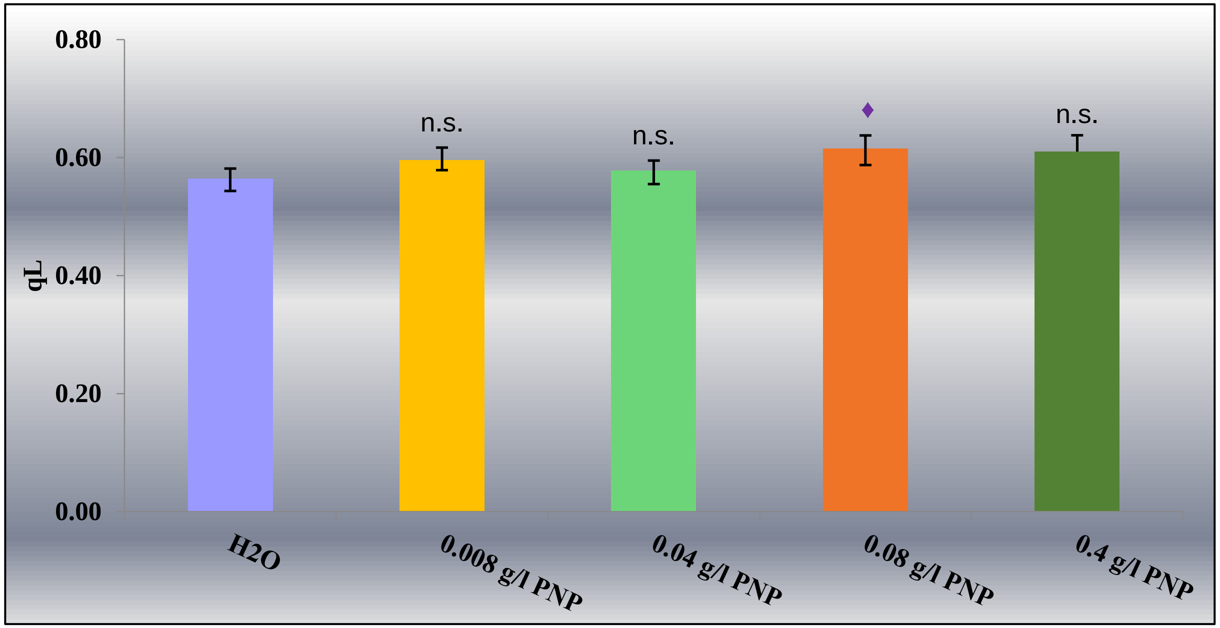
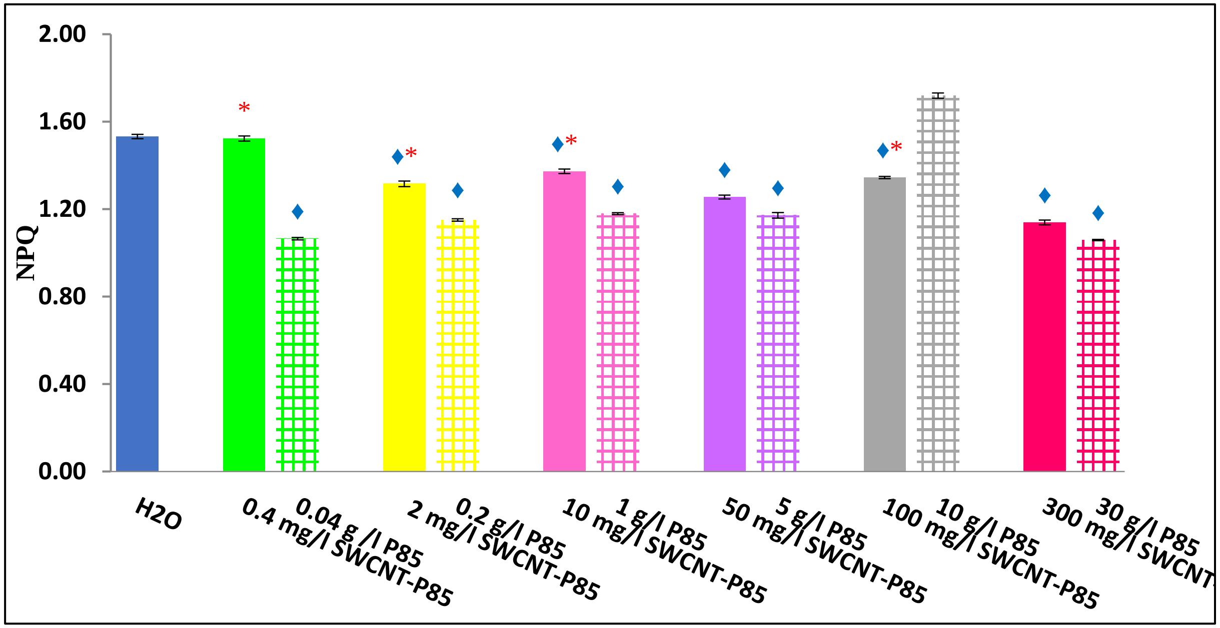


