

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Influence of growth stimulators on germination energy and ability of scots pine seeds (*Pinus Sylvestris* L.).

Valentina Iu Ostroshenko, and Valentina V Ostroshenko*.

Department of Forest Plantations, Primorskaya State Academy of Agriculture 44, Bliukhera Street, Ussurisk, Primorye Territory, 692510, Russia.

ABSTRACT

The paper presents the outcomes of the research study of the impact made by the Krezatsin growth stimulator on improvement of sowing qualities of seeds of Scots pine (*Pinus sylvestris* L.), a tree species growing in the southern forest sites of the Far East region. Results. The germination energy was 73.6-91.3%; the laboratory germinating ability was 81.4-98.9%. The biometric parameters of seedlings have been improved. The most effective improvement of seedling parameters have been observed at the solution concentrations of $1 \cdot 3 \cdot 10^{-3}$ - $1 \cdot 5 \cdot 10^{-3}$.

Keywords: sowing qualities of seeds, growth stimulators, Krezatsin, germination energy, laboratory germinating ability, seedling length and weight

**Corresponding author*

INTRODUCTION

Forests of the Far East region take vast territories. Conifers cover over 76% of the total forest area. The tree species composition is as follows: Korean pine (*Pinus koraiensis* Sieb. et Zucc.), Amur larch (*Larix amurensis* B. Kolesn.), white-barked Khingan fir (*Abies nephrolepis* Maxim.), Scots pine (*Pinus sylvestris* L.).

Scots pine is a boreal cenoelement within the areas with a continental and severely continental climate. This tree species of great economic value is one of the best known and widely involved in conifer breeding in the world. It can be used for different economic purposes and is always in good demand. Under conditions of mountainous, vulnerable and hard recovering ecological systems of the Far East, Scots pine is one of the best species used for forest improvement in the purposes of gully afforestation, slope stabilization, sand binding, field-protective and roadside forest belts. This tree species is considered promising for plant introduction, landscape gardening and amelioration due to its fast growth, resistance to droughts and cold [1-5].

Due to its high economic value, biological and ecological parameters and a lot of benefits Scots pine needs protection and more active work on its reproduction. However, seed years in Scots pine stands of the mentioned region occur in three-four years of low yields [6, 2, 4, 5]. After such a storage period the seed germination energy reduces. The treatment of seeds with growth stimulators can improve sowing qualities of seeds [7-17].

Growth stimulators are the substances promoting or inhibiting plants' growth and development. They can be both natural and synthetic. However, user's manuals for growth stimulators are intended for agriculture. As for silviculture, they are used in this sphere experimentally. The first experiments on growth stimulator application carried out in the European part of Russia [18, 7, 9, 14-17, 19] and then in Siberia and in the Far East [20, 8, 10-13] showed effectiveness of these products. The laboratory germinating ability of seeds improved. Seedlings grew vigorously and demonstrated a high level of survival. Further research into this investigation area is appropriate.

This paper overviews the outcomes of research into effective application of the Krezatsin growth stimulator in silviculture. The previous studies state that the Krezatsin growth-regulating product ensures plants' resistance to difficult environments and diseases, improves the laboratory and field germinating ability of seeds, promotes root formation, a seedling growth, a biomass increase and the yield of standard planting materials from an area unit [18, 20, 9, 21, 10-16, 19, 22].

The goal of research is to study a stimulating impact of the Krezatsin water solutions and to reveal the doses stimulating a seed germination energy and laboratory germinating ability as well as length and weight increments of Scots pine seedlings (*Pinus sylvestris* L.), which is one of the widespread tree species in the Far East having great economic value. The goal intends attainment of the following objectives:

- Scots pine seed harvesting;
- Scots pine seed soaking in the Krezatsin solutions of different concentrations;
- seed germination in vitro;
- analyses of the impact made by the Krezatsin growth stimulator on the seed germination energy and laboratory germinating ability, on the Scots pine seedling length and weight increments.

