

УДК 547.914

## Study of Plant Growth Promoting Activity and Chemical Composition of Pine Bark after Various Storage Periods

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Received 24.11.2008, received in revised form 15.12.2008, accepted 22.12.2008

*Composition and content of terpene compounds in pine bark (Pinus sylvestris) after various storage periods were studied by GLC-MS. Resin acids were found to be the main diterpenoic compounds in the bark. Content of dehydroabietic acid in initial bark is 0.6 g/kg and it decreases three-fold after one year of storage. High activity of pine bark after various storage periods towards risogenesis of wheat (Triticum aestivum) was discovered. Strong correlation ( $r = 0.89$ ) between growth promoting activity of pine bark and content of dehydroabietic acid in it was found.*

*Keywords: terpene compounds, pine bark, GLC-MS, diterpenoic compounds, dehydroabietic acid, plant growth promoting activity.*

### Introduction

The use of plant growth stimulants is a promising field in modern agrochemistry. They attract even more interest if they are made of cheap and easily available waste materials. Tree bark can be such a material. Annual accumulation of waste bark in Russia attains 20-30 million tons. Only 10 % of this amount is processed into commodities and millions of tons are kept in waste piles that last many years [1, 2]. Thus the problem of waste bark disposal is very important.

Chemical composition of fresh pine bark is studied in detail in papers [3, 4]. However, changes in pine bark composition in the process of its storage is practically unresearched. The present paper discusses the possibility of obtaining plant growth stimulants by hot water extraction from old pine bark after various periods of its storage. Correlations between the plant growth promoting activity and changes in chemical composition of pine bark after various storage periods are also studied.

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## Experimental

Bark of pine (*Pinus sylvestris*) sized to 3-5 mm (over 60 %) was used as the initial material for studies. The bark was moistened to 60 % humidity and was stored under aerobic conditions at 18-21 °C without any additives for 3, 6, 9 and 12 months. Elemental analysis of bark samples dried at 105 °C were accomplished by mass-spectrometry and atomic emission spectroscopy methods.

To study the growth-promoting activity water extracts of pine bark were prepared as follows: the sample of bark sized down to 0.5-1 mm was immersed into boiling water (bark : water ratio 1 : 20) and was let cool down for an hour during which the mixture was occasionally stirred. Then it was filtered through a paper filter. The obtained water extracts were used to treat the seeds of soft spring wheat "Novosibirskaya 15" variety. These experiments were carried out according to GOST 12038-84 [5] using rolls of filter paper loaded with 100 seeds kept for 7 days at 21-25 °C. Germinating ability and number of roots formed were then determined. For control experiment seeds treated with boiled tap water were used.

To study the chemical composition of pine bark after various storage periods and also to isolate the diterpenoid compounds which according to [6] have a growth-promoting activity a sample of bark sized down to 0.5-1 mm was extracted in a 250 ml flask by hexane (bark : hexane ratio 1 : 20), the mixture was stirred every hour during daytime. Total extraction duration was 1.5 months. Then the extract was filtered and evaporated under vacuum to 1 ml residual volume. Determination of the chemical composition of bark after various storage periods was accomplished using the GLC-MS spectrometer (GCD Plus, Hewlett Packard, USA). Capillary column HP-5S, length 30 m, i.d. 0.25 mm. Carrier gas (helium) feed 1 ml/min. Sample

lead-in temperature 250 °C. Initial column temperature 80 °C, final temperature 320 °C, elevation rate 8 °C/min, 30 min isothermic mode. Scanned mass range 45-450 m/z). Obtained mass-spectra were identified by comparing with data of Finigan MAT NIST Library for GCQ/ICIS database (Finigan DB). Mass spectra of identified terpene compounds of pine bark are identical to the database spectra (m/z values are listed, and peak intensity is given in parentheses):  $\alpha$ -pinen - 136 (11), 121 (15), 105 (8), 93 (100), 77 (21), 67(7), 53 (7), 41 (14); verbenol - 152 (7), 152 (7), 119 (29), 109 (100), 94 (71), 91 (50), 81 (57), 69 (71), 55 (43); 3-carene - 93 (100), 136 (14), 121 (17), 105 (13), 77 (36), 67 (10); camphene - 136 (11), 121 (57), 107 (29), 93 (100), 79 (46), 77 (29), 67 (31), 53 (13); longifolene - 204 (17), 189 (33), 161 (92), 147 (26), 135 (43), 121 (36), 119 (43), 94 (100); abietic acid - 302 (30), 285 (29), 239 (40), 213 (17), 197 (21), 121 (50), 105 (86), 91 (100); kauren-18-carboxylic acid - 302 (21), 287 (36), 241 (21), 187 (21), 133 (50), 119 (54), 105 (91), 91 (100); dehydroabietic acid - 300 (23), 285 (73), 239 (100), 197 (48), 159 (15), 107 (85), 129 (38), 91 (37);  $\beta$ -sitosterol - 414 (13), 255 (13), 163 (33), 145 (58), 121 (60), 107 (85), 81 (100), 67 (75); stigmast-4-en-3-one - 412 (7), 370 (7), 289 (7), 229 (21), 149 (24), 124 (100), 95 (33), 55 (50).

