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ECOTOXICOLOGICAL EFFECTS OF DIFFERENT CONCENTRATIONS OF ALKALINE METAL SALTS AND AN ACID ON THE SEED GERMINATION OF *PINUS NIGRA* SSP. *PALLASIANA*

ERSIN YÜCEL*

Anadolu University, Faculty of Sciences, Department of Biology, 26470 Eskişehir-TURKEY

Abstract

Ecotoxicological effects of different concentrations of alkaline metal salts (NaCl, KNO_3) and strong acid (H_2SO_4) on the germination of *P. nigra* ssp. *pallasiana* (Black Pine) seeds of different origins were investigated. Seeds were exposed to four different concentrations of NaCl, KNO_3 and H_2SO_4 (0.05% to 3%). The results revealed that seed germination rate and germination speed of the Black Pine seeds collected from 14 origins responded differently to the toxicity of NaCl, H_2SO_4, and KNO_3. The results indicated that, while low NaCl concentrations (0.5 to 1%) had no effects on seed germination rates, high concentrations (2 to 3%) had significant inhibitory effects, all concentrations reduced germination speed of the seeds. 0.5 to 2% concentrations of KNO_3 had no effects, but 3% concentration had significant inhibitory effect on germination rate, it reduced germination speed as well. H_2SO_4 had significant inhibitory effects, reduced both germination percentage and speed, inhibiting or preventing germination altogether.

Introduction

Pinus nigra Arnold. has a wide geographical distribution over three continents; Africa, Europe and Asia (Mirov, 1967). There are five subspecies of *P. nigra* and one of these five subspecies, Black Pine (*P. nigra* Arn. ssp. *pallasiana* (Lamb.) Holmboe) has a natural distribution over the Balkans, Turkey, the Crimea, the southern Carpathian Mountains, Cyprus and Syria (Richardson, 1998).

The forests in Turkey cover a total land area of 20.2 million hectares and Black Pine (Anatolian Black Pine) has a distribution covering a land area of 2.2 million hectares and there are many varieties and geographical within this wide distribution in Turkey. Among the varieties emerging from these studies are differences in seedling germination characteristics (Yücel 2000a), resistance to winter-cold (Larsen & Suner, 1986), growth and survival rates (Kalıpsız, 1959), growth, branching and resistance to damage caused by freezing temperatures (Wilcox & Miler, 1975) resistance to drought and frost as well as morphological characteristics (Alptekin, 1986). Research has also been done on variations seed morphology, seed processing technology and storage periods (Atay, 1959).

Rapid industrialisation, population increase and urbanisation bring important environmental problems in their wake. One of the most critical of these is that which is posing a deadly threat to our forests; acid rain. With the complexity and extensiveness of acid rains, among the most affected of living things are our flora (Liao *et al*., 2005). The

* Corresponding author: bitkilerim@gmail.com, Tel.: 90 (222) 335 05 80 /Ext.5722; Fax:+ 90 (222) 320 49 10)

most severe effects of acid rain are felt in Central Europe, where industrialisation is most intense. The forests of these regions are now reported to be home to large quantities of sulphur (30-35 kg/ha/year) and nitrogen (15-30 kg/ha/year) (Karakaş, 1998). Salinity and alkalinity are particularly threatening to Turkey soils. Due to increasing salinity, 0.6% of Turkey's land area is no longer accommodating to cultivation. As a result, the risk of further salinification and consequent barrenness in currently fertile soils requires serious and urgent consideration. Forest fires, violent erosion and salinisation are causing ever-increasing damage to forest soils. There have been 18.808 outbreaks of fire in the past ten years (1988-1997), causing the destruction of 124.466 hectares of forest in Turkey (Anon., 1998). 58.74% of Turkey's land area now faces the threat of violent soil erosion (Çepel, 1998). One of the trees most widely-used for forestation and erosion-control is the Black Pine. The wideness of its natural distribution and of its use in forestation make comprehensive studies of the Black Pine a necessity.