OBJECTS AND METHODS OF RESEARCH

The object of this study is Scots pine seeds (*Pinus sylvestris* L.) harvested in the south of the Primorye Territory. The stimulating impact of the Krezatsin growth stimulator on sowing qualities of seeds was investigated in vitro in compliance with the effective state standards [23]. For experiments on germination we selected seeds without any visual external damage. The researchers from Siberian State Technological University, O.P. Kovylina et al., [8] used in their experiments on germination of Siberian larch seeds soaking of seeds in the Energen (potassium humate) and Epin water solutions during 20-24 hours [8]. As for this study, we also soaked seeds in the water solutions of the growth stimulators during 20 hours. The experiments consisted of seven variations (the solution concentrations were as follows: $1 \cdot 10^{-3}$, $1 \cdot 2 \cdot 10^{-3}$, $1 \cdot 3 \cdot 10^{-3}$, $1 \cdot 4 \cdot 10^{-3}$, $1 \cdot 5 \cdot 10^{-3}$, $1 \cdot 6 \cdot 10^{-3}$ mL/L and a control solution). The control part of seeds was soaked in distilled water. The volume proportion of the seeds and the solution was 1:5. All the experiments were carried out in four

replications. The seeds specially prepared for experiments were placed in Petri plates, one hundred seeds per each plate. The seeds were put on the wet bed made of four layers of absorbent paper cut according to the Petri plate size. Seed germination was performed in the TC-80 thermostat (an electrical dry-air thermostat). The Petri plates were placed in the thermostat chamber. The seed germination beds were maintained wet by adding some distilled water time by time.

The germination temperature was set as 22-24° C. Seedlings were counted, measured and weighted on the fifth, seventh, 10th and 15th days [23].

At each counting normally germinated and decayed seeds were removed from the beds; the numbers of germinated and decayed seeds and ungerminated seeds left on the bed were recorded for each replication individually. The germination energy was defined on the fifth and seventh days of the germination process. On the final counting day we recorded the numbers of sound, untypically germinated, decayed, softened, germless and empty, infested seeds among the seeds remained on the bed [23]. The obtained data were recorded in the analysis inventory. The seedling length was measured with a micrometer, the weight with electronic scales. The experimental data were processed by the Microsoft Excel application [24].

RESULTS

The Krezatsin growth stimulator is a synthetic adaptogene and immune stimulant. It promotes root formation and improves the resistance of living organisms to adverse environment factors such as high and low temperatures, droughts, etc. This product is safe for people and animals, accelerates the growth, development and ripening of agricultural plants by 7-10 days; increases crop productivity and plant resistance to diseases and spring and autumn frosts; increases sugar and vitamin contents in fruits, extends fruit shelf life; prevents bosom and seed-bud falls of all plant species [22].

The product has low toxicity and no mutagenic effect; it is recommended as a pre-sowing, root and foliar nutrient for plants, safe for humans, animals and useful insects. As it is environmentally friendly, the Krezatsin is listed in the inventory of the pesticides and agricultural chemicals approved for use in the Russian Federation. The product is freely soluble in water and alcohol; it is sold without any restrictions [14, 25]. However, user's manuals for growth stimulators are intended only for agricultural crops. Within the forestry sector they are still applied experimentally.

The study has stated that the germination energy and germinating ability depend on stimulator solution concentrations.

The speed of seed germination within the first week of experiments was the highest, and then, within the second week of germination it decreased (Table 2). The most active germination was observed on the first counting day (the fifth day of the experiment). At the higher solution concentrations ($1 \cdot 10^{-3}$ and $1 \cdot 2 \cdot 10^{-3}$) the number of germinated seeds varied within the range of 70,0-70,3%; at the concentrations of $1 \cdot 3 \cdot 10^{-3}$ – $1 \cdot 5 \cdot 10^{-3}$ it was 73,3-71,5%, respectively. At the lower solution concentrations down to $1 \cdot 6 \cdot 10^{-3}$ the number of germinated seeds decreased confidently to 61.3%. The similar dynamics of seed germination was also observed on the seventh day of their germination.

