Impact of developed coniferous biomass extract formulations on soil biological activity and quality

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INTRODUCTION

Plant pathogens induced considerable economic losses in agricultural production industry; therefore, more attention should be paid to development and implementation of environmentally friendly techniques of plant protection. Since 2010, we are working on the development of new environmentally friendly plant protection products against pathogenic fungi and bacteria causing diseases of 3 plants. Several plant protection products on base of coniferous trees biomass were produced in cooperation between the Latvian State Forest Research Institute "Silava" and the Institute of Biology, University of Latvia. **General aim of the research was to develop new environmentally friendly plant protection product, usable in organic farming and integrated pest management. The specific aim of this study was to evaluate impact of new plant protection products on soil biological activity and quality.**

Materials and methods

Extracts and formulations

Spruce bark extract (dry mass 30 %) and pine bark extract (dry mass 26 %) was prepared in Latvian State Forest Research Institute "Silava". Bark was crushed with extrusion-type grinder M-1. The resulting mass was fractionated using sieves, and a fraction with particles size 0.5-1.0 mm was used for further production. Extraction was done with "Büchi" Universal Extraction System B-811 in Soxhlet regime. Ethanol 96% (vol.) was used as a solvent (Table 1). Formulations of bark extracts were developed in the Institute of Biology of University of Latvia. Formulations consisted of: bark ethanol extract 67%, water 26,8%; binding agent Trifolio S – Forte (Trifolio-M GmbH, Germany) 3.2 %, emulsifier Tween-80 (Scharlau, Spain) 2.5 %; KOH 0.4 %; stabilizer 0.1 % and preservative 0.02%.

Spruce bark extract formulation which show anti-fungal activity *in vitro* (Minova et al., 2015) and in field trials on fruit crops strawberries and raspberries (Laugale et.al., 2013, Jankevica et al. 2018.) were selected for studies of impact on soil biological activity. Soil biological activity was analysed for three different soil samples that had not been treated with preparations and three soil samples after the treatment and incubation period. The rate of treatment was 500 L ha^{-1} of working solution (2%).

Soil analysis

- **Soil respiration** was determined by the amount of CO_2 released from the soil. The CO_2 emitted was determined by the titration method.
- The **biomass of microorganisms in soil** was calculated according to the ISO 1420-1: 1997.
- **Fluorescein diacetate (FDA) hydrolysis assays** was used to measure the enzyme activity of microbes in a soil samples, released fluorescein was measured by spectrophotometry (464nm).
- Method for determination of **dehydrogenase activity of soil** with iodonitrotetrazolium chloride was used. Reduced iodonitrotetrazolium formazan (INTF) was measured by spectrophotometry (464 nm) and expressed in g of formazan per 100 g of dry soil.
- **Soil microbiological analysis** - total number of bacteria grown on GPA medium and number of microscopic fungi grown on Sabouraud agar was determined and expressed as the number of colony forming units (CFU) per gram of dry soil.

Statistical analysis

Single factor ANOVA and *F*-test.

Fig.1. Commercial strawberry fields on the Pure Horticultural Research Station

Fig.2. Spruce bark ethanol extract formulation used in test

Table 1. Characteristics of plant extracts used for development of plant protection product formulations

Source	Solvent for extraction	Plant extraction method	pH	Dry mass (%)	Properties
Spruce bark	96% (vol.) ethanol	In Soxhlet apparatus	3.8	30	Thick dark product
Pine bark	96% (vol.) ethanol	In Soxhlet apparatus	3.6	26	Thick brownish product

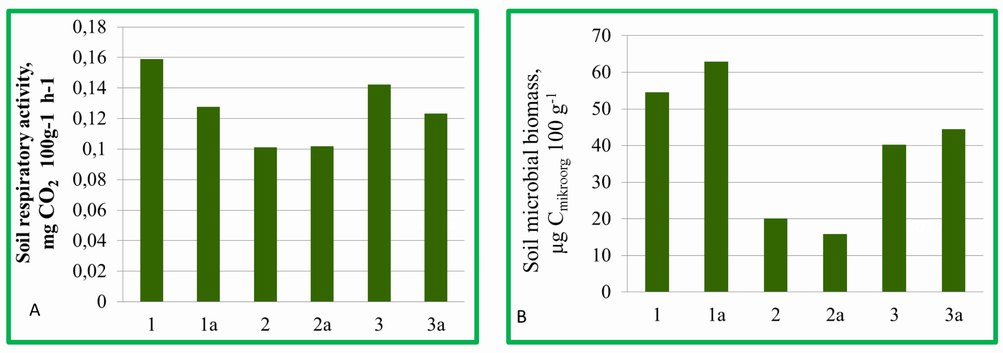

Fig. 3 (A, B). Soil respiratory activity and soil microbial biomass

1, 2, 3 samples not treated with the preparation. 1a, 2a, 3a samples treated with the preparation.

