

Research Article

Experimental Research of Reliability of Plant Stress State Detection by Laser-Induced Fluorescence Method

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Experimental laboratory investigations of the laser-induced fluorescence spectra of watercress and lawn grass were conducted. The fluorescence spectra were excited by YAG:Nd laser emitting at 532 nm. It was established that the influence of stress caused by mechanical damage, overwatering, and soil pollution is manifested in changes of the spectra shapes. The mean values and confidence intervals for the ratio of two fluorescence maxima near 685 and 740 nm were estimated. It is presented that the fluorescence ratio could be considered a reliable characteristic of plant stress state.

1. Introduction

Fluorescence analysis is a widely used high-sensitivity method that is applied in many scientific and technical fields. A viable application of the technique is the analysis of plant state [1–14]. External factors can cause plants stress and make their growth abnormal. Stress conditions are difficult to detect by visual observation during the early growth stages of a plant; however, the laser-induced fluorescence method is effective in the remote detection of plant stress state.

Chlorophyll is the basic fluorescent component of green leaf in the red and far-red regions. The fluorescence spectrum of a green leaf at room temperature exhibits two maxima in the red band (680–690 nm) and in the far-red band (730–740 nm) [1, 6, 8, 15]. The fluorescence spectrum of a stressed plant is deformed in comparison with that of a plant in a nonstressed state. This effect is caused by disturbing the photosynthetic process of a plant under stressed conditions. The fluorescence spectrum depends on different factors such as excitation wavelength, type of stress factor, and plant species.

There are wide experimental data on the fluorescence spectra of various plant species, both stressed and nonstressed, excited at wavelength ranges of 266–635 nm [2, 10, 11, 15, 16]. However, a number of points remain to be investigated. One such point is the reliability of the plant state

detection based on the differences in fluorescence spectra of samples of a plant species, grown under identical conditions, except that some samples were stressed and the others were not.

In this paper, experimental results of the analysis of fluorescence spectra variation of different samples of a plant species in both normal and stressed states are presented.

2. Materials and Methods

2.1. Laboratory Setup Description. The fluorescence spectra were excited at a wavelength of 532 nm. It is common to use lasers with wavelengths at 337, 335, and 532 nm for fluorescence excitation in experimental research. The laser source used in this study was selected because of the advantages offered by the solid-state YAG:Nd laser at the wavelength of 532 nm (for remote sensing equipment development), in comparison with both the nitrogen gas laser at the wavelength of 337 nm and the solid-state YAG:Nd laser at the wavelength of 355 nm (the third harmonic of the YAG laser has lower pulse intensity than its second harmonic).

The laboratory configuration used to measure fluorescence spectra is shown in Figure 1.

An EKSPLA NL210 solid-state YAG:Nd laser with diode pumping and frequency doubling was used as the source of

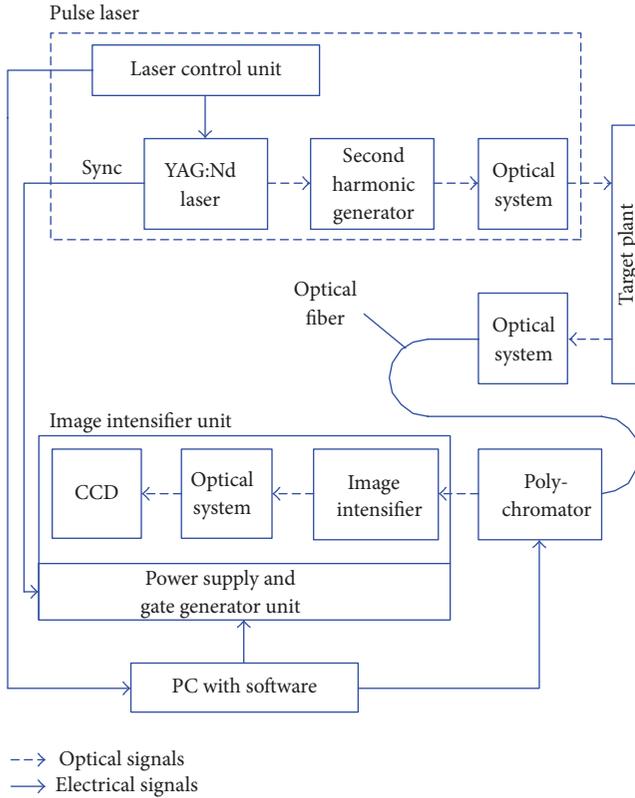


FIGURE 1: Laboratory configuration for laser-induced fluorescence experiments.