At all the solution concentrations the germination energy and seed germinating ability exceeded the rates of the control part. The most effective concentrations were $1 \cdot 10^{-3}$ – $1 \cdot 5 \cdot 10^{-3}$ as at these levels the seed germination energy was 85.3-91.3% (i.e. 25.8-34.7% more that of the control).

Activating the seed germination energy caused the improvement of their germinating ability: at the solution concentrations of $1 \cdot 10^{-3}$ – $1 \cdot 2 \cdot 10^{-3}$ the germinating ability increased by 88.1-89.0% (Quality Class II); at the solution concentrations of $3 \cdot 10^{-3}$ – $1 \cdot 5 \cdot 10^{-3}$ by 96.1-98.9% (Quality Class I) [26]. At the lower solution concentration of $1 \cdot 6 \cdot 10^{-3}$ the effectiveness of the product impact on the seed germination decreased and was as follows: the germination energy - 73.6%, the germinating ability - 81.4%, which exceeded the control by 8.5% and 4.0%, respectively.

Table 1: The impact of the Krezatsin growth stimulator on the germination energy and laboratory germinating ability of Scots pine seeds (*Pinus sylvestris* L.)

Date of seedling counting, days	Control (distilled water)	Solution concentration, mL/L					
		$1 \cdot 10^{-3}$	$1 \cdot 2 \cdot 10^{-3}$	$1 \cdot 3 \cdot 10^{-3}$	$1 \cdot 4 \cdot 10^{-3}$	$1 \cdot 5 \cdot 10^{-3}$	$1 \cdot 6 \cdot 10^{-3}$
Number of seeds vs solution concentration, pcs / % in relation to the control							
1	2	3	4	5	6	7	8
5th	52.3±2.1	70.0 ± 5.7	70.3 ± 4.8	73.3 ± 2.7	74.8 ± 2.2	71.5 ± 2.8	61.3 ± 2.3
		+33.8	+34.4	+40.2	+43.0	+36.7	+17.2
Confidence t_m	25.4	12.2	14.6	27.2	34.3	25.9	26.9
7th	15.5±0.7	15.3 ± 0.8	15.8 ± 0.5	15.8 ± 0.9	16.5 ± 1.6	15.8 ± 0.3	12.3 ± 1.4
		-1.3	+1.9	+1.9	+6.5	+1.9	-20.6
Confidence t_m	23.8	20.4	32.9	18.4	10.6	58.5	8.5
10th	6.5±1.6	1.5 ± 0.5	1.6 ± 0.2	5.8 ± 0.3	4.3 ± 0.1	6.8 ± 1.0	5.5 ± 1.2
		-76.9	-75.4	-10.8	-33.8	+4.6	-15.4
Confidence t_m	4.2	3	8.9	18.1	84	7.2	4.6
15th	4.0±0.4	1.3 ± 0.3	1.3 ± 0.3	4.0 ± 0.2	1.8 ± 0.5	2.0 ± 0.1	2.3 ± 0.5
		-32.5	-32.5	-	-55	-50	-57.5
Confidence t_m	9.8	3.9	5.2	26.7	3.8	50	4.8
Germination energy, %	67.8	85.3	86.1	89.1	91.3	87.3	73.6
Germinating ability, %	78.3	88.1	89.0	98.9	97.4	96.1	81.4
Ungerminated seeds, pcs	22	12	11	2	3	4	19
Among ungerminated seeds:							
normal	4	2	2	-	-	-	7
decayed	1	1	-	-	-	-	-
softened	1	-	1	-	-	-	-
germless	5	5	7	1	3	4	6
abnormally germinated	11	4	1	1	-	-	6
wormy	-	-	-	-	-	-	-

Note: The solution concentrations: $1 \cdot 10^{-3}$ - 1mL of the product per 1L of water, $1 \cdot 2 \cdot 10^{-3}$ - 1mL/2L, $1 \cdot 3 \cdot 10^{-3}$ - 1mL/3L, $1 \cdot 4 \cdot 10^{-3}$ - 1mL/4L, $1 \cdot 5 \cdot 10^{-3}$ - 1mL/5L, $1 \cdot 6 \cdot 10^{-3}$ - 1mL/6L.

