Agronomy Research **16**(5), 2056–2067, 2018
https://doi.org/10.15159/AR.18.189

Environmental risk assessment studies on new plant protection products which have been elaborated from coniferous tree bark

L. Jankevica[1,*], O. Polis[2], A. Korica[2], I. Samsone[1], V. Laugale[3] and M. Daugavietis[2]

[1]University of Latvia, Institute of Biology, Department of Experimental Entomology and Microbiology, Miera street 3, LV-2169 Salaspils, Latvia
[2]Latvian State Forest Research Institute 'Silava', Rīgas street 111, LV-2169 Salaspils, Latvia
[3]Latvia University of Life Sciences and Technologies, Institute of Horticulture, Graudu street 1, LV- 3701 Ceriņi, Dobele District, Latvia
[*]Correspondence: liga.jankevica@lu.lv

Abstract. Nowadays there are still various chemical pesticides being applied in the course of ensuring plant protection. Since 2010, we have been working on the development of new, environmentally-friendly plant protection products which will provide an effective tool against pathogenic fungi and bacteria which cause disease in crop plants. The specific aim of this study was to evaluate a risk assessment for new plant protection products that have been elaborated on the basis of coniferous tree bark. Various products were tested which are extracted during the processing of wood bark from pine (*Pinus sylvestris* L.) and spruce (*Picea abies* (L.) Karst.). Ethanol extracts were formulated and applied during these experiments. Two formulations, which showed anti-fungal activity *in vitro* and in field trials on fruit crops (involving strawberries and raspberries) were selected for the risk assessment studies. The impact was studied of formulation treatment on crop plants and soil biological activity, and the accumulation of residues of active substances in crop plants and soil. The application of new formulations did not show any negative effect on the chlorophyll content and the chlorophyll fluorescence of plant leaves. The results showed that pine and spruce bark extract formulations contain active compounds (coumaric acid, quercetin, epicatechin, and ferulic acid) within the range of 5.1–5.9 mg kg^{-1} and 11.1–443.9 mg kg^{-1} respectively. The amount of active substances which were determined in most cases was higher in the spruce bark extract formulation when compared to the pine bark extract formulation. Our results confirmed the presence of active compounds – epicatechin, quercetin, and coumaric acid – in strawberry fruits which remained untreated and in those that were treated with spruce ethanol extract formulation. Untreated raspberry fruits contained all four active substances within the range of 81–5,300 µg kg^{-1}. We observed a significant increase of coumaric acid and quercetin in raspberries after their having been treated with spruce bark extract formulation in a 2% concentration, $P < 0.05$, and did not find any negative impact for spruce bark extract formulations when used on soil microbial biomass.

Key words: coumaric acid, epicatechin, ferulic acid, pine bark ethanol extract, raspberry, residues in soil and plants, spruce bark ethanol extract, strawberry, quercetin.

INTRODUCTION

Plant pathogens induce considerable economic losses in the agricultural production industry; therefore, it is felt that more attention should be paid to the development and implementation of environmentally-friendly techniques. Pest management is one of the major tasks to be associated with growing berries, one which occasionally involves 70% of total growing expenses (Prits & Handley, 1998). Fungal diseases occur in flowers, fruits, leaves, crowns, and roots, reducing yields and the quality of fruit (Paulus, 1990). Grey mould (caused by the fungal pathogen *Botrytis cinerea* Pers.), phytophthora crown rot (caused by *Phytophthora cactorum* (Lebert and Cohn) J. Schrot.), anthracnose fruit rot (caused by *Colletotrichum acutatum* J H Simmonds), strawberry leaf spot (caused by *Mycosphaerella fragariae* (Tul)), verticillium wilt (caused by *Verticillium dahliae* Kleb.), and fruit rot (caused by *Rhyzopus* sp.) are amongst the most widespread diseases which can affect raspberries and strawberries (Paulus, 1990). Most of the fungicides that have been developed for fungal diseases control are site-specific inhibitors with a high risk of resistance development. These problems result in the necessity for alternative methods to be developed, methods which must be safe and are able to replace fungicide treatments. Several studies have been carried out which focus on the investigation of the antifungal effect of different components from coniferous trees (Micales et al., 1994; Zarins & Daugavietis, 1998; Hong et al., 2004; Laugale & Daugavietis, 2009; Zarins et al., 2009; Co et al., 2012; Gabaston et al., 2017). For the most part, the effectiveness of products that have been obtained from resin and tree needles have been evaluated.