Ecotoxicological effects of different some alkaline metal salts (NaCl, KNO_3,) and strong acid (H_2SO_4) concentrations on the germination of Black Pine (*P. nigra* ssp. *pallasiana*) seeds were examined. The aim was to determine ecotoxicological differences of various Black Pine origins.

Materials and Methods

Seeds were collected from 14 different naturally areas of Turkey in December. The weight of 1000 pure seeds (Bonner, 1974) was found and the experiments took place in March. The experiments were carried out in plant growth chambers (MLR-350 Model Sanyo, Japan). For the duration of the experiments a constant temperature (+25°C) and photo-period of 8 hours light, 16 hours darkness were maintained. For each origin, four main treatment series were tested (NaCl, H_2SO_4, KNO_3 and control group). In each series, seeds were treated with 0.5%, 1%, 2% and 3% concentrations of NaCl, H_2SO_4, and KNO_3. They were treated with the salt, nitrate of acid for the entire 50 days. For the control group pure distilled water was used. For each concentration in each experiment series 100 dark-coloured seeds were used in repetitions of four. Germination tests were performed with the four replicates in a Petri dish (9cm diameter lined with two discs of filter paper). Experiments were terminated on the 50th day, owing to a complete cessation in germination. Seeds were considered germinated when the radicle was touching the filter paper. Determining the germination speed is as much important as determining the seed germination percentage. For this reason, germination speed was calculated for each series of experiments according to Yücel (2000b), and interspecific Ecotoxicological differences were studied. All the data from the experiment were statistically analyzed using the Anova; and all statistical significance was set at the level of $P<0.05$.

Results

The Black Pine (*P. nigra* ssp. *pallasiana*) seeds used in this study were collected from areas of natural distribution in Turkey; Afyon, Bursa, Konya, Kütahya and Eskişehir (Table 1). Of the coloured seeds the heaviest 1000 seed weight was that of Bursa/Ovakorusu (25.19gr) and the lightest was that of Bilecik/Pazaryeri (17.17g); of the