The solution concentrations made a significant impact on the seedling increments in length and weight. The higher solution concentrations ($1 \cdot 10^{-3}$ and $1 \cdot 2 \cdot 10^{-3}$) restrained the seedling growth in length; it decreased in comparison to the control by 5.9-26.7%. The lower solution concentrations within the range of $1 \cdot 3 \cdot 10^{-3}$ – $1 \cdot 5 \cdot 10^{-3}$ activated the seedling growth which exceeded the control by 11.1-38.9%. The further lowering of the solution concentrations (down to $1 \cdot 6 \cdot 10^{-3}$) caused the decrease of the seedling length in comparison to the control by 5.6-13.3% (Table 2).

The high concentration of the product ($1 \cdot 10^{-3}$) also restrained the seedling weight increment; it confidently decreased in comparison to the control by 8.7-13.0%. As the solution concentration was lowering from $1 \cdot 2 \cdot 10^{-3}$ to $1 \cdot 5 \cdot 10^{-3}$, biometrical parameters of seedlings improved; and depending on the solution concentration the growth exceeded the control by 1.4-42%. The further lowering of the solution concentrations (down to $1 \cdot 6 \cdot 10^{-3}$) caused a slight increase of the seedling weight, up to 6.0-8.3% (Table 3, Figure 1,2).

Table 2: The impact of the Krezatsin growth stimulator on the seedling growth in length in the Scots pine (*Pinus sylvestris* L.) seed germination experiments

Date of seedling counting, days	Control (distilled water)	Solution concentration, mL/L					
		$1 \cdot 10^{-3}$	$1 \cdot 2 \cdot 10^{-3}$	$1 \cdot 3 \cdot 10^{-3}$	$1 \cdot 4 \cdot 10^{-3}$	$1 \cdot 5 \cdot 10^{-3}$	$1 \cdot 6 \cdot 10^{-3}$
Average seedling length, cm / % in relation to the control							
1	2	3	4	5	6	7	8
5th	1.6±0.1	1.4 ± 0.1	1.4 ± 0.1	1.9 ± 0.1	1.9 ± 0.1	1.8 ± 0.1	1.6 ± 0.1
Confidence t_m	17.8	-12.5	-12.5	+18.8	+18.8	+12.5	-
7th	1.8 ±0.1	1.4 ± 0.1	1.6 ± 0.1	2.0 ± 0.1	2.5 ± 0.3	2.1 ± 0.1	1.9 ± 0.1
Confidence t_m	36	-22.2	-11.1	+11.1	+38.9	+16.7	+5.6
10th	1.7 ±0.1	1.6 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	2.2 ± 0.1	2.3 ± 0.1	1.8 ± 0.1
Confidence t_m	24.3	-5.9	-	+11.8	+29.4	+35.3	+5.9
15th	1.5 ±0.1	1.4 ± 0.1	1.1 ± 0.4	1.7 ± 0.1	1.9 ± 0.1	2.0 ± 0.1	1.7 ± 0.1
Confidence t_m	37.5	-6.7	-26.7	+13.3	+26.7	+33.3	+13.3
Confidence t_m	37.5	46.7	3.1	24.3	17.3	50	24.3

Note: The solution concentrations: $1 \cdot 10^{-3}$ - 1mL of the product per 1L of water, $1 \cdot 2 \cdot 10^{-3}$ - 1mL/2L, $1 \cdot 3 \cdot 10^{-3}$ - 1mL/3L, $1 \cdot 4 \cdot 10^{-3}$ - 1mL/4L, $1 \cdot 5 \cdot 10^{-3}$ - 1mL/5L, $1 \cdot 6 \cdot 10^{-3}$ - 1mL/6L.

Figure 1: The impact of the Krezatsin growth stimulator on the seedling length in the Scots pine (*Pinus sylvestris* L.) seed germination experiments.

