In vivo monitoring of polystyrene microplastics effect on lentil seed germination and seedling growth using Biospeckle Optical Coherence Tomography

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1. Introduction

The effect of microplastics on plant growth and seed germination is getting worldwide attention due to the widespread usage, mismanagement, having an unrevealed impact. Waste plastics degraded to microplastics (MPs) (diameter < 5 mm) and nanoplastics (diameter < 100 nm) by natural forces, thus widely spread in environment [1]. Several studies reported the adverse effect of MPs on seed germination and plant growth [2]. In general, the effect of seed germination and seedling growth from MPs are assessed by destructive post-harvesting conventional measurements that require a relatively long period of time. Hence, there is an urgent requirement for novel methods that can monitor the effect of MPs on seed germination and seedling growth at an early stage. Therefore, in this study, the effect of polystyrene microplastics (PSMPs) on lentil (*Lens culinaris*) seed germination and seedling growth was monitored using ultrahigh accurate Biospeckle Optical Coherence Tomography (bOCT), introduced by our team. bOCT is a non-contact, non-destructive, *in vivo* monitoring technique to visualize the change of internal activity of a biological object [3]. In our previous studies, the effect of different Zn concentrations and alumina NPs on seed germination was reported using bOCT [4].

2. Experiments and Discussion

A schematic diagram of the fiber-based custom-made OCT system used for the experiments is shown in Fig.1. A superluminescent (SLD) diode having wavelength 836.1 nm and 55.2 nm bandwidth was used as a light source. The axial resolution (depth resolution) was estimated to be 6µm.

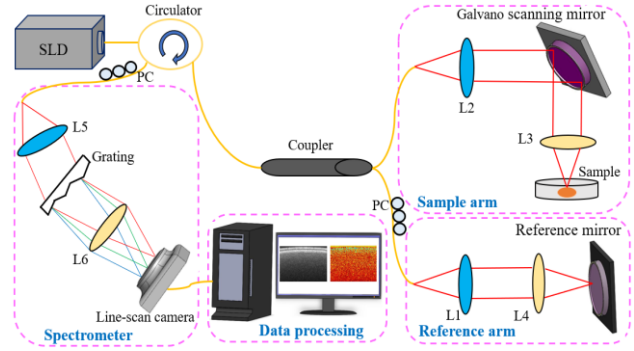

Fig.1 - Schematic of experimental system

In bOCT, from the acquired OCT structural images, the biospeckle contrast (γ), which is defined as the ratio of the standard deviation of the intensity at each pixel along the temporal axis to the mean value of pixels across total time of the scan was calculated according to the following equation.

$$\gamma(x,y) = \frac{1}{< I_{OCT}(x,y) >}\left[\frac{1}{N}\sum_{j=1}^{N}\{I_{OCT}(x,y,t_j) - < I_{OCT}(x,y) >\}^2\right]^{\frac{1}{2}}$$

$$< I_{OCT}(x,y) >= \frac{1}{N}\sum_{j=1}^{N} I_{OCT}(x,y,t_j)$$

Where x, y represents the pixel coordinates, j is the scan number, and N indicates the total number of scans. The presence of larger temporal variation or larger speckle contrast indicates larger changes in time within the seeds. Hence, the change of magnitude of speckle contrast could be a valid parameter that can predict the biological activity on seeds under the presence of an external agent. In the experiments, 50 and 100 mg/L PSMPs concentration with the particle size of 5.2 µm were used to investigate effects on the germination of the lentil seeds. For each sample, 6 seeds were placed on a filter paper in 9 cm Petri dishes and 10 ml from each MPs concentration was added. Thereafter, the Petri dishes were kept in a growth chamber at 27° C temperature, relative humidity of >70%, and constant illumination of 4000 lux. The bOCT observations were taken at 6h, 12h, 24h of exposure, and the bOCT contrast image after 24 h exposure is shown in Fig.2.

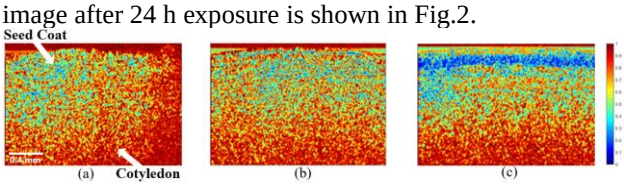

Fig.2. Comparison of bOCT images of seeds under PSMPs exposure after 24 h. control (a); 50 mg/L PSMPs (b); 100 mg/L PSMPs (c); Scale bar represents 0.4 mm.

The average local contrast of the bOCT images was calculated using six ROIs. A significant difference was observed for average normalized contrast, as shown in Fig.3, due to inhibition of nutrient and water uptake through the physical blockage of pores of the seed coat.

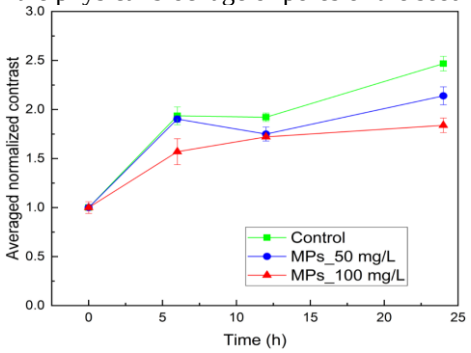

Fig.3. Averaged normalized contrast of bOCT image under PSMPs exposure

3. Conclusion

Here, we proposed the bOCT to monitor the effect of PSMPs on lentil seed germination. The results imply that the tested concentrations have a negative influence on seed germination, whereas a clear reduction of the internal activity of seeds was observed within 6 h of exposures. Thus, the proposed method could be reliable and capable enough to observe the effect of PSMPs on lentil seed germination *in vivo*, at an early stage before the germination.

References

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