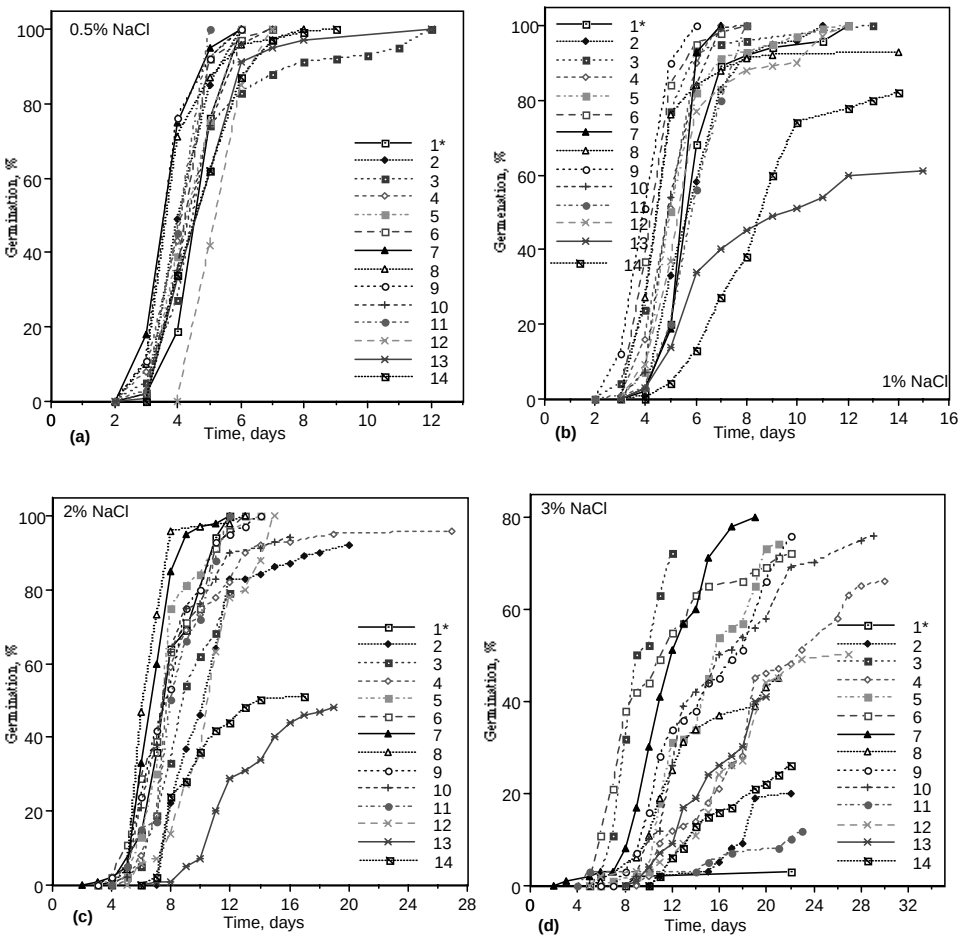

Figure 1. Toxic effects of NaCl on seed germination of Black Pine [(*) origin no, (a) 0.05%, (b) 1%, (c) 2%, (d) 3%].

light-coloured seeds the heaviest were those of Kütahya/Baliköy (19.81g) and the lightest, those of Bilecik/Pazaryeri (5.88g) (Table 2).

Because it is known that 80% of Black Pine seeds are dark-coloured and 20% light-coloured (Saatçioğlu, 1971), and that of light-coloured seeds only 16.5% full, while of the dark-coloured seeds 79.9% full (Atay, 1959), only dark-coloured seeds were used in this study. The findings obtained by the study consistent with existing literature. Dark-coloured seeds (21.65±2.17) were found to be significantly heavier than light-coloured seeds (14.61±3.33) ($P \leq 0.0001$) (Table 2). A significant correlation was not found between seed weight and germination percentage (Correlation=0.115; R^2=0.013) or germination speed (Correlation= -0.331; R^2=0.11). Seed germination rate of the Black Pine origines responded different to the toxicity of NaCl, H_2SO_4, and KNO_3.

Table 1. Seed-collection localities (in Turkey).

Origine No.	Locality	Altitude (m)	Aspect
1	Afyon-Ahırdağ	1350	N-NW
2	Afyon-Çataloluk	1510	DN
3	Bilecik-Pazaryeri	1100	N
4	Bursa-Dağakça	1050	NW
5	Bursa-İnegöl-Boğazova	1200	E-S
6	Bursa-Mustafa Kemal Paşa-Burhandağ	1000	W-NW
7	Bursa-Mustafa Kemal Paşa-Devecikonak	950	S
8	Bursa-Keles-Sorgun	1350	N
9	Bursa-Orhaneli-Çatak	1000	S
10	Bursa-Ovakorusu	950	N
11	Konya-Beyşehir-Anamas	1320	N-SE
12	Kütahya-Tavşanlı-Baliköy	1500	N
13	Kütahya-Domaniç	1400	N-NE
14	Eskişehir-Mihalıççık-Çatacık	1450	N

Table 2. Seed weight (*n*:1 000).

Origine No	Dark-coloured seeds (g)	Light-coloured seeds (g)
1	22.71	15.56
2	20.69	13.50
3	17.17	5.79
4	20.32	14.12
5	21.87	15.11
6	23.05	17.98
7	19.68	13.50
8	18.98	14.56
9	23.97	15.76
10	25.19	12.62
11	22.65	15.68
12	23.86	19.81
13	21.90	18.16
14	20.10	12.46
Sum of Sqr.	6620.61	3133.14
Mean±Std.Dev.	21.65±2.17	14.61±3.33

Toxic effects of NaCl on seed germination: Seeds of all origins germinated completely in 0.5% NaCl (Figure 1a). The earliest germination was completed within four days for origin 11, the longest was 12 days for origins 3 and 13. In the 1% NaCl series all origins except 8, 13 and 14 (93%, 61% and 81%) achieved 100% germination (Figure 1b). Germination speed was slowed and its duration extended, starting a day later than the control group. Origins 13 and 14 are distinguishable from the rest by their lower germination percentage and speed. Marked differences emerge among the 2% NaCl series, regarding germination percentage and speed (Figure 1c). The lowest germination percentages belonged to origins 13 (48%) and 14 (51%). With the exception of origins 2 to 4 and 10, all the others achieved 100% germination. Germination began during days 3 to 7 and was completed during days 12 to 27.