Coniferous bark is one of the by-products of forest exploitation which can also be used in the production of plant protection products. Various active substances have been isolated from coniferous bark. For instance, Pan & Lundgren (1995) isolated 28 phenolic compounds from spruce root bark. It is known that coniferous needles and bark contain a wide variety of phenolic compounds with antibacterial, antifungal, antioxidant, and metabolic activities (Richter & Wild, 1992; Co et al., 2012; Salem et al., 2016). Studies which have been carried out on the chemical composition of coniferous bark taken from trees which have been grown in Latvia had been carried out by Verovkins et al. (2008). The major phenolic compounds which are contained in spruce needles and bark are catechin, epicatechin, vanillic acid, *p*-coumaric acid, *o*-coumaric acid, and ferulic acid, with possible others (Iravani & Zolfaghari, 2014; Sadeghi et al., 2014).

In recent years, several plant protection products which have been produced from the biomass of coniferous tree were produced in cooperation between the Latvian State Forest Research Institute 'Silava' and the Institute of Biology, University of Latvia. Various products were tested which are extracted during the processing of wood from pine (*Pinus sylvestris* L.) and spruce (*Picea abies* L.). Different solvents (ethanol, butanol, sodium carbonate, sodium hydroxide, and water) were used for extraction purposes (Jankevica et al., 2013; Laugale et al., 2013). Between 2011 and 2017 several laboratory and field investigations were carried out in order to be able to test the effectiveness of extracts and formulations against important diseases which can infect berry crops.

The laboratory experiments (involving radial growth tests) showed that ethanol extract formulations from coniferous bark are the most effective, and these significantly inhibited the mycelial growth of phytopathogenic fungi: *Botrytis cinerea*, *Colletotrichum acutatum, Phytophthora cactorum,* and *Mycosphaerella fragariae*, at the highest dosage level of 20 g L^{-1} resulted in the complete and total mycelial growth

inhibition of fungi. *B. cinerea, C. acutatum,* and *P. cactorum* did not differ from the conventional fungicide, Signum® (Minova et al., 2015). In field trials on strawberries and raspberries the extract formulations showed significantly lower levels of effectiveness than fungicide Signum®. The application of spruce bark extract in concentrations of 1% significantly reduced the development of leaf spots in 2012, and in both 1% and 2% concentrations in 2013, compared to the untreated control (Volkova et al., 2014). A risk assessment of new plant protection products and active substances needs to be tested to see how they match up to Regulation (EC) No 1107/2009.

The aim of the research was to develop a new, environmentally-friendly plant protection product, one which is usable in organic farming and integrated pest management, by carrying out an environmental risk assessment of new plant protection products which have been developed on the basis of coniferous tree bark.

MATERIALS AND METHODS

Bark extracts and formulations

Spruce bark extract (containing dry matter at an amount of 30%) and pine bark extract (containing dry matter at an amount of 26%) were prepared at the Latvian State Forest Research Institute 'Silava'. Bark was crushed with an M-1 extrusion-type grinder. The resulting mass was fractionated using sieves, and a fraction with particles size of between 0.5–1.0 mm was used for further production. The extraction was carried out using a 'Büchi' B-811 Universal Extraction System and the Soxhlet regime. Extraction was carried out in three and-a-half hours, which is a sufficient amount of time (according to our previous experience) for complete extraction. Ethanol at 96% (volume) was used as a solvent (Table 1). A determination of phenols was based on an optical density measurement of coloured oxidation products, which was obtained using a Folin-Ciocalteu reagent (tungstic acid in an alkaline medium results in a blue colour). Gallic acid was used as a reference substance (Pasqualini et al., 2003; Mechnikova et al., 2007). The density of the blue-coloured substances and reference substance (gallic acid) was measured at 765 nm. The concentration of the total flavonoids was measured using a differential spectroscopy method. Optical densities of the coloured substances after their reaction with aluminium chloride were measured at 410 nm. A Genesys 10 UV scanning spectrophotometer was used for optical density measurements.

Table 1. The characteristics of those plant extracts being used for the development of plant protection product formulations

Source	Solvent for extraction	Plant extraction method	pH	Dry matter (%)	Content of flavonoids in dry matter (%)	Content of phenols in dry matter (%)	Properties
Spruce bark	96% (vol) ethanol	in Soxhlet apparatus	3.8	30	1.2	32.3	thick dark product
Pine bark	96% (vol) ethanol	in Soxhlet apparatus	3.6	26	1.2	20.9	thick brownish product

Extracts were formulated to improve adhesion to the plant. Formulations of bark extracts were developed at the University of Latvia's Institute of Biology. The formulations consisted of: bark ethanol extract 67.0% (dry matter 26.0% or 30.0%); water 26.78%; a binding agent, Trifolio S – Forte (Trifolio-M GmbH, Germany) 3.2%; an emulsifier, Tween-80 (Scharlau, Spain) 2.5%; KOH 0.4%; stabiliser 0.1%; and a preservative at 0.02%. When forming the preparations, KOH was added in order to normalise the pH content. The pH value for the formulations that were developed was at 7.5 ± 0.2. Before use dilutions of 1%, 2%, and 4% of the extract formulation were prepared using warm, clean tap water.

