INFLUENCE OF SF EXTRACTS OF *REYNOUTRIA SACHALINENSIS* ON FLUORESCENCE INDUCTION OF HIGHER PLANTS

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ABSTRACT

The supercritical fluid processing shows numerous advantages compared to traditional organic solvent and water extraction. It was concluded to study the influence of supercritical fluid (SF) extract of *Reynoutria sachalinensis* on higher plants and to compare the results with data obtained for water and organic extracts from this plant. Earlier it was shown that organic extract from the giant knotweed, *Reynoutria sachalinensis* (F. Schmidt) Nakai (also known as the main compound of commercial product Milsana®) and water plant extract from this plant were an effective fertilizers protecting plants from powdery mildew fungi, when applied preventively. In this work the influence of SF extracts of *Reynoutria sachalinensis* on fluorescence induction of bean leaves was studied. The supercritical carbon dioxide with 2% and 10% of ethanol as a modifier and pure carbon dioxide were used for extraction process. Fluorescence induction of control plants and plants cultivated by supercritical fluid extracts of *Reynoutria sachalinensis* was studied in laboratory experiments. The obtained data show an increase in photosynthetic activity for treated plants.

INTRODUCTION

Scientists all over the world are looking for a natural fertilizers and fungicides that will be harmless to beneficial insects and animals. Earlier it was shown that water extract of giant knotweed *Reynoutria sachalinensis* and formulated bioprotectant concentrate Milsana® based on organic extract of *Reynoutria sachalinensis* protect mono- and dicotyledone crops from phytopathogenic fungi [1, 2, 3]. The basic mechanism of the protective action of these extracts is associated with the enhancement of the natural defense response of treated plants (induced resistance) [4,5]. For example, application of the extracts from *R. sachalinensis* induced the increase in peroxidase, polyphenoloxidase, and chitinase activities in cucumber leaves [1], and stimulated biosynthesis of phytoalexins in inoculated plants [6]. Earlier, the stimulant effect of these preparations on the photosynthetic activity in leaves was proven [7,8].
As the supercritical fluid processing shows numerous advantages compared to traditional organic solvent and water extraction, it was concluded to study the influence of supercritical fluid extracts of *Reynoutria sachalinensis* on higher plants and to compare the results with

data obtained for water and organic extracts from this plant. The method used in the study is based on the registration of slow fluorescence induction of the leaves allowing to monitor the photosynthetic processes *in situ*. This method was chosen as chlorophyll *a* fluorescence induction (FI) is now a widespread method used in photosynthesis research. This is because FI is non-invasive and highly sensitive, fast and easily measured, it requires relatively inexpensive equipment, and it contains important information about the photosynthetic apparatus [9].

MATERIALS AND METHODS

Acrospires of *Vicia faba* L. were grown in a greenhouse conditions under natural light. For treatment of the plants by spraying several preparations were used. Water and supercritical fluid extracts were obtained from *Reynoutria sachalinensis*, grown in the middle part of Russia. Water extract was prepared fresh prior to spraying by soaking dried plant powder. To prepare the extract, 2 g of plant powder were added to 100 ml of distilled water at 50°C. The suspension was stirred for one hour without further heating. After that the plant particles were filtered off.

Extractions with supercritical fluid CO_2 were performed in laboratory-scale system SFE-1000M1-2- FMC50 by Thar Instruments, Inc. /USA/. The scheme of the system is shown in Figure 1. Three types of experiments were carried out to obtain SF extracts used in these researches: with clear CO_2, with CO_2 and 2% of ethanol as modifier, with CO_2 and 10% of ethanol as modifier. For spraying of the plants obtained SF extracts were diluted in distilled water to the final concentration of 2%.