In the 3% NaCl series, differences between origins were more marked and significant, while germination was further delayed and inhibited (Figure 1d). The lowest germination percentage (3%) was achieved for origin 1 and the highest (80%) for origin

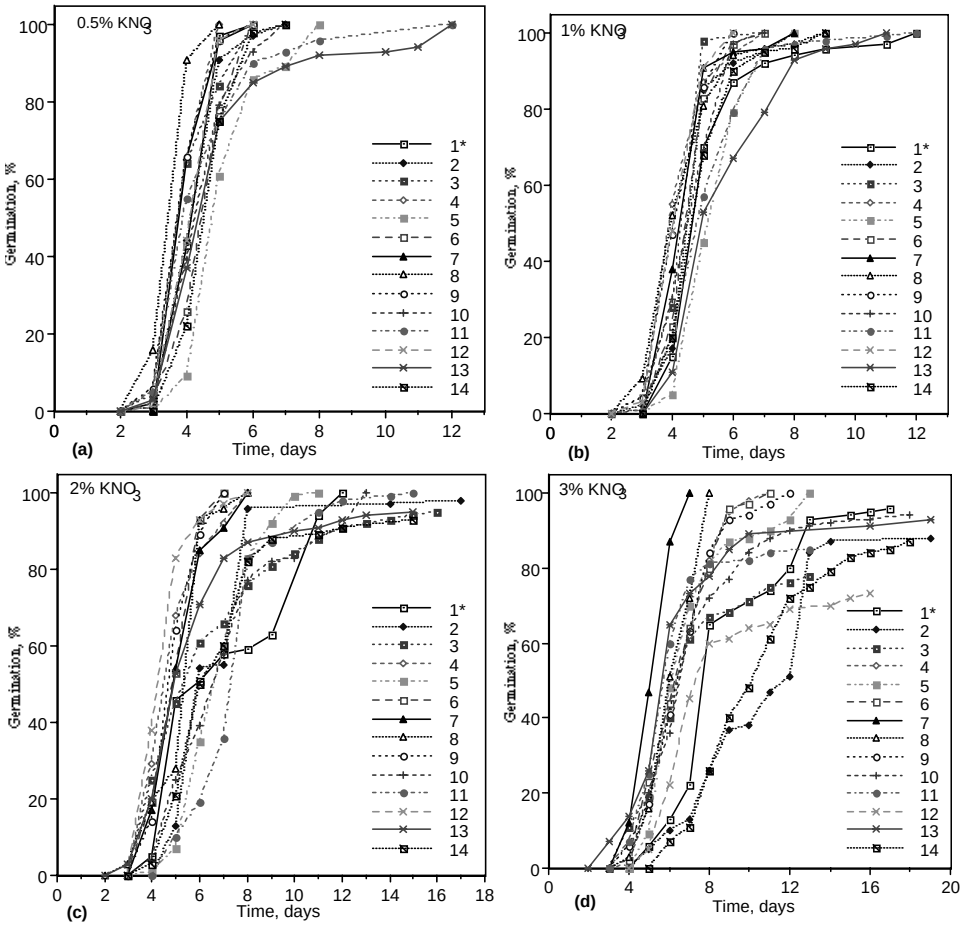

Figure 2. Toxic effects of KNO_3 on seed germination of Black Pine [(*) origin no, (a) 0.05%, (b) 1%, (c) 2%, (d) 3%].

7, with germination beginning during days 3 to 11 and was completed during days 12 to 30.