Measurements of chlorophyll content and chlorophyll α fluorescence

The influence was evaluated of two coniferous extracts on the plant photosynthetic parameters on strawberry plants, cv. 'Senga Sengana', propagated *in vitro*. Plantlets were removed from the medium and were planted into plastic pots which contained commercial peat with mineral nutrients. The plants were kept in a growth chamber at 25 °C, photoperiod 18hrs, relative humidity 60 ± 5%. The light was provided by fluorescent lamps with 200 μmol m^{-2} s^{-1} of light intensity. Two month old plants were used for the experiments. A working solution was made up with 1% and 2% concentrations of each coniferous extract's formulations and this was spread over ten plants. The control plants were sprayed with tap water. A visual inspection of the treated plants was carried out. The chlorophyll content and chlorophyll fluorescence levels were measured on ten leaflets at 48, 72, and 168 hours after treatment.

Chlorophyll α fluorescence was measured on the abaxial side of the leaves by using the fast fluorometer PEA (Hansatech, England). The leaves were placed into clips, being darkened for twenty minutes, and then illuminated for five seconds using red diodes (peak 650 nm, the maximum PPFD on the leaf surface was 3,000 μmol m^{-2} s^{-1}). The samples were characterised by the parameter Fv/Fm. Chlorophyll content was measured by a SPAD-502 chlorophyll meter (Konica- Minolta, Osaka, Japan). Ten consecutive readings were taken across the surface of each leaf. The SPAD-502 determines the relative amount of chlorophyll by measuring the absorbance of the leaf in two wavelength regions – blue (400–500 nm) and red (600–700 nm). Using these absorbance levels, the meter calculates a numerical SPAD value which is proportional to the amount of chlorophyll present in the leaf. The mean value was calculated using the internal function of the chlorophyll meter. The data was statistically analysed using the Student's *t-test* (level of significance: $P \leq 0.05$).

Field trials

Field research was carried out in cooperation with scientists from the Pure Horticultural Research Centre and the Latvian Plant Protection Research Centre. The trials were conducted in 2012 and 2013 within the grounds of Ķekavas Dārzs Ltd (Ķekava, Latvia) on one-year-old strawberry plantings (*Fragaria* × *ananassa* Duch.) using cv. 'Induka' (2012) and cv. 'Rubin' (2013) and on the grounds of the Pure Horticultural Research Centre (Pure, Latvia) on six year-old primocane raspberry plantings (*Rubus idaeus*) cv. 'Gerakl' (2013), according to the European and Mediterranean Plant Protection Organisation guidelines (EPPO 1996; EPPO 2012). Before making a start on preparing any of the formulation, we tested the impact of all of the additives in a 1% concentration. No significant impact was observed on chlorophyll content, chlorophyll

α fluorescence, or transpiration (Samsone, unpublished). Field experiments were carried out with spruce ethanol extract preparations because the spruce extracts contained more active substances. There were three experimental plots created for the spruce bark ethanol extract formulation (concentrations of 1%, 2%, and 4%) and a control without treatment. We applied the formulation with its respective levels of concentration on strawberries at an interval of seven to eight days, from the beginning of flowering (24 May 2012 and 28 May 2013) until the maximum level was reached for fruit harvesting (29 June 2012 and 1 July 2013). The rate of treatment was 500 L ha^{-1} of the working solution. On the raspberry plantings we applied solutions at concentrations of 1% or 2% (600 L ha^{-1}) with an interval of seven days, from the beginning of flowering (27 July 2013). We used a randomised block design with four replicates per treatment and untreated plants as a control. A visual inspection of the plants was carried out at each treatment stage. The yield was harvested between two and three times a week. At the end of the 2013 season, four subsamples were taken from each sampling plot. Subsamples of berries were mixed in order to obtain a representative sample and this was delivered to the laboratory for a determination of the residues of active substances.