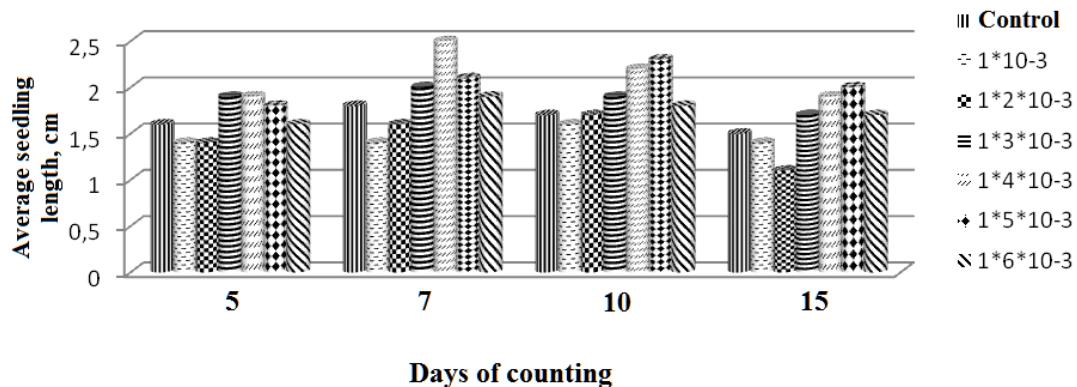


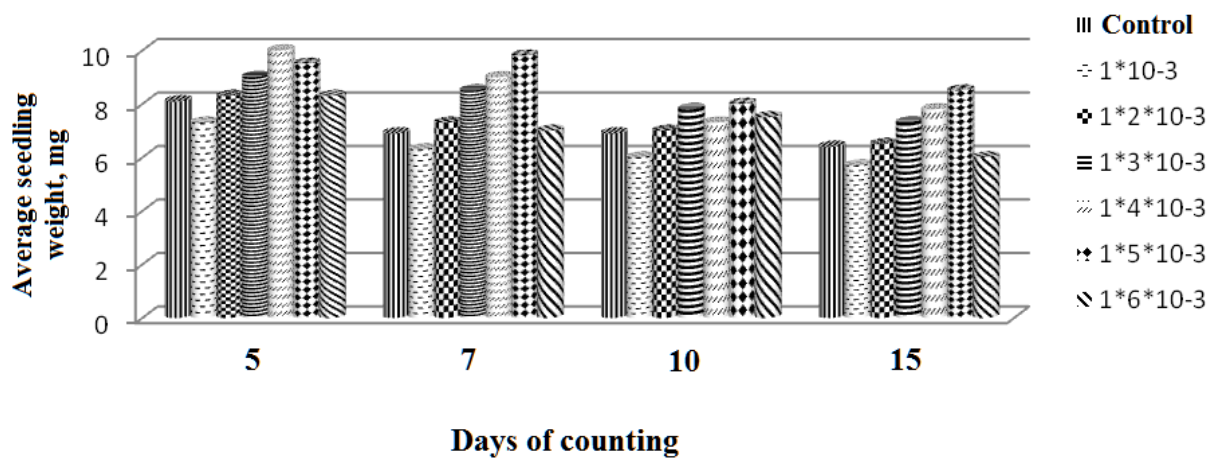
Table 3: The impact of the Krezatsin growth stimulator on the seedling weight increment in the Scots pine (*Pinus sylvestris* L.) seed germination experiments.

Date of seedling counting, days	Control (distilled water)	Solution concentration, mL/L					
		$1 \cdot 10^{-3}$	$1 \cdot 2 \cdot 10^{-3}$	$1 \cdot 3 \cdot 10^{-3}$	$1 \cdot 4 \cdot 10^{-3}$	$1 \cdot 5 \cdot 10^{-3}$	$1 \cdot 6 \cdot 10^{-3}$
Average seedling weight, mg / % in relation to the control							
1	2	3	4	5	6	7	8
5th	8.1±0.2	7.3 ± 0.3	8.3 ± 0.5	9.0 ± 0.7	10.0 ± 0.9	9.5 ± 0.7	8.3 ± 0.9
		-9.9	+2.5	+11.1	+23.5	+17.3	+2.5