Toxic effects of KNO_3 on seed germination: In the 0.5% and 1% KNO_3 series completely germination was found for all origins (Figure 2a, b). Germination had begun on day 3 and was completed by day 12. In the 2% KNO_3 series 100% germination was achieved for all origins apart from origins 2,3,12 and 14. The lowest germination percentage (93%) was observed for origin 14 (Figure 2c). Germination had begun during days 3 to 5 and was completed during days 7 to 17. In the 3% KNO_3 series the lowest germination percentage achieved was 73% (for origin 12) and 100% was the highest (Figure 2d).

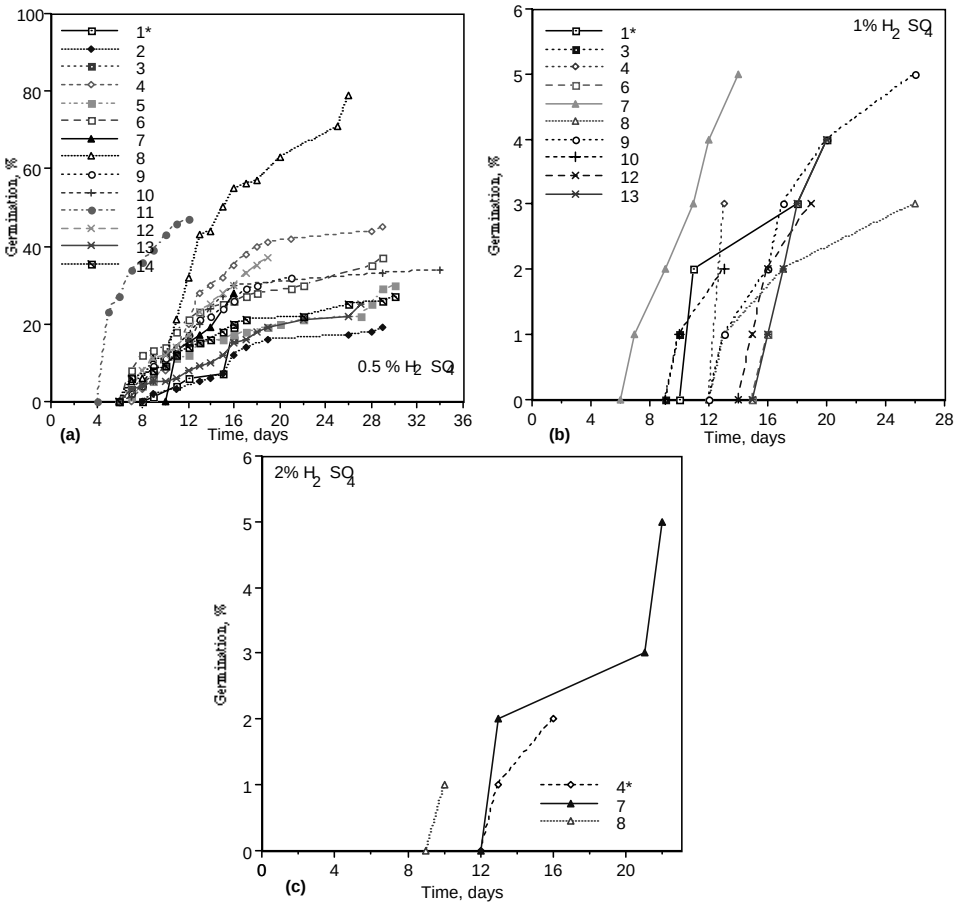

Figure 3. Toxic effects of H_2SO_4 on seed germination of Black Pine [(*) origin no, (a) 0.05%, (b)1%, (c) 2%].

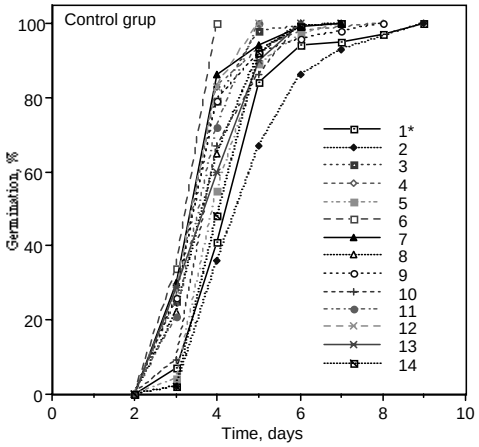

Figure 4. Control group on seed germination of Black Pine [(*) origin No.].