A determination of active substances in the preparation, plant, and soil

We selected four active compounds – coumaric acid, quercetin, epicatechin, and ferulic acid – which were easy detectable in extracts and which can be used as model substances to show the dispersion and accumulation of the prepared formulations in the production (berries) and in the soil. Berry samples were frozen and stored prior to the start of the analysis. Before the analysis started, the samples were unfrozen, and 50 g of samples were taken and homogenised. All samples were prepared according to the following procedure: 10 mL acetonitrile was added to 5 g of sample and this was shaken using a laboratory shaker for a total of ten minutes; 4 g of anhydrous magnesium sulphate and 0.5 g of sodium chloride were added, and then vigorously shaken for one minute; the mixture was centrifuged for ten minutes at 3,000 rpm; 5 mL of extract was evaporated into a dry mass in a nitrogen flow at a temperature of 40 °C; dry residue was dissolved in 200 µL water and an acetonitrile mixture of 8:2, v/v with 0.1% formic acid, and this was used for high resolution HPLC-MS/MS detection. HPLC-MS/MS measurements were carried out with the Waters Alliance 2690 system, which was connected to a Quattro LC mass spectrometer (Waters). Chromatography analysis was carried out with a Luna C18 column (100 mm × 2.0 mm, particle size 5 µm, 100Å pore size; Phenomenex) at a temperature of 40 °C with an injection volume of 50 µL. The mobile phase flow rate was 0.3 mL min^{-1}. The mobile phase composition was as follows: 'A' – 0.1% of formic acid solution in water; 'B' – 0.1% of formic acid solution in acetonitrile. The time taken for chromatography was 25 mins. A Quattro LC mass spectrometer is equipped with an ESI source in negative mode with the following parameters: 2.5 kV capillary voltage, 150 °C source temperature, 350 °C desolvation temperature, 600 L h^{-1} desolvation gas flow, and 30 L h^{-1} cone gas flow. Statistical analyses were carried out using the R 2.14.1 software. To be able to determine significant differences, the resultant date was submitted to a one-way ANOVA, followed by Tukey's honest significant difference test ($p < 0.05$).

The impact of active substances on soil microbial biomass

Soil samples were collected for an analysis of microbial biomass from the field trial location at the end of the 2013 season. The sampling procedure, transportation, and storage were carried out according to ISO 10381-6 (2009). Four soil subsamples from each sampling plot were taken to a depth of 0–10 cm. The subsamples were mixed and analysed as one sample. The substrate-induced respiration method (ISO 14240-1, 1997) was used to determine soil microbial biomass or soil microbial carbon (SMC).

RESULTS AND DISCUSSION

The impact was evaluated of the use of the formulation treatment on crop plants and of the residues of active substances which have accumulated in crop plants and in the soil. The effect of coniferous extracts on a plant's physiological state was characterised by the chlorophyll content and chlorophyll *a* fluorescence. It has been reported that conifers produce many compounds which may influence other plant growth (Wilt et al., 1993; Aliloo et al., 2012; Cádiz-Gurrea et al., 2014); for example, pine needle inhibitory compounds belong to substances that hinder photosynthesis (Nektarios et al., 2005). The Fv/Fm ratio is used as a stress indicator and describes the potential yield of the photochemical reaction. According to our findings, none of the coniferous bark extracts that were used for the treatment of plants showed any negative effect on the Fv/Fm chlorophyll fluorescence ratio. The results showed that the value of chlorophyll fluorescence (parameter Fv/Fm) was in the range of 0.82–0.84 in all treatments, expressing a high potential activity for photosystem II. The application of spruce and pine bark extract formulations of 1%, 2%, and 4% concentrations did not show any negative effect on the chlorophyll fluorescence of plant leaves (Table 2).

Table 2. Chlorophyll concentration (SPAD units) and chlorophyll *a* fluorescence parameter, Fv/Fm, on strawberry leaves at 48, 72, and 168 hours after treatment with 1% and 2% pine and spruce bark extract formulations (mean ± standard deviation)

Formulation, concentration	Chlorophyll concentration (SPAD units)			Chlorophyll *a* fluorescence parameter Fv/Fm		
	48 hrs after treatment	72 hrs after treatment	168 hrs after treatment	48 hrs after treatment	72 hrs after treatment	168 hrs after treatment
Spruce bark extract, 1%	40.6 ± 2.2	40.1 ± 2.2	40.0 ± 2.0	0.84 ± 0.01	0.83 ± 0.03	0.83 ± 0.01
Spruce bark extract, 2%	41.4 ± 2.9	41.2 ± 2.7	40.9 ± 2.8	0.84 ± 0.01	0.83 ± 0.01	0.83 ± 0.01
Pine bark extract, 1%	38.6 ± 3.3	39.9 ± 1.5	39.4 ± 2.2	0.84 ± 0.01	0.83 ± 0.01	0.83 ± 0.01
Pine bark extract, 2%	34.0 ± 2.6*	40.2 ± 3.6	40.9 ± 3.2	0.84 ± 0.01	0.83 ± 0.01	0.83 ± 0.01
Control	40.5 ± 2.8	40.0 ± 3.3	40.0 ± 2.8	0.83 ± 0.01	0.82 ± 0.01	0.82 ± 0.01

* test, $P < 0.05$.

We observed, on the first days following treatment with the 2% pine bark ethanol extract formulation, that the chlorophyll content of the leaves decreased ($P < 0.05$), and slightly increased after 72 hrs in comparison to non-treated leaves (Table 2). During the visual plant inspection on the day of spraying, in variants in which spruce and pine park

extracts of a 4% concentration had been applied, brown spots were detected on the plant leaves. Since the chlorophyll fluorescence parameter Fv/Fm shows an optimum value, it cannot be assumed that the physiological state of the plants would have deteriorated due to the treatment.