Obtained experimental results were processed by regression and correlation analysis methods.

## Results and Discussion

Pine bark contains all necessary elements which can be available to plants after bark mineralization. Element content decreases with the order (element content mg/kg is given in parentheses): Ca (5397) > K (913) > Al (681) > Mg (524) > Fe (407) > P (290) > Na (165) > Mn (116) > Zn (17) > Cu (15) > B (4.40) > Ti (4.00) > Ni (1.00) > As (1.00) > Cr (0.90) > Pb (0.90) > Li (0.85) > V (0.75) > Cd (0.20) > Sr (0.14) > Ba (0.13) > Mo

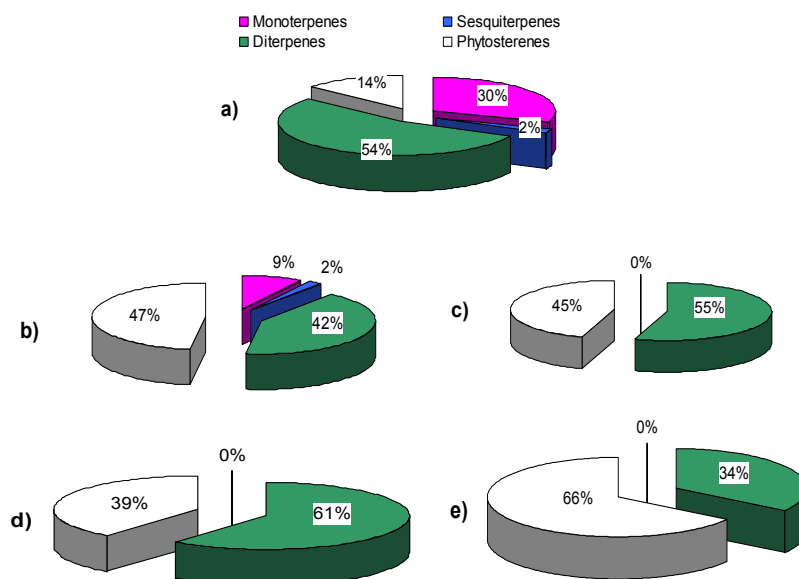


Fig. 1. Changes in terpene compounds content in hexane extract of pine bark after various storage periods: a) fresh bark, b) 3 months, c) 6 months, d) 9 months, e) 12 months

(0.08) > Cs (0.02) > Be (0.01). These results show that content of toxic elements is low compared to maximum permissible concentrations [7].

Total yield of hexane extract of initial pine bark and bark stored for 3, 6, 9 and 12 months was 4.5, 2.5, 2.5, 2.3 and 4.2 % of air-dry bark sample mass respectively. GLC-MS analysis data show that diterpenoic compounds are predominant in extracts of bark stored for 3-9 months (42-61 % of total terpene compounds amount), and only after a one year of storage its content decreases to 34 % (Fig. 1).

The following compounds in hexane extracts were identified by the GLC-MS method: bicyclic monoterpenes –  $\alpha$ -pinen, 3-carene, camphene, verbenol; tricyclic sesquiterpene – longifolene; tricyclic diterpenes – abietic acid, dehydroabietic acid, kauren-18-carboxylic acid.

$\alpha$ -pinen can be noted as one of monoterpenoic compounds prominent in the fresh bark extract (10 %), but its content decreases by 3.4 times after 3 months of storage. It can be result of its volatilization during the bark was stored under aerobic conditions as well some oxidation to

verbenol is possible. Insignificant amounts of other monoterpenoic compounds (verbenol, 3-carene, camphene) were discovered. After 6 months of bark storage monoterpenes are not found in extracts.

Diterpenes are predominant among the identified terpeneoic compounds. Abietic acid which is found in fresh bark is unstable and easily oxidized [8]. After 3 and more months of bark storage it is not found in the extracts. The predominant diterpene is dehydroabietic acid which is not oxidized by oxygen and is more stable. Its content in hexane extract can make up 16 %. After 9 months of bark storage the amount of dehydroabietic acid remains high and only in extract of bark after one year of storage its amount decreases by three times. Depending on the bark storage duration probability of dehydroabietic acid identification in hexane extracts according to Finigan DB spectra is 89 - 93 %.