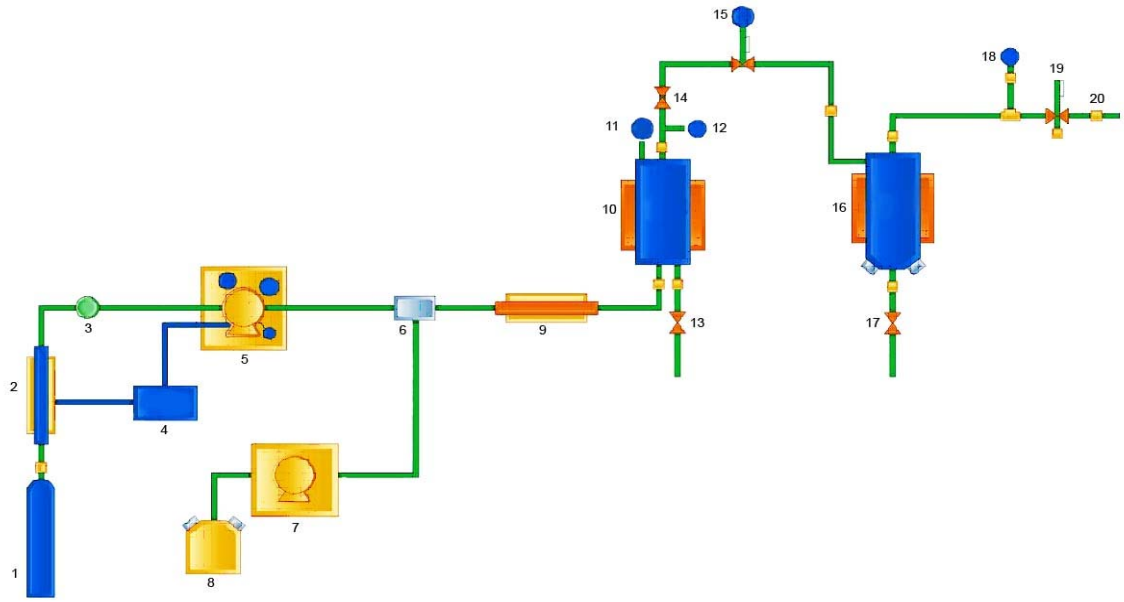

Figure 1. The scheme of the laboratory-scale system for supercritical fluid extraction - 1000M1-2- FMC50 by Thar Instruments, Inc. /USA/. 1) CO_2 cylinder, 2) cooling heat exchanger, 3) flowmeter, 4) cooling bath, 5) CO_2 pump, 6) mixer, 7) co-solvent pump, 8) solvent reservoir, 9) heating heat exchanger, 10) extraction vessel with heat jacket, 11) gauge, 12) temperature sensor, 13) extraction vessel pressure-relief valve 14)) extraction vessel output valve, 15) automated BPR, 16) cyclone separator with heat jacket, 17) separator pressure-relief valve, 18) gauge, 19) manual BPR, 20) exhaust.

For treatment of the plants with commercial liquid formulation of *R. sachalinensis*, Milsana®, the preparation was diluted with water to final concentration of 0.5%. Formulated bioprotectant concentrate Milsana® was kindly granted by Dr. A. Schmitt (Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Control, Darmstadt, Germany).
All preparations used in these researches were diluted with water to final concentration of approximately 10^{-4}% of active compounds. Control plants were sprayed with water.
To measure slow fluorescence induction, the leaf was initially adapted to darkness for 5 min and then exposed to wide-band blue light (50 W/m^2) using a slide projector (LETI-55) as light source. Fluorescence emission at the wavelength λ=686 nm (the maximum of chlorophyll fluorescence band) was isolated with a monochromator SF-4 and monitored by a data recording system that included a FEU-79 photomultiplier and amplifier. Earlier, it was shown that ratio $(F_M-F_T)/F_T$ (F_M = maximal value, F_T = stationary level of fluorescence) correlated with the rate of photosynthetic O_2 production per mg chlorophyll [10].

RESULTS

Spraying of plants with SF and water extracts resulted in an increase in $(F_M-F_T)/F_T$ values on the second day after treatment. Typical curves of slow fluorescence induction are presented in Figure 2.