fluorescence excitation. Laser light was transmitted by means of the optical system to the target plant located at a distance of 1 m from the optical system. The apparent diameter of the laser beam on the plant sample was approximately 25 mm. The laser spot has covered 15–20 plants. The fluorescent radiation of the plants was collected from the same spot size together with the reflected laser light by the optical system and directed into the optical fiber. The optical fiber was used to transmit light to the input of the polychromator. The reflected light from the laser beam was prevented from entering a polychromator by using an NF01-532U Semrock filter. Fluorescent radiation from 595 to 800 nm was detected. An M266 Solar LS polychromator was used as the spectral device and all transitions within the polychromator fully automated (i.e., the swapping of diffraction grids and optical filters and slit width selection).

The fluorescence spectrum was detected using a highly sensitive detector (Matrix-430k-ns Deltatekh) based on CCD array with an image intensifier. The image intensifier (generation II+, diameter 18 mm) has quantum efficiency 15% at the wavelength 550 nm. The image was transferred by the optical system from the image intensifier to the CCD. The image was converted into a digital array and transmitted to the computer. Special software developed with LabVIEW National Instruments was used to control the setup. The major specifications of the setup are presented in Table 1.

The experiment included equipment calibration as a preparatory step. The polychromator was calibrated by

TABLE 1: Specifications of laboratory setup.

Specifications	Value
Laser pulse energy, mJ	2.1
Laser wavelength, nm	532
Laser pulse duration, ns	<7
Laser repetition rate, Hz	<500
Laser beam spread, mrad	<3
Spectral band of registration, nm	595–800
Spectral resolution, nm	6
Diameter of optical detection system, mm	15
Distance to sample, m	1

wavelength using a calibration light source based on a mercury-argon lamp (SL2 StellarNet Inc.) with a linear spectrum. The test was performed at the wavelength of 546.07 nm. Calibration of sensitivity of the registration system was performed using a light source based on halogen lamp (DH-2000-CAL Ocean Optics Inc.) with a continuous spectrum. Known spectrum of the lamp was acquired for sensitivity calculation.

2.2. Plant Samples. The experimental research of laser-induced fluorescence spectra was performed using easy to keep fast-growing plant species, that is, salads, watercress, mustard, common borage, cucumbers, and lawn grass. The experimental measurements of fluorescence spectra of watercress (*Lepidium sativum*) and lawn grass (that comprised a mixture of 30% perennial ryegrass (*Lolium perenne*), 65% creeping red fescue (*Festuca rubra*), and 5% sheep's ovina (*Festuca ovina*)) are presented in this paper. The research was conducted on plants in their normal state and under the influence of stress factors, for example, mechanical damage (leaf cutting and laying, root system damage), root system overwatering, and soil pollution (copper sulfate, CuSO_4 , ferric sulfate, FeSO_4 , and sodium chloride, NaCl).

2.3. Normal and Stress Conditions. The plants in normal state were grown in favorable condition for their development. The watercress plants have height of approximately 4 cm, and the lawn grass plants 8 cm.

By the leaf cutting of the watercress, the half of one leaf of each plant was dissected. The leaf laying was conducted using 7×7 cm flat plate with 200 g weight during approximately 1 min. For root system damage in seedlings pots was cut a slit at the depth of 2 cm, the root system has been damaged through the slit by means of utility knife, and then the slit was closed.

The overwatering stress condition was implemented by placing the pot of the watercress sample in a watering can. The level of water in the watering can was always slightly below the level of soil in the plant pot; thus, it was not visually obvious that the root system of the plant sample was constantly in overwatered soil.