Confidence t_m	36.8	29.2	17.3	12.7	10.9	14.6	9.7
7th	6.9±0.3	6.3 ± 0.3	7.3 ± 0.3	8.5 ± 1.9	9.0 ± 0.7	9.8 ± 0.5	7.0 ± 1.5
		-8.7	+5.8	+23.2	+30.4	+42.0	+1.4
Confidence t_m	28.8	25.2	29.2	4.4	12.7	20.4	4.8
10th	6.9±0.3	6.0 ± 0.4	7.0 ± 0.4	7.8 ± 1.3	7.3 ± 0.9	8.0 ± 0.4	7.5 ± 0.7
		-13	+1.4	+13.0	+5.8	+15.9	+8.7
Confidence t_m	24.6	14.6	17.1	6.2	8.5	19.5	11.5
15th	6.4±0.3	5.7 ± 1.2	6.5 ± 0.7	7.3 ± 0.9	7.8 ± 0.9	8.5 ± 0.7	6.0 ± 0.9
		-10.9	+1.6	+14.1	+21.9	+32.8	-6.2
Confidence t_m	24.6	4.8	10	8.5	9.1	13.1	6.5

Note: The solution concentrations: $1 \cdot 10^{-3}$ - 1mL of the product per 1L of water, $1 \cdot 2 \cdot 10^{-3}$ - 1mL/2L, $1 \cdot 3 \cdot 10^{-3}$ - 1mL/3L, $1 \cdot 4 \cdot 10^{-3}$ - 1mL/4L, $1 \cdot 5 \cdot 10^{-3}$ - 1mL/5L, $1 \cdot 6 \cdot 10^{-3}$ - 1mL/6L.

Figure 2: The impact of the Krezatsin growth stimulator on the seedling weight length in the Scots pine (*Pinus sylvestris* L.) seed germination experiments.



CONCLUSIONS

The experiments revealed the positive influence of the Krezatsin growth stimulator on the seed germination energy and laboratory germinating ability as well as on the further seedling growth of Scots pine growing in the Primorye Territory. The study resulted in the following conclusions.

The Krezatsin stimulator is an active agent of root formation. In the experiments on Scots pine seed germination (*Pinus sylvestris* L.) the product demonstrated a high level of effectiveness in improving the seed germination energy and laboratory germinating ability.

The speed of seed germination measured on the counting days can be described as high on the first dates of germination period and as lower by the end of the germination period specified by the effective GOST standard.

In the perspective of the Scots pine biology and cost efficiency of silviculture activities it is recommended to apply the Krezatsin under production environment in the concentrations of $1 \cdot 2 \cdot 10^{-3}$ - $1 \cdot 5 \cdot 10^{-3}$.

REFERENCES

- [1] Koriakin V.N. Reference book for inventory of the Far East resources, Khabarovsk, Russia, 2010, pp. 34-35.
- [2] Urusov V.M., Lobanova I.I., Varchenko L.I. Conifers of the Russian Far East are valuable objects of study, protection, breeding and use, Vladivostok, Russia, Dalnauka, 2007, pp.232,227.