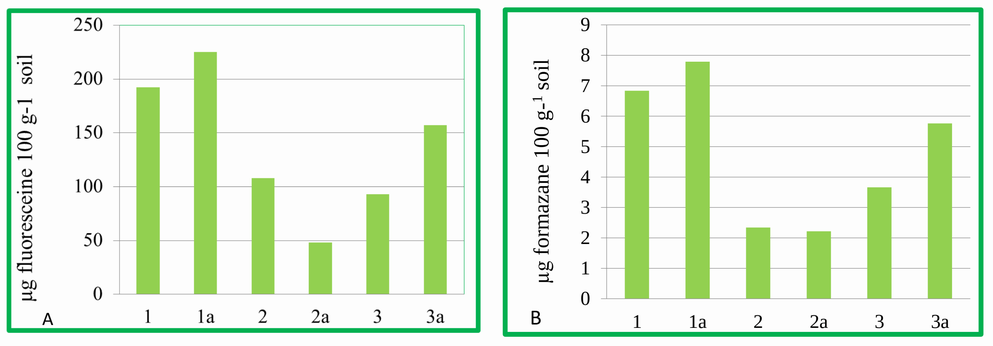

Fig. 4 (A, B). Enzymatic activity of microbes in soil (A) and dehydrogenase activity (B) of soil samples untreated and treated with 2% preparation produced from spruce bark ethanol extract . 1, 2, 3 samples not treated with the preparation; 1a, 2a, 3a samples treated with the preparation.

Results and discussion

We found the highest soil respiration rate for samples 1 and 3, but for the second soil sample the lowest respiration rate, which remains the same for the treated sample (Fig.3 A). Samples 1 and 3 showed a slightly lower respiration rate in the post-incubation period. However, differences in respiration rate are not statistically significant. Therefore, the preparations used cannot be considered to have a significant effect on the activity of soil microorganisms.

Statistically significant changes in the biomass of microorganisms (Fig. 3 B) were found only for the first sample ($F = 8.03 < F_{critical} = 7.70$)

Significant changes in FDA hydrolysis intensity were observed only in the second soil sample, the activity was significantly reduced after treatment of preparation. It is possible that the preparation used may in some cases have a greater effect on microorganisms in biologically inactive soils. This is evidenced by the results of dehydrogenase and FDA hydrolysis intensities (Fig. 4 A, B).

The changes in the total number of bacteria in the analyzed soil samples were not significant (Fig. 5 A). Larger changes were observed in the number of microscopic fungi (Fig. 5 B).

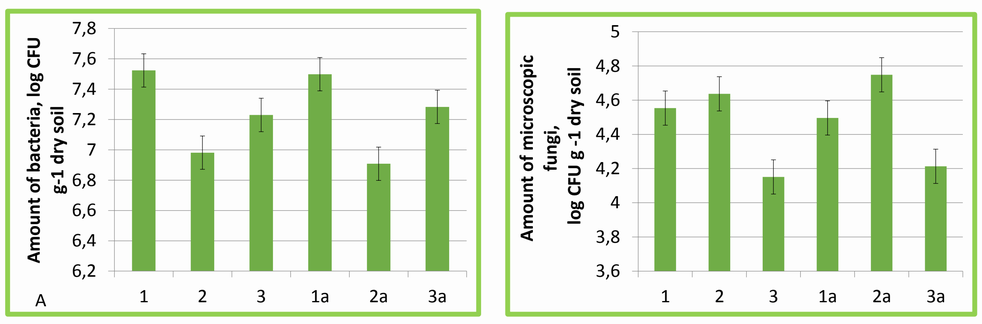

Fig. 5 (A, B). Number of bacteria and microscopic fungi in soil samples, treated und untreated with spruce bark extract preparation. 1, 2, 3 samples not treated with the preparation. 1a, 2a, 3a samples treated with the preparation.

Conclusions

- The treatment with the preparation has not a significant influence on the biomass of microorganisms in the soil, however, there is a tendency that the significance of the effect depends on the properties of the analysed soil.
- The tendency of soil respiration intensity decrease after the use of spruce bark extract preparation was observed.
- The total number of bacteria did not change significantly under the influence of the preparation.
- Significant changes in the number of microscopic fungi were observed for the second soil sample, which generally had the lowest biological activity.

Acknowledgement

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References

ISO 14240-1: 1997. Soil quality - Determination of soil microbial biomass - Part 1: Substrate-induced respiration method. International Standard Organization. 4 pp.

Jankevica, L., Polis, O., Korica, M., Laugale, V., Daugavietis, M. 2018. Environmental risk assesment studies of new plant protection products elaborated from coniferous trees bark. *Agronomy Research*, 16 (5), 2056 – 2067 .

Laugale V., Jankevica L., Samsone I., Halimona J., Seskena R., Metla Z., Lepsis J., Rancane R. & Daugavietis M. 2013. Preliminary studies on development of a new environmentally friendly plant protection product against grey mould. *Proc. Latv. Acad. Sci. B.* 67: 199–202.

Minova, S., Sešķēna, R., Voitkāne, S., Metla, Z., Daugavietis, M. & Jankevica, L. 2015. Impact of pine (*Pinus sylvestris* L.) and spruce (*Picea abies* (L.) Karst.) bark extracts on important strawberry pathogens. *Proceeding of Latvian Academy of Science*. Section B. 69 (1-2), 62–67. DOI: 10.1515/prolas-2015-0008.