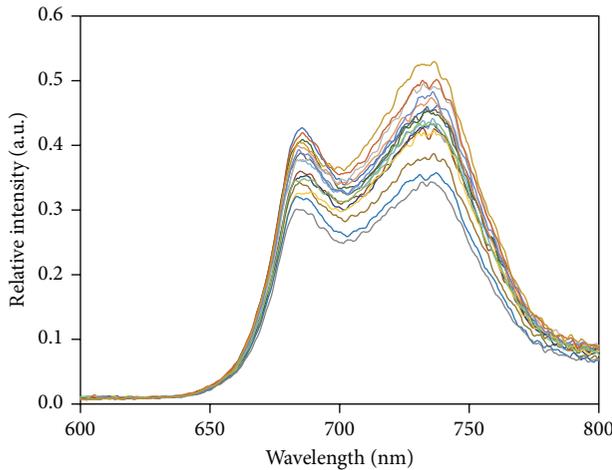


FIGURE 2: Fluorescence spectra of different watercress samples in normal state.

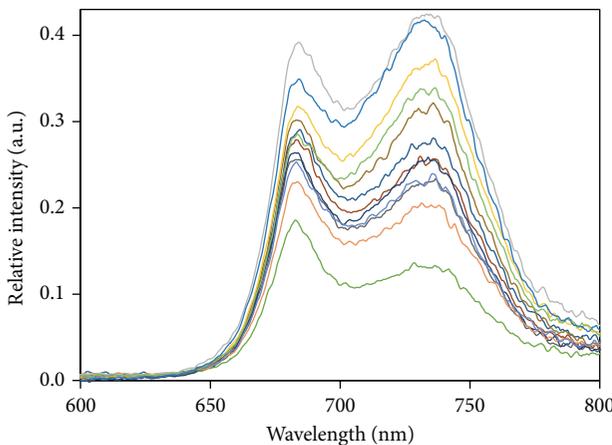


FIGURE 3: Fluorescence spectra of different watercress samples in stressed state caused by leaf laying.

3. Results and Discussion

3.1. Fluorescence Spectra of Different Watercress Samples in Normal State. The fluorescence spectra of different samples of watercress grown under normal conditions are shown in Figure 2. The different plots in Figure 2 correspond to different plant samples that were planted at the same time and grown under the same conditions. The measurements were conducted in 16 days after planting.

As it can be seen in Figure 2, there are insignificant changes in the shapes of the fluorescence spectra from one sample to another, despite the differences in spectra intensity.

3.2. Fluorescence Spectra of Watercress Stressed by Mechanical Damage. The fluorescence spectra of different samples of watercress stressed by leaf laying mechanical damage are shown in Figure 3. There have been several experimental researches on the fluorescence spectra of plants in stressed states caused by different types of mechanical damage [9, 13, 14], but few or none investigated the fluorescence spectra at an excitation wavelength of 532 nm [9].

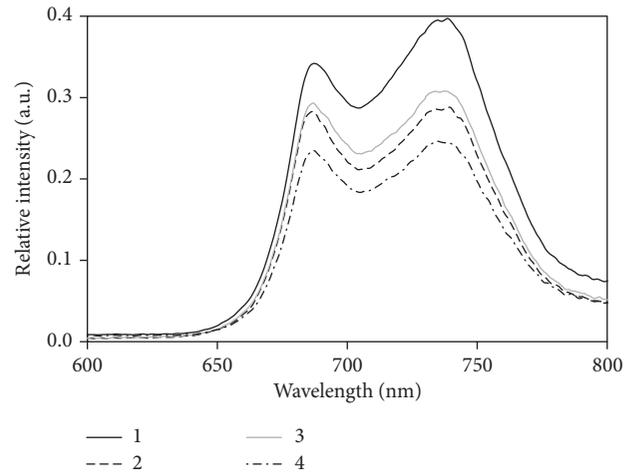


FIGURE 4: Averaged fluorescence spectra of watercress samples in normal and stressed conditions: (1) normal state, (2) leaf laying stress, (3) leaf cutting stress, and (4) root system damage stress.