***Fig.7. qL - fraction of open photosystem II centers**, indicator of the redox state of photosystem II .*

Treated plants with **SWCNT-P85** solutions have more open photosystem II reaction centers at all studied concentrations, besides the 2 mg/l treated sample.

The effect of **PNP** solutions does not cause signifficant changes for this parameter with the exception of the slight increase at 0.08 g/l treatment.

Photosystem II functionality



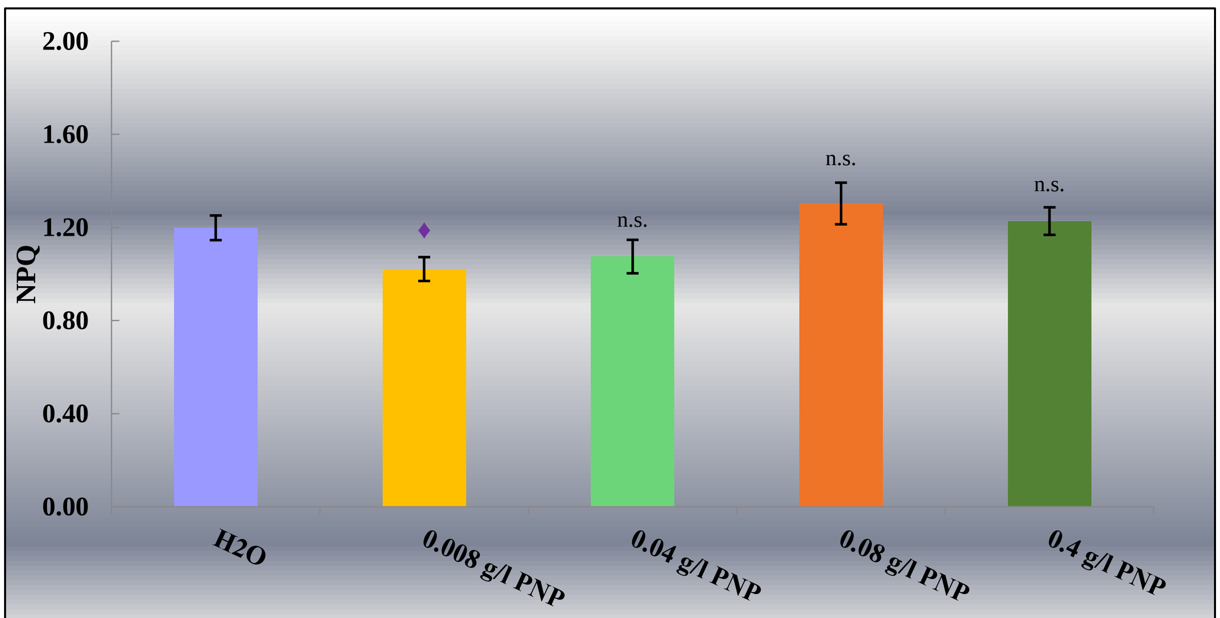


Fig.8. NPQ- non-photochemical quenching of chlorophyll a fluorescence–protective mechanism against excess light intensity

SWCNT-P85 solutions cause concentration-depended effect, leading to maximal reduction of values at 300 mg/l **SWCNT-P85.**

Strong decrease in NPQ is observed for all tested **P85** solutions.

PNP application slightly but not significantly increase NPQ in plants, treated with the highest concentrations.

CONCLUSIONS

- **Concentration-dependent effect of SWCNT-P85 treatment on synchronized germination is observed.**
- **There is no clear effect on chlorophyll content in all treatments with exception of lower doses of P85.**
- **Leaf temperature is higher for treatments with 10-50 mg/L SWCNT-P85 and above 1 g/L P85.**
- **The data reveal that while PNP do not induce significant changes in photosystem II functionality, SWCNT-P85 exert different effects that depend on the applied concentration of P85 and nanotubes.**
- **It appears that those effects are not only due to increased number of photosystem II centers but to the decreased extent of non-photochemical quenching of chlorophyll a.**
- **The data strongly suggest that seeds priming with SWCNT-P85 results in nanotubes translocation towards the photosynthetic organelles which makes them suitable objects for further development of cargo-loaded nanoparticles.**

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