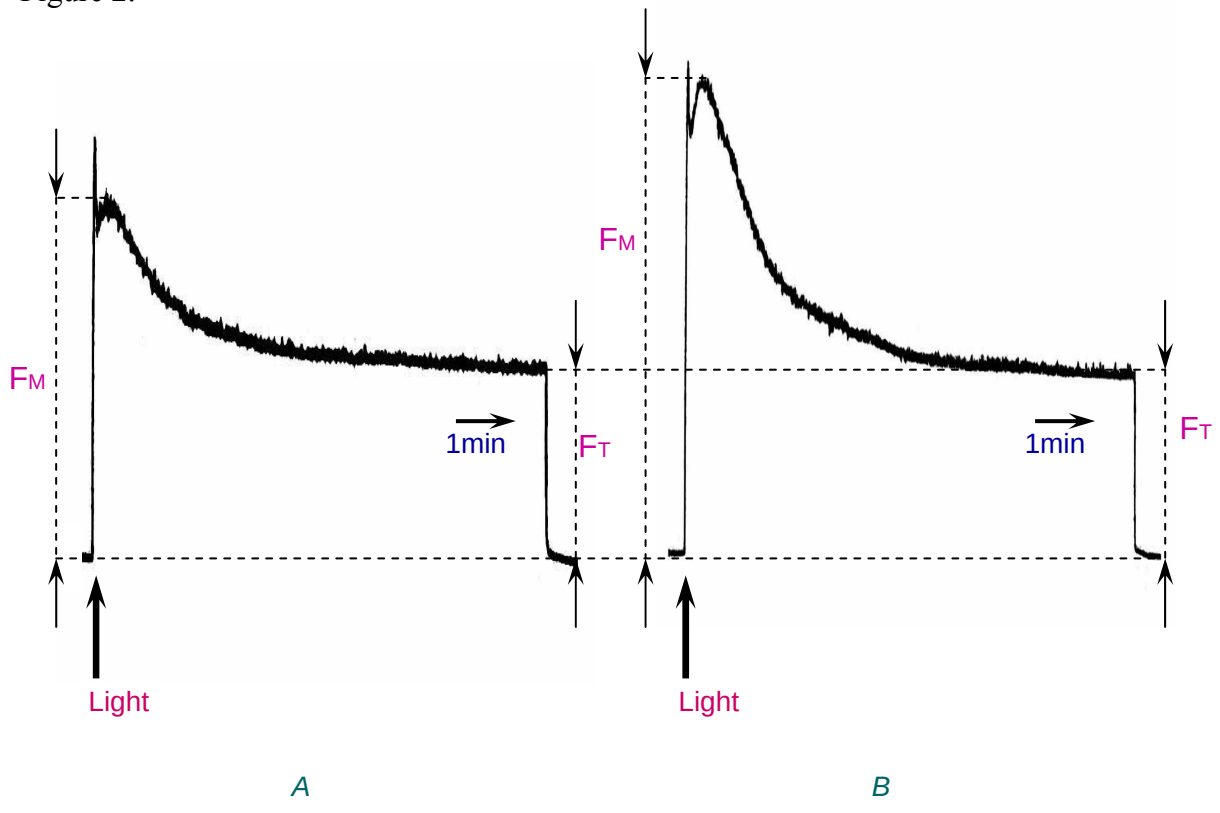

Figure 2. Slow fluorescence induction typical curves of bean leaves. A) Control plants, B) Plants treated with SF extract of *Reynoutria sachalinensis* (supercritical CO_2 + 2% of C_2H_5OH as solvent).

The increase in $(F_M-F_T)/F_T$ values indicates the stimulation of photosynthetic activity in bean leaves. In accordance with generally accepted conception [9, 11], high values of fluorescence intensity at first seconds of illumination are bound with reduction of primary acceptor of photosystem 2. At the following period regulatory processes become operative and provide the optimal functioning of the system, this leads to fluorescence decrease. he stimulant effect of all preparations was recorded on the ninth day after treatment (Table 1).

Table 1. $(F_M-F_T)/F_T$ values of slow fluorescence induction of bean plants after treatment with preparations from *Reynoutria sachalinensi*s.

Treatment	Days after treatment	
	2	9
Control (H_2O)	100%	100%
SF extract *R. sachalinensis* (CO_2 + 10% C_2H_5OH)	120%	118%
SF extract *R. sachalinensis* (CO_2 + 2% C_2H_5OH)	137%	132%
SF extract *R. sachalinensis* (CO_2)	123%	111%
Water extract *R. sachalinensis*	125%	106%
Formulated bioprotectant concentrate Milsana®	85%	124%

The increase of $(F_M-F_T)/F_T$ values can be bound with acceleration of electron transport at the expense of quinine compounds receipt to the cells of the leaves. These quinine compounds can play a role of additional electron acceptors on the reduction side of photosystem 2.
The treatment with SF extract obtained with addition of 2% of ethanol appeared to be more effective than treatment with other preparations. It was also marked that plants, treated with this preparation looked more strongly and were more inconvertible to dehydration.
It can be noted that spraying with water extract also leaded to significant increase of $(F_M-F_T)/F_T$ parameter on the second day after treatment, but this effect wasn't so long, on the ninth day the values for this preparation were practically equal to control plants values.

CONCLUSION

It was showed that treatment of the plants with SF extracts of *Reynoutria sachalinensis* favorably influence on photosynthetic apparatus of the plants and assist a stimulant effect by increasing the photosynthetic activity of the bean acrospires.

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