The fluorescence spectra of watercress in a stressed state caused by leaf laying fluctuate considerably (Figure 3) and differ from those of watercress in a normal state (Figure 2). A similar difference is found between the fluorescence spectra of watercress in normal and stressed states when the stress is caused by mechanical damage of root system.

The differences between the fluorescence spectra of plants under normal and stressed conditions are illustrated clearly by averaging the measurements of the fluorescence spectra. Figure 4 displays the averaged fluorescence spectra of watercress in a normal state (plot 1) and stressed state by the mechanical damage of leaf laying (plot 2), leaf cutting (plot 3), and root system damage (plot 4). Plot 1 in Figure 4 corresponds to the averaged fluorescence spectra over the result of 20 measurements. Plots 2, 3, and 4 in Figure 4 correspond to the averaged fluorescence spectra over 11 measurements for each stress factor; thus, a single measurement corresponds to the measurement of a single fluorescence spectrum of a plant sample in definite time intervals from 20 to 40 min from the start of the stress factor influence.

It is clearly illustrated in Figure 4 that the shapes of the laser-induced fluorescence spectra of watercress in stressed conditions were caused by various types of mechanical damage change significantly. The ratios of fluorescence intensity in the red region (680–690 nm) and far-red region (730–740 nm) increase in stress conditions.

3.3. Fluorescence Spectra of Watercress in Stress State Caused by Overwatering. The laser-induced fluorescence spectra of watercress in stressed state caused by overwatering are comparable with those presented in Figures 3 and 4. Figure 5 shows the fluorescence spectra of watercress in stressed state caused by overwatering during 24 days (different spectra correspond to different measurements and plant samples).

As it is clearly illustrated in Figure 5 the fluorescence spectra of the watercress in a stressed state caused by overwatering during 24 days differ from those of watercress in a normal state. Furthermore, the spectra of the stressed samples

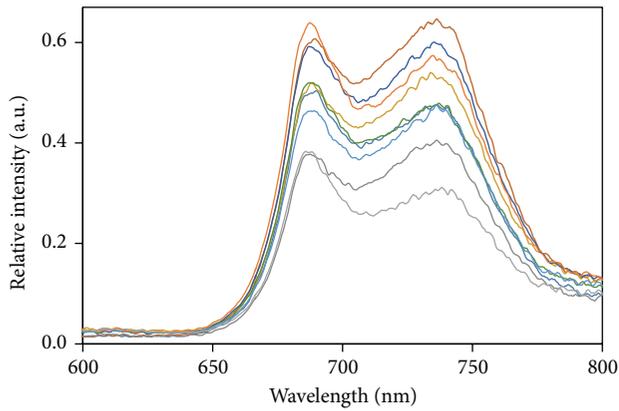


FIGURE 5: Fluorescence spectra of different watercress samples in stressed state caused by overwatering during 24 days.

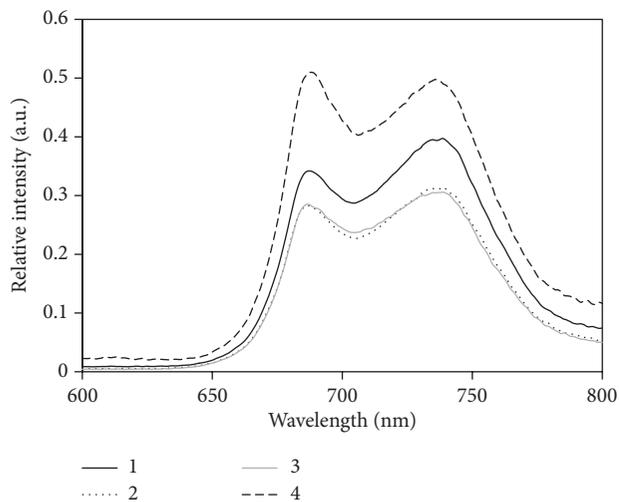


FIGURE 6: Fluorescence spectra of different watercress samples in stressed state caused by overwatering: (1) normal state, (2) 11-day overwatering, (3) 17-day overwatering, (4) 24-day overwatering.

fluctuate considerably, comparable with the fluorescence spectra of the watercress stressed by mechanical damage.