It is known that abietic acid has growth-promoting activity [6]. Do other diterpenoic compounds have this quality? Hereinafter

Table 1. Influence of pine bark water extracts (WE) on germination of wheat seeds, «Novosibirskaya 15» variety

Seed treatment variant	Germinating ability, %	Number of roots formed, % of control experiment
Water – control experiment	84	100
WE of fresh bark	93	115
WE of bark after 3 months storage	90	109
WE of bark after 6 months storage	89	109
WE of bark after 9 months storage	90	113
WE of bark after 12 months storage	85	101

Table 2. Content of terpenes in extracts of pine bark after various storage periods and number of wheat roots formed

Bark storage duration, months	Compound content in extract, % and content in initial bark, g/kg (in parentheses)								Number of wheat roots per 100 seeds (control experiment)
	mono-terpenes	sesqui-terpenes	diterpenes	phyto-sterenes	abietic acid	dehydroabietic acid	kauren-18-carboxylic acid		
Fresh bark	14.60 (0.54)	0.88 (0.03)	26.06 (0.97)	6.58 (0.24)	2.71 (0.10)	16.19 (0.60)	7.16 (0.27)		260
3	4.57 (0.17)	1.05 (0.04)	21.86 (0.81)	25.09 (0.93)	0	18.75 (0.69)	3.11 (0.12)		299
6	2.01 (0.07)	0	16.55 (0.61)	13.59 (0.50)	0	12.89 (0.48)	3.66 (0.14)		284
9	0	0	16.60 (0.61)	10.64 (0.39)	0	16.60 (0.62)	0		284
12	0	0	5.32 (0.20)	10.15 (0.38)	0	5.32 (0.20)	0		294
									262

Table 3. Correlations between a number of wheat roots formed during germination *versus* terpenoid compounds content in bark extracts

Compounds	r	r <sup>2</sup>
Monoterpenes	0.63	0.40
Sesquiterpenes	0.49	0.24
Diterpenes	0.93	0.86
Phytosterenes	0.32	0.10

**Note:** here and hereinafter r – correlation coefficient, r<sup>2</sup> – determination coefficient.

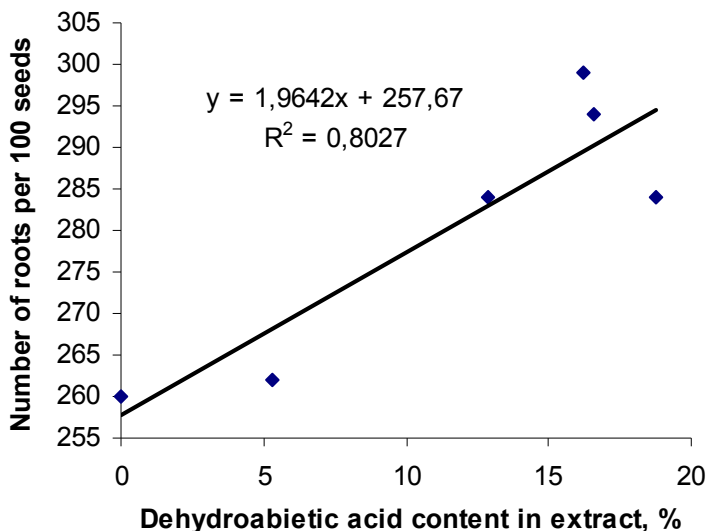


Fig. 2. Dependence of number of wheat roots formed *versus* dehydroabietic acid content in extract

described results of correlation analysis indicate that dehydroabietic acid also has this quality.

When treated by water extracts of pine bark after various storage periods seeds germinating ability increases by 6-11 %. Evidently, this extract stimulates cell division which results in 9 - 15 % increase of roots number during germination of wheat seeds compared to control experiment.

Table 2 presents data on terpenoid compounds content in extract and number of wheat roots formed *versus* bark storage duration. These results demonstrate that with increase of bark storage duration the content of monoterpenes and diterpenes decreases gradually. The most stable diterpenoid is dehydroabietic acid which is present in bark throughout the time of research.

Table 3 presents correlation dependencies of number of wheat roots formed during germination *versus* terpenoid compounds content in bark extracts.

Correlation dependencies of number of roots formed *versus* content of monoterpenes, sesquiterpenes and phytosterenes are defined as weak and medium. Strong correlation dependence ( $r = 0.93$ ) is observed for number

of roots formed *versus* content of diterpenes. Diterpenoid compounds are precursors of gibberellins. Obtained results agree with data of other researchers [9] which indicate that diterpenes are intermediates in biosynthesis of gibberellins. Metabolic path of gibberellins synthesis, determined by use of  $^{14}\text{C}$  compounds can be represented by the following schematic: acetate  $\rightarrow$  melavonate  $\rightarrow$  numerous reformations  $\rightarrow$  kaurene (diterpene)  $\rightarrow$  gibberellin [9].

Among identified diterpenoid compounds in extracts are: abietic acid, dehydroabietic acid and kauren-18-carboxylic acid. Which of these diterpenes has the stronger influence on the growth-promoting effect? To answer this question correlation dependencies of number of wheat roots formed *versus* content of individual diterpenoids in extracts were studied (Table 4).

The strongest correlation ( $r = 0.89$ ) is observed for dependence of number of roots formed *versus* amount of dehydroabietic acid in an extract (Fig. 2):

$$y=1.9642x + 257.67; r^2=0.8027,$$

where  $y$  – total number of wheat roots formed,  $x$  – dehydroabietic acid content in extract, %.

Correlation dependencies of number of roots formed versus content of abietic acid or kauren-18-carboxylic acid are weaker.

Thus the results of the present research demonstrate that bulk waste material of wood

processing industry – pine bark can be utilized to obtain promising natural plant growth stimulants, and that the most active component that determines the growth-promoting activity is dehydroabietic acid.

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