Volkova et al. (2014) reported that in field trials the treatments with spruce biomass extract had no significant effect on strawberry yield and fruit size; however the highest concentration (4%) of the extract had a negative influence on fruit taste and aroma, and it slightly reduced the average size of the berries, although the reduction was not statistically significant.

Selected major components – coumaric acid, quercetin, epicatechin, and ferulic acid – were determined by using the HPLC-MS/MS method in newly developed formulations (Fig. 1, a and b). The results showed that pine and spruce bark extract formulations contained all four active substances in the range of 5.9–35.1 mg kg^{-1} and 11.1–443.9 mg kg^{-1} respectively (Table 3). The amount of determined active substances in most cases was higher in the spruce bark extract formulation than they were in the pine bark extract formulation. The amount of epicatechin in the spruce bark extract formulation was ten times higher than the amount of other substances. Therefore we used the spruce ethanol extract formulation in field trials.

Table 3. Amount of active substances (mg kg^{-1}) in the developed bark extract formulations as determined by the HPLC-MS/MS method (relative standard deviation 2%)

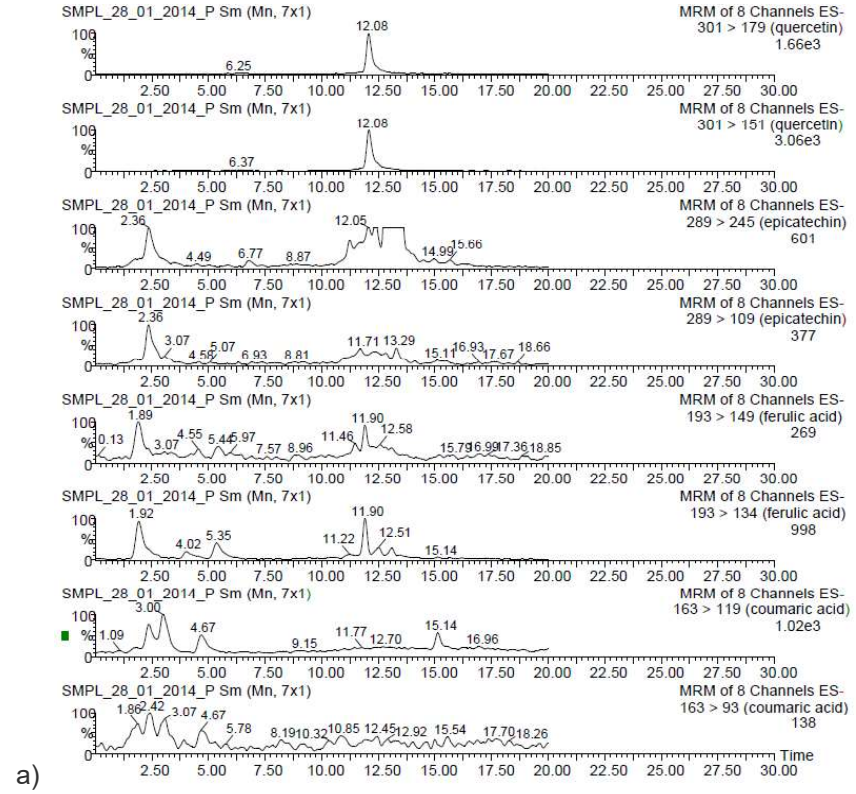
Active substance	Substance volume (mg kg^{-1})	
	Pine bark extract formulation	Spruce bark extract formulation
Epicatechin	35.1	**443.9**
Ferulic acid	32.9	11.1
Quercetin	9.2	43.1
Coumaric acid	5.9	40.6

Using the High Performance Liquid Chromatographic (HPLC) method, flavonoids (kaempferol, quercetin, and myricetin) and phenolic acids (p-coumaric, caffeic, ferulic, p-hydroxybenzoic, gallic, and ellagic acids) were detected in nineteen berries, including strawberries and raspberries (Hakkinen et al., 1999). Research by Hakkinen & Törrönen (2000) shows that the phenolic contents in strawberries are between 421–544 mg kg^{-1}, and flavonols (quercetin, myricetin, and kaempferol) and phenolic acids (ellagic, *p*-coumaric, caffeic, and ferulic acids) are major components. Our results confirmed the presence of the active compounds, epicatechin, quercetin, and coumaric acid, in strawberry fruits, both untreated and those which had been treated with spruce bark extract formulations (Table 4).