- [3] Urusov V.M., Lobanova I.I., Varchenko L.I. Conifer trees and bushes of the Russian Far East. Geography and biology (biology, variability, ecology, geography of gymnosperms), Vladivostok, Russia: Dalnauka. 2004, p.63.
- [4] Usenko, N.V. Trees, bushes and lianas of the Far East, Khabarovsk, Russia, Khabarovsk Book Publishing, 1969, pp.56-59.
- [5] Usenko, N.V. Trees, bushes and lianas of the Far East, Reference book 3rd ed., Khabarovsk, Russia, Priamurskie Vedomosti Publishing House, 2010, p.27-28.
- [6] Grozdov, B.V. Dendrology, Moscow, USSR, 1952, p.88.
- [7] Vakulenko, V.V. Growth regulators. Plant protection and quarantine 2004; 1: 24-46.
- [8] Kovylin O. P., Kovylin N. V., Kenya E. S., Poznahirko P. Sh. The study of growth regulator influence on germination of Siberian larch seeds. Urgent problems of the forestry complex 2014; 38: 93-97.
- [9] Kolganova I.S., Taran S.S. Innovative techniques for cultivation of sycamore maple seedlings in the Rostov-on-Don region. Modern Science: urgent problems and solutions 2013; 1: 13-17.
- [10] Ostroshenko V.V., Ostroshenko L.Yu., Klyuchnikov D.A. et al. The influence of growth stimulators on germination energy and laboratory germinating ability of Scots pine seeds (*Pinus sylvestris* L.). Izvestiya of Samara Research Centre of the Russian Academy of Science. Vol.17 2015; 6: 242-247.
- [11] Ostroshenko V.V., Ostroshenko L.Yu. The influence of the Krezatsin growth stimulator on the growth and development of Manchurian apricot seedlings (*Armeniaca mandshurica* (Maxim.)). Vestnik of KrasGAU 2011; 2: 56-60.
- [12] Ostroshenko V.V., Ostroshenko L.Yu., Ostroshenko V.Yu.. Application of the Krezatsin growth stimulator in cultivation fir tree seedlings (*Abies*). Vestnik of KrasGAU 2015; 5: 184-189.
- [13] Ostroshenko V.Yu.. History of research growth stimulators and their application in silviculture. Philosophy of modern nature management in river Amur basin. Proceedings of the V International Research Conference 5, Khabarovsk, Russia, Pacific National University, 2016, pp. 81-84.
- [14] Pentelkina, N.V. Environmentally friendly technologies using growth stimulators. Ecology, science education 2002; 3: 69-71.
- [15] Pentelkin, S.K. Outcomes of studying stimulators and polymers in silviculture within the recent 20 years. Forestry information 2003; 11: 16, 34-35.
- [16] Pentelkin S.K., Pentelkina, N.V. The economic value of the Krezatsin growth stimulator in cultivation of fir tree species planting materials. Urgent problems of the forestry complex 2006; 13: 95-98.
- [17] Chilimov A.I., Petelkin S.K. Issues of growth stimulators application in forestry. Forestry 1995; 6: 11-12.
- [18] Bagaev S.S., Korenev I.A., Tretyakov V.V. The study of the influence of bio-effecting agents on Norway spruce cultivation in the Kostroma region. [Research trends in the 21st century: theory and practice](#). Vol. 2, 5-3 2014; (10-3): 11-14.
- [19] Chukarina A.V. Testing the Krezatsin in cultivation of Crimean pine seedlings in steppe environments (in the Rostov-on-Don region). Urgent problems of the forestry complex 2013; 33: 115-118.
- [20] Boyandina T.E. Application of growth stimulators in cultivation of one-year cherry seedlings. Selection, seed production and use of technology in cultivating fruit and berry, vegetable crops and potatoes. 2017; XIX: 38-43.
- [21] Kravchenko R.V., Radchevskij P.P., Troshin L.P., Prah A.V. Effectiveness of the Immunocitophyte, Krezatsin and HB-101eco growth stimulators for cultivation of Saperavi grapes. Polythemagraphic network e-journal of the Kuban State Agrarian university, 2014; 95: 429-442.
- [22] Shapoval O.A., Vakulenko V.V., Prusakova L.D. Plant Growth regulators. Plant protection and quarantine 2008; 12: 53-88.
- [23] GOST 13056.6-97. Seeds of tree and bush species. Detection of germinating ability. – Substitution of ГOCT13056.6-75., Moscow, Russia, 1998, pp. 4-7, 23.
- [24] Doev, S.K. Mathematical methods for processing and analysis of silvicultural information for full-time and part-time students of the Forestry Institute in Major 250100 Forestry, Primorskaya State Academy of Agriculture, Ussuriysk, Russia, 2011, pp. 44-45.
- [25] State catalogue of pesticides and agricultural chemicals approved for use in the Russian Federation, Moscow, Russia, 2015, pp. 691-694.
- [26] GOST 14161-86. Seeds of conifer tree species. Sowing qualities. Technical specifications. – Effective from 1986-27-03, Moscow, USSR, 1986, p. 6.