Table 3. The total average effect of NaCl, KNO_3 and H_2SO_4 over germination percentage and germination speed of 14 origins.

Consentration (%)		Germination (%)		Germination speed	
NaCl	0.5	100^{e}		21^{c}	
	1	95^{e}	84^{b}	17^{b}	14^{b}
	2	90^{d}		11^{b}	
	3	51^{c}		7^{a}	
KNO_3	0.5	100^{e}		21^{c}	
	1	100^{e}	98^{c}	20^{c}	18^{b}
	2	97^{e}		16^{b}	
	3	92^{d}		15^{b}	
H_2SO_4	0.5	34^{b}		8^{a}	
	1	2^{a}	9^{a*}	6^{a}	4^{a*}
	2	1^{a*}		2^{a*}	
	3	0		0	
Control		100^{e}	100^{c}	24^{d}	24^{c}

*Within each column, means with the same letter are not significantly (P<0.05)

Table 4. The total average effect of NaCl, KNO_3 and H_2SO_4 over germination and speed germination of different consentrations (0.5%, 1%, 2%, 3%).

Origine	NaCl		KNO_3		H_2SO_4	
No.	**Germ. (%)**	**Germ. speed**	**Germ. (%)**	**Germ. speed**	**Germ. (%)**	**Germ. speed**
1	76^{b}	14^{b}	99^{c}	16^{b}	8^{b}	4^{b}
2	78^{b}	13^{a}	97^{b}	19^{c}	6^{a}	2^{a}
3	88^{c}	15^{b}	93^{a*}	18^{c}	5^{a*}	7^{d}
4	91^{d}	14^{b}	100^{c}	20^{e}	17^{d}	7^{d}
5	94^{d}	14^{b}	100^{c}	14^{a*}	10^{b}	2^{a*}
6	93^{d}	16^{c}	100^{c}	18^{c}	13^{c}	4^{b}
7	95^{d}	16^{c}	100^{c}	20^{e}	12^{c}	7^{d}
8	86^{c}	16^{c}	100^{c}	20^{e}	28^{e}	7^{d}
9	92^{d}	16^{c}	100^{c}	19^{c}	12^{c}	4^{b}
10	93^{d}	14^{b}	100^{c}	18^{c}	12^{c}	6^{d}
11	78^{b}	14^{b}	100^{c}	17^{b}	16^{d}	5^{b}
12	88^{c}	12^{a}	100^{c}	19^{c}	13^{c}	5^{b}
13	63^{a*}	12^{a}	99^{c}	17^{b}	10^{b}	4^{b}
14	65^{a}	12^{a*}	98^{b}	16^{b}	9^{b}	6^{d}

*Within each column, means with the same letter are not significantly (P<0.05). Germ. = Germination

Toxic effects of H_2SO_4 on seed germination: In the 0.5% H_2SO_4 series 19 to 79% germination was achieved, although germination was delayed and inhibited in all series and germination percentages and speeds were lower than the control group (Figure 3). In 1% H_2SO_4 series germination was totally inhibited for origins 2, 5,11 and 14, while for the others it was delayed and decreased significantly (1 to 5%) compared to the control group (Figure 3b). In 2% H_2SO_4 series a low germination percentage (1 to 5%) was seen for origins 4, 7 and 8, while germination was totally inhibited for all other origins (Figure 3c). In 3% H_2SO_4 series germination was totally inhibited in all origins.

In the control group germination: In the control group germination began on day 3 and 100% germination was observed (Figure 4). The fastest germination was observed for origin 6 and the slowest for origin 2.

Discussion

In this study, Ecotoxicological effects of NaCl, KNO_3 and H_2SO_4 on germination of seeds of one of Turkey's most important forest trees, Black Pine, from various origins was investigated. There are many geographical variations, showing morphological differences within the wide natural distribution of Black Pine (Wright & Bull, 1962). While there are known to be important differences among the variations in terms of resistance to winter (Röhring, 1966), there is little known about the existence or otherwise of ecophysiological differences among these subspecies and race (Larsen & Suner, 1986). Although Black Pine shows distribution over quite a wide area, as the most widely planted species, it is important to know whether or not resistance to extreme conditions, such as acid and salt, exists in any origins.