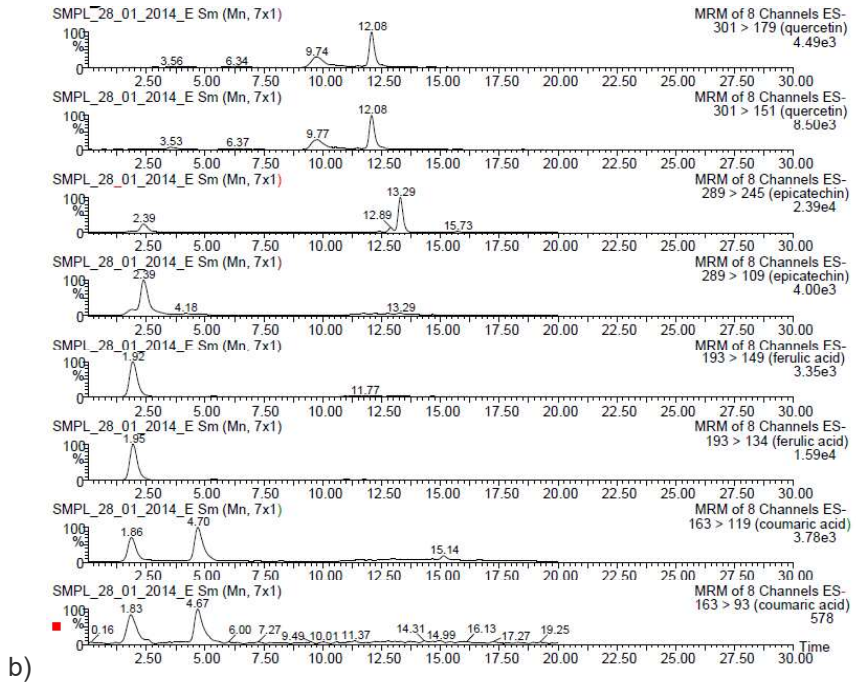
Table 4. The amount of active substances (determined by the HPLC-MS/MS method) in strawberry fruits in field trials, both untreated and those treated with spruce bark extract formulations in 1%, 2%, and 4% concentrations

Formulation, concentration	Substance amount (μg kg^{-1})			
	Quercetin	Epicatechin	Ferulic acid	Coumaric acid
Control	77 a	5,000 a	< 100	49 a
Spruce bark extract formulation, 1%	47 b	5,400 a	< 100	**30** c
Spruce bark extract formulation, 2%	45 b	3,900 b	< 100	51 a
Spruce bark extract formulation, 4%	88 a	2,900 c	< 100	43 b

Those values which have the same suffix letters within the columns are not significantly different at $P < 0.05$.



a)



b)

Figure 1. The chromatographic profile of the bark extract formulations: a – the pine bark ethanol extract formulation; b – the spruce bark ethanol extract formulations.

We observed significant differences in the levels of phenolic compounds in strawberry fruits after their treatment with different concentrations of the spruce extract formulation, $P < 0.05$ (Table 4). It is already known that phenolic content in strawberries is slightly affected by cultivation technique, cultivars, the ripening stage, and the growing conditions (Hakkinen & Törrönen, 2000; Huang et al., 2012).

The red raspberry is characterised by higher concentrations of phenolic acids in comparison to flavonols. Untreated red raspberries showed a high concentration of epicatechin, at 5,300 μg kg^{-1}, and ferulic acid, at 654 μg kg^{-1}. Quercetin and *p*-coumaric acid were found at lower concentrations (Table 5). These results are in accordance with those reported by Hakkinen et al (1999).

Table 5. The amount of substances (as determined by the HPLC-MS/MS method) in raspberry fruits from the untreated control field and fields which had been treated with working solutions of spruce bark extract formulation at a 1% and 2% concentration

Formulation, concentration	Substance amount (μg kg^{-1})			
	Quercetin	Epicatechin	Ferulic acid	Coumaric acid
Control sprayed with water	111 b	5,300 a	654 b	81 b
Spruce bark extract formulation, 1%	114 b	4,846 c	645 b	84 b
Spruce bark extract formulation, 2%	**138** a	5,000 b	**675** a	**199** a

Those values which have the same suffix letters within the columns are not significantly different at $P < 0.05$.

We observed a significant increase of coumaric acid, ferulic acid, and quercetin in raspberries after their treatment with a spruce bark extract formulation of 2% concentration, $P < 0.05$ (Table 5). Considering the fact that the average samples were tested and knowing that the content of the phenols in berries is affected by environmental factors, the planting technology, and the ripening stage, the scattering results for the amount of active substances cannot be limited to the effect of the extracts.

We did not find quercetin, epicatechin, ferulic acid, or coumaric acid in soil samples from the control fields or those fields which had been treated with bark extract formulations (of 1%, 2%, and 4% concentrations).

It is already known that soil microbial communities can significantly influence the productivity and overall quality of the agricultural ecosystem due to the roles they play in nutrient cycling, detoxification processes, and soil aggregate stability, among other functions (Lovaisa et al., 2017). We studied the impact of the bark extract formulations on soil biological quality. The substrate-induced respiration method was used for a determination of soil microbial biomass or SMC content. The determined microbial biomass in untreated soil samples fell in the range of between 0.40 mg kg^{-1} and 0.54 mg kg^{-1}. The results from soil samples which had been treated with a 2% spruce extract preparation, following the normal incubation period, did not differ significantly from the figures for untreated soil. Our data can be seen to be comparable with that which was obtained by Lovaisa et al (2017) in one year-old strawberry fields. The next step will be the determination of the impact of bark extract formulations on soil invertebrates and on *Spirodela polyrhiza* (L.) growth.