Figure 6 shows the laser-induced fluorescence spectra of watercress averaged over the number of plant samples and measurements (18 measurements for watercress in normal condition and 9 measurements for watercress in stressed condition).

Plot 1 in Figure 6 corresponds to the averaged fluorescence spectrum of watercress in a normal state. Plots 2, 3, and 4 in Figure 6 correspond to the averaged fluorescence spectra of watercress in stressed condition caused by overwatering during 11, 17, and 24 days, respectively. It can be clearly seen that the influence of the stress factor (overwatering in this case) accumulates gradually over the time of abnormal watering, increasing the fluorescence intensity.

The results presented in Figures 2–6 are in agreement with those of other experimental researches [8, 12] on plants under nitrogen stress and soil pollution using a fluorescence excitation source at the wavelength of 532 nm.

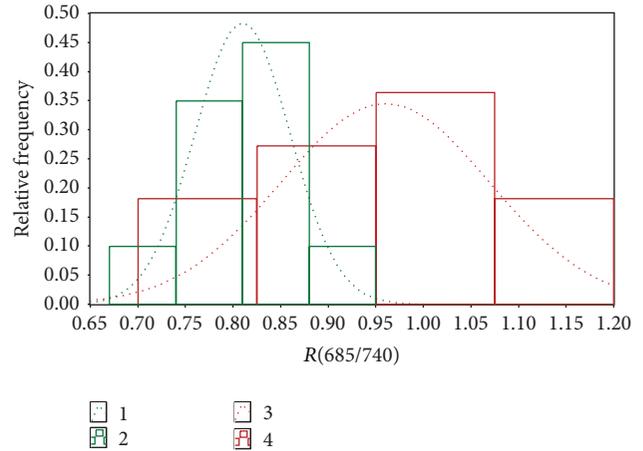


FIGURE 7: Histograms of distribution of fluorescence ratio, for watercress in a normal state and in a stressed state caused by leaf laying: (1) histogram for normal state, (2) histogram approximation for normal state, (3) histogram for stressed state, and (4) histogram approximation for stressed state.

3.4. Fluorescence Ratio. The ratio of fluorescence intensities in the 680–690 and 730–740 nm spectral bands is widely used in experimental research to characterize the fluorescence spectrum shape. Analysis of experimental data indicated that the ratio of fluorescence intensities near 685 and 735 nm can be used to characterize plant stress state.

Histograms of distribution of fluorescence intensities ratio (R) at 685 and 740 nm in narrow spectral bands with bandwidths of 10 nm, for watercress in a normal state in 16 days after planting and in a stressed state caused by leaf laying, are shown in Figure 7. Histograms were approximated by Gaussian function.

The mean value of the fluorescence ratio is 0.81 and the standard deviation is 0.05 for the watercress plants in a normal state. The mean value of the fluorescence ratio is 0.96 and the standard deviation is 0.11 for the watercress plants in a stressed state caused by leaf laying.

Histograms of distribution of the fluorescence intensities ratio at 685 and 740 nm for watercress in normal condition in 16 days after planting and in a stressed condition after 24 days of overwatering are shown in Figure 8.

The mean value of the fluorescence ratio is 0.81 and the standard deviation is 0.05 for watercress plants in a normal state. The mean value of the fluorescence ratio is 0.97 and the standard deviation is 0.07 for watercress in a stressed state caused by overwatering during 24 days.

As it is shown in Figures 7 and 8, it is possible to mistake, using a single measurement of fluorescent ratio R , defining whether a plant is under normal or stressed conditions because the distributions are overlapped. A far reliable method for defining the condition of a plant consists in using mean value of the fluorescence ratio, even in the case of small set of measurements.