Salinity affects the fertility of soil to the effect that production is significantly reduced (Joshi *et al*., 2004; Hamid *et al.*, 2008). While plants' tolerance of salt has a relation to their growth, high salt-concentrations inhibit germination (Zapata *et al*., 2004; Khan *et. al.*, 2006). In order to reduce the economic losses incurred through the decreased fertility of salty soils, causing, at the first instance, the inhibition of germination, it is important to identify the most suitable seed-types and origins (Yücel, 2000a). In this study it was established that 0.5 to 1% concentrations have no significant effect on germination but that 2 to 3% concentrations inhibit germination and that these findings are statistically significant at 0.05 reliability level (Table 3). Nevertheless, at all salt concentrations germination-speed was lower than for the control and, because this difference was found to be statistically significant, it was concluded that salt decreases germination speed. When origins are correlated with the sensitivity to NaCl, there is significant difference among origins (Table 4).

KNO_3 is held to be one of the growth-regulating and germination-stimulating substances (Puppala, *et al.*, 2002; Sarwar *et. al*., 2006). The difference between the germination percentage of KNO_3 series and control group was found to be statistically significant (Table 3). It was found to be significant when all origins were compared in terms of the sensitivity to KNO_3; it was revealed that the differences of germination percentage between the origins are significant at 0.05 level (Table 4). Further, the coefficient of germination speed of all KNO_3 series being found to be lower than the control group, it was established that this substance decreases germination speed and the difference is statistically significant.

The result of rapid urbanisation and industrialisation, acid rain has recently emerged as a serious environmental issue. The growth and germination of *P. sylvestris* L. and *P. strobes* L. are known to be harmed by pH levels over 3 (Percy, 1986) and H_2SO_4 is a known inhibitor or terminator of germination even at low concentrations (Yücel, 2000 b,c). This study obtained similar findings; H_2SO_4 was seen to have inhibited germination at all concentrations, with the germination percentage and speed falling significantly at low concentrations (0.5 to 1%) and stopping altogether at higher concentrations (Table 3). The difference was significant when different origins were compared with in terms of their sensitivity to H_2SO_4 (Table 4).

In all KNO3 and NaCl series the germination percentage and speed was shown to be higher than for equivalent concentrations of H_2SO_4 and these differences were statistically significant. Comparing germination percentages, no significant difference

was found between 0.5 to 2% NaCl and KNO_3 concentrations, although for the 3% NaCl series the germination percentage and speed were established to be lower than the KNO_3 series at the same concentration, and the difference was found to be significant. Low concentration (0.5 to 1%) of NaCl and KNO_3 did not have any effect on seed germination percent, however at high concentration of these solutions, seed germination is inhibited, and at all concentration of H_2SO_4, germination is inhibited.

As an overall comparison between origins, of the effects of NaCl, KNO3 and H_2SO_4 the applications on Black Pine seeds germination percentages and speeds, a significant ($p \leq 0.05$) difference was found. It was established that origins 13 and 14 had the lowest germination percentages and speeds, while origins 7 and 8 had the highest.

References

Alptekin, Ü. 1986. Anadolu Karaçamı (*Pinus nigra* ssp. *pallasiana*) nın coğrafik varyasyonları. İ.Ü. *Orman Fak. Derg. Seri, A* 3 (2): 132-154.

Anon. T.C. 1998. Orman Bakanlığı orman yangınları ile mücadele faliyetleri 1997 yılı değerlendirme raporu. *Ankara*, p. 376.

Atay, İ. 1959. Karaçam'ın (*Pinus nigra* var. *pallasiana*) tohumu üzerine araştırmalar. İ.Ü. *Orman Fak. Derg. Sayı, A* 9 (1), 48-92.