CONCLUSIONS

The newly developed formulations which are based on pine and spruce bark ethanol extracts did not negatively influence crop plants at concentrations of 1% and 2%. However, in some cases the amount of chlorophyll increases, although after two days this returns to its initial level.

The newly developed formulations which are based on pine and spruce bark ethanol extracts contain active substances in detectable quantities – coumaric acid, quercetin, epicatechin, and ferulic acid.

Some significant changes were observed in the amount of coumaric acid, epicatechin, and quercetin in strawberries after their treatment by different formulations, and in a significant increase of coumaric acid and quercetin in raspberries after their treatment with a spruce bark extract formulation of a 2% concentration.

The coniferous tree bark preparations which had been developed contained natural compounds that are present in the environment. Therefore their application did not leave any significant influence on soil quality levels that were stated using soil microbial biomass as an indicator. The next stage will be to explore the impact on soil invertebrates and on *Spirodela polyrhiza* (L.) growth.

ACKNOWLEDGEMENTS. We would like to acknowledge I Pugajeva, *Dr Chem*, from the Institute of Food Safety, Animal Health, and Environment (BIOR) for conducting the HPLC-MS/MS measurements, and Dr O Treikale and J Volkova Msc from the Latvian Plant Protection Centre for arranging and conducting the field studies. This study has been supported by ERDF 2010/0249/2DP/2.1.1.1.0/10/APIA/VIAA/168 and LU grant 2016/AZ81.

REFERENCES

Aliloo, A.A., Shahabivand, S., Farjam, L. & Heravi, S. 2012. Allelopathic Effects of Pine Needle Extracts on Germination and Seedling Growth of Ryegrass and Kentucky Bluegrass. *Advances in Environmental Biology* **6**(9), 2513–2518.

Cádiz-Gurrea, M.L., Fernández-Arroyo, S. & Segura-Carretero, A. 2014. Pine Bark and Green Tea Concentrated Extracts: Antioxidant Activity and Comprehensive Characterization of Bioactive Compounds by HPLC-ESI-QTOF-MS. *Int J Mol Sci*. **15**(11), 20382–20402.

Co, M., Fagerlund, A., Engman, L., Sunnerheim, K., Sjöberg, P.J.R. & Turner, C. 2012. Extraction of antioxidants from spruce (*Picea abies*) bark using eco-friendly solvents. *Phytochem. Anal.* **23**, 1–11.

EPPO. 1996. *Botrytis cinerea* on strawberries. *Guidelines for Efficacy Evaluation of Plant Protection Products*. PP1/016.

EPPO. 2012. Conduct and reporting of efficacy evaluation trials, including good experimental practice. PP1/181. *Bulletin OEPP/EPPO Bulletin* **42**, 382–393.

Gabaston, J., Richard, T., Biais, B., Waffo-Teguo, P., Pedrot, E., Jourdes, M., Corio-Costet, MF. & Mérillon, JM. 2017. Stilbenes from common spruce (*Picea abies*) bark as natural antifungal agent against downy mildew (*Plasmopara viticola*). *Industrial Crops and Products* **103**, 267–273.

Häkkinen, S., Heinonen, M., Kärenlampi, S., Mykkänen, H., Ruuskanen, J. & Törrönen, R. 1999. Screening of selected flavonoids and phenolic acids in 19 berries. *Food Res.Internat.* **32**(5), 345–353.

Häkkinen, A.S. & Törrönen, R. 2000. Content of flavonols and selected phenolic acids in strawberries and *Vaccinium* species: influence of cultivar, cultivation site and technique. *Food Research International* **33**(6), 517–524.

Huang, W., Zhang, H., Liu, Y. & Li, C.H. 2012. Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. *J. Zhejiang Univ.Sci. B*. **13**(2), 94–102.

Hong, E.J., Na, K.J., Choi, I.G., Choi, K.C. & Jeung, E.B. 2004. Antibacterial and antifungal effects of essential oils from coniferous trees. *Biol. Pharm. Bull*. **27**(6), 864–866.

Iravani, S. & Zolfaghari, B. 2014. Phytochemical analysis of *Pinus eldarica* bark. *Res. Pharm. Sc.* **9**(4), 243–250.

ISO 10381-6 2009. Soil quality – Sampling – Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory. International Standard Organization. 6 pp.