The mean values (with 95% confidence intervals) of the experimental laser-induced fluorescence spectra of watercress under different stress conditions (leaf cutting, leaf

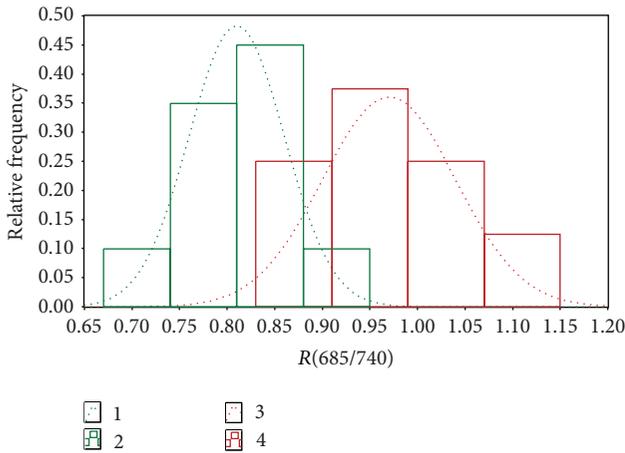


FIGURE 8: Histograms of distribution of fluorescence ratio, for watercress in a normal state and stressed state caused by overwatering for 24 days: (1) histogram for normal state, (2) histogram approximation for normal state, (3) histogram of stressed state, and (4) histogram approximation for stressed state.

laying, root system damage, and root system overwatering during 11, 17, and 24 days) are shown in Figure 9.

Columns 1, 3, 5, 7, 9, and 11 in Figure 9 correspond to the plants in a normal state and columns 2, 4, 6, 8, 10, and 12 correspond to plants in a stressed state (2: leaf laying, 4: leaf cutting, 6: root system damage, 8: overwatering during 11 days, 10: overwatering during 17 days, and 12: overwatering during 24 days).

The changes of fluorescence spectra for plants in stress conditions described above are typical not only for watercress but also for other plants in stress conditions caused by different impact. The effect of soil pollution on lawn grass is considered below.

The aggregated statistical results (mean values and 95% confidence intervals) of the experimental laser-induced fluorescence spectra of lawn grass under different stress conditions caused by soil pollution (copper sulfate, CuSO_4 , ferric sulfate, FeSO_4 , and sodium chloride, NaCl) are shown in Figure 10.

Columns 1, 3, 5, 7, and 9 in Figure 10 correspond to lawn grass in a normal state (experimental research was conducted six weeks after planting, directly before the soil was polluted). Columns 2, 4, 6, 8, and 10 in Figure 10 correspond to lawn grass in a stressed state; measurements were performed 2 weeks after the initial influence of the stress factor for columns 2 and 6 and 4 weeks after the initial influence of the stress factor for columns 4, 8, and 10. The stress factor was soil pollution by sodium chloride, NaCl (5 g per plant sample, columns 2 and 4), ferric sulfate, FeSO_4 (3 g per plant sample, columns 6 and 8), and copper sulfate, CuSO_4 (2 g per plant sample, column 10).

It is clearly illustrated in Figures 9 and 10 that the fluorescence ratio (R) is characterized by stable and sufficient difference. The confidence intervals of the fluorescence ratio for plants under normal and stressed states were not large (≤ 0.1 in the majority of the cases). The sum of confidence intervals of the fluorescence ratio (R) for plants under normal and stressed states is not usually more than the difference

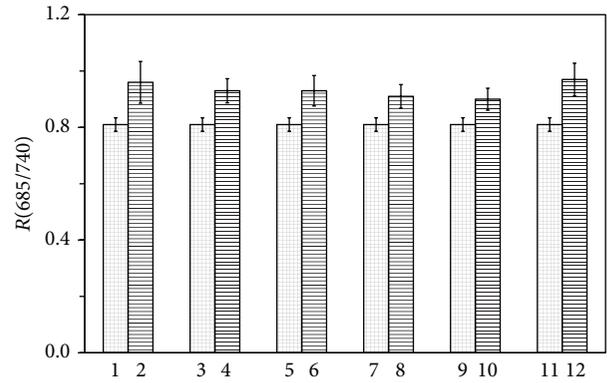


FIGURE 9: Fluorescence ratio (R) mean values and 95% confidence intervals of watercress in normal conditions (columns 1, 3, 5, 7, and 9) and under stressed conditions caused by mechanical damage and overwatering (columns 2, 4, 6, 8, 10, and 12).