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

Jankevica, L., Samsone, I., Minova, S., Seskena, R., Halimona, J., Metla, Z., Laugale, V., Rancane, R., Daugavietis, M. & Zarins, I. 2013. Elaboration of new environmentally friendly plant protection product from coniferous trees biomass against plant diseases. In *13th SGEM GeoConference on ecology, economics, education and legislation*. Sofia, Bulgaria, pp. 353–360.

Laugale, V. & Daugavietis, M. 2009. Effect of coniferous needle products on strawberry plant development, productivity and spreading of pests and diseases. *Acta Horticulture* **842**, 239–242.

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.

Lovaisa, N.C., Guerrero-Molina, M.F., Delaporte-Quintana, P.G., Alderete, M.D., Ragout, A.L., Salazar, S.M. & Pedraza, R.O. 2017. Strawberry monocropping: Impacts on fruit yield and soil microorganisms. *Journal of Soil Science and Plant Nutrition* **17**(4), 868–883.

Mechnikova, G. Ya. Stepanova, T.A. & Zaguzova, E.V. 2007. Quantitative determination of total phenols in strawberry leaves. *Pharm. Chem. J*. **41**(2), 97–100.

Micales, I.A., Han, I. S., Davis, I.L. & Young, R.A. 1994. Chemical composition and fungitoxic activities of pine cone extractives. Biodeterior **4**, 317–332.

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. *Proc. Latv. Acad. Sc.* Section B. **69**(1–2), 62–67. doi: 10.1515/prolas-2015-0008

Nektarios, P.A., Economou, G. & Avgoulas, C. 2005. Allelopathic effects of *Pinus halepensis* needles on turfgrasses and biosensor plants. *HortScience* **40**(1), 246–250.

Pan, H. & Lundgren, L.N. 1995. Phenolic extractives from root bark of *Picea abies*. *Phytochemistry* **39**, 1423–1428.

Pasqualini, V., Robles, C., Garzino, S., Greff, S., Bpousquet-Melou, A. & Bonin, G. 2003. Phenolic compounds content in *Pinus halepensis* Mill. needles: A bioindicator of air pollution. *Chemosphere* **52**, 239–248.

Paulus, O. 1990. Fungal diseases of strawberry. *HortScience* **25**(8), 885–888.

Prits, M. & Handley, D. 1998. Strawberry production guide. In: Northeast Resource: Agriculture, and Engineering Service, 88 pp.

Regulation (EC) No 1107/2009. https://www.ecolex.org/details/legislation/regulation-ec-no-11072009-of-the-european-parliament-and-of-the-council-concerning-the-placing-of-plant-protection-products-on-the-market-and-repealing-council-directives-79117eec-and-91414eec-lex-faoc103352/.

Richter, C.M. & Wild, A. 1992. Phenolic Compounds in Needles of Norway Spruce Trees in Relation to Novel Forest Decline. II. Studies on Trees from a Site in the Northern Black Forest. *Biochem. Physiol. Pflanzen* **188**, 305–320.

Sadeghi, A.M., Fallah, H.H., Tajalizadekhoob, Y., Mirarefin, M., Sharifi, F., Taheri, E., Saeednia, S., Larijani, B. & Fakhrzadeh, H. 2014. Determination of phenolic compounds in *Pinus eldarica* by HPLC. *Journal of Medicinal Plants* **13**(49), 22–33.

Salem, M.Z.M., Elansary, H.O., Elkelish, A.A., Zeidler, A., Ali, H.M., Mervat, E.H. & Yessoufou, K. 2016. *In vitro* bioactivity and antimicrobial activity of *Picea abies* and *Larix decidua* wood and bark extracts**.** *BioRes.* **11**(4), 9421–9437.

Zarins, I. & Daugavietis, M. 1998. Conifer foliage extractive substances in plant protection. *Baltic Forestry* **4**(2), 31–39.

Zarins, I., Daugavietis, M. & Halimona, J. 2009. Biological activity of plant extracts and their application as ecologically harmless biopesticide. *Sodininkystė ir Daržininkystė* **28**(3), 269–280.

Verovkins, A., Neiberte, B., Šāble, I., Zaķis, Ì. & Shulga, G. 2008. Latvijas raksturīgāko koku sugu mizas ķīmiskais komponentsastāvs [Chemical composition of Latvian wood species bark]. *Latvijas Ķīmijas žurnâls* **2**, 195–201 (in Latvian).

Volkova, J., Laugale, V., Lepse, L., Baženova, A., Jankevica, L. & Daugavietis, M. 2014. Evaluation of spruce biomass extract for control of grey mold (*Botrytis cinerea*) in field-grown strawberries. *Environmental and Experimental Biology* **12**, 89–93.

Wilt, F.M., Miller, G.C. & Everett, R.L. 1993. Measurement of monoterpene hydrocarbon levels in vapor phase surrounding single-leaf pinyon (*Pinus monophylla* Torr. & Frem.: Pinaceae) understory litter. *Journal of chemical ecology* **19**(7), 1417–1428.