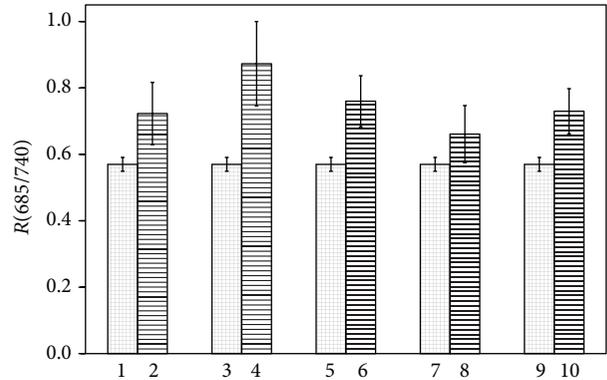


FIGURE 10: Fluorescence ratio (R) mean values and 95% confidence intervals of lawn grass under normal conditions (columns 1, 3, 5, 7, and 9) and stressed conditions caused by different soil pollutants (columns 2, 4, 6, 8, and 10).

between ratio R for plants in a normal state and ratio R for plants in a stressed state caused by various factors (mechanical damage, overwatering, and soil pollution).

This means that fluorescence excitation at 532 nm wavelength and the ratio of fluorescence intensities in the red (685 nm) and far-red (740 nm) bands can be used as signatures of plant stress state caused by various factors.

4. Conclusions

By the processing of the experimental results of fluorescence spectra (induced by a 532 nm wavelength laser) of plants in normal and stressed states caused by mechanical damage, overwatering, and soil pollution the following conclusions can be postulated.

- (i) The fluorescence spectra of different samples of a plant species revealed repeatability of the spectra shapes. Ratio R of the fluorescence intensity at 685 and 740 nm demonstrated sufficient stability. However, it is possible to mistake defining the plant stress state (normal or stressed) using only single measurement of ratio R . We proposed more reliable method

to define plant condition using mean value of R ratio, which is suitable for small set of measurements.

- (ii) The difference between the mean value of ratio R for a plant in a normal state and that in a stressed state, in the majority of cases, is greater than the difference between ratio R values for different samples of one plant species.

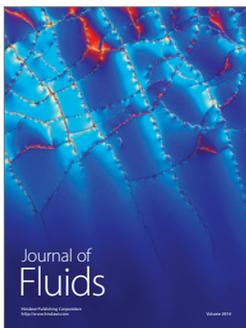
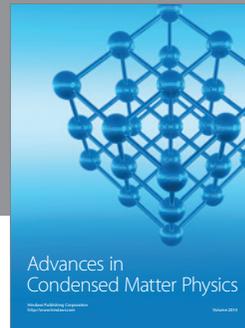
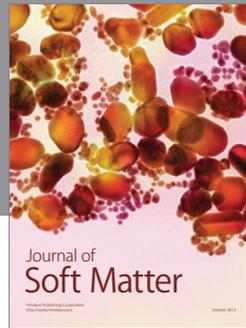
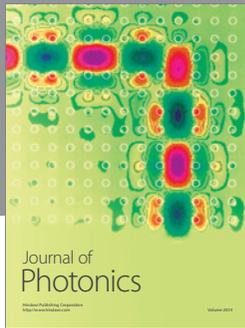
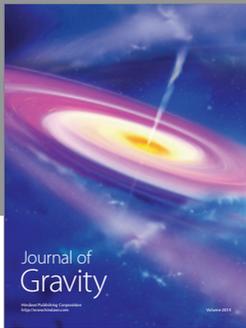
The experimental results obtained allow us to develop a remote laser system for detecting plant stress state. However, to ensure the reliability of the measurements, it is necessary to calculate the mean value of ratio R for several measurements for several plants.

Competing Interests

The authors declare that they have no competing interests.

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