Bonner, F.T. 1974. Seed testing. In Seed of woody plants in the United States. *Agriculture Handbook No. 450*. For. Service USDA Washington.

Çepel, N. 1998. Ormanların erozyon üzerindeki etkileri. In Çevre ve İnsan. Anodolu Üniversitesi Yayınları No. 1017, *Eskişehir*, 143-171.

Hamid, M., M.Y. Ashraf, U.K. Rehman and M. Arashad. 2008. Influence of salicylic acid seed priming on grovth and some biochemical attributes in weat grown under saline condition. *Pak. J. Bot.*, 40(1): 361-367.

Joshi, A.J., B.S. Mali, and H. Hinglajia. 2004. Salt tolerance at germination and early growth of two forage grasses growing in marshy habitats. *Env. Exp. Bot.*, 1-8.

Kalıpsız, A. 1959. Türkiye'de Karaçam (*Pinus nigra* ssp. *pallasiana*) hasılat tablosu. İ.Ü. *Orman Fak. Derg. A 9* (2), 110-137.

Karakaş, M. 1998. Asit yağmurları, *Tabiat ve İnsan* 32: 23-26.

Khan, M.A. and S. Zia. 2007. Alleviation of salinity effect by sodium hypochlorite on seed germination of *Limonium sttocksii. Pak. J. Bot.*, 39(2): 503-511.

Larsen, J.B. and A. Suner. 1986. Karaçam (*Pinus nigra*) orijinleri arasındaki kuraklığa ve dona dayanma farklılıkları. Ormancılık *Arş. Enst. Derg.* 63: 93-109.

Liao BH, Z.H. Guo and J.L. Probst. 2005. Soil heavy metal contamination and acid deposition: experimental approach on two forest soils in Hunan Southern. China. *Geoderma,* 127: 91-103.

Mirov, N.T. 1967. *The genus Pinus*, The Ronald Press Company, New York, p. 602.

Percy, K. 1986. The effects of simulated acid rain on germinative capacity, growth and morphology of forest tree seedlings, *New Phytol.*, 104: 473-484.

Puppala, N., L James and J. Fowler. 2002. Lesquerella seed pretreatment to improve germination. *Indust. Crops Prod.*: 64-69.

Richardson, D. M. 1998. *Ecology and Biogeoraphy of Pinus*, Cambridge University Press, Cambridge, England, p. 527.

Röhring, E. 1966. Die schwarzkiefer (*Pinus nigra*) und ihre formen, II. ernste ergebnisse von provenienzversuchen. *Silva Genetica*, 15: 21-26.

Saatçioğlu, F. 1971. Orman ağacı tohumları. Sermet Matbaası, İstanbul, p. 242.

Sarwar, N., S. Yousaf and F.F. Jamıl. 2006. Induction of salt tolerance in chickpea by using simple and safe chemicals. *Pak. J. Bot.*, 38(2): 325-329.

Wilcox, M.D. and J.T. Miller. 1975. *Pinus nigra* provenance variation and selection in *Nev Zealand. Silva Genetica,* 24: 132-140.

Wright, J. and I. Bull. 1962. Geographic variation in European Black Pine. *Forest Sci.*, 8: 23-32.

Yücel, E. 2000a. Ecological properties of *Pinus nigra* ssp. *pallasiana* var. *şeneriana. Silvae Genetica,* 49/6, 264-277.

Yücel, E. 2000b. Effects of different salt (NaCl), nitrate (KNO_3) and acid (H_2SO_4) concentrations on the germination of some Salvia species seeds, *Seed Sci. Technol.*, 28: 853-860.

Yücel, E. 2000c. Effects of different salt (NaCl), nitrate (KNO_3) and acid (H_2S0_4) concentrations on the germination of *Pinus sylvestris* ssp. *hamata* seeds, In (ed.) Gözükırmızı, N., Proceedings of the 2nd Balkan Botanical Congress, Plant of The Balkan Peninsula: Into the Next Millennium Volume II, Istanbul, Turkey, Pp: 129-136.

Zapata, J.P., M. Serrano, M.T. Pretel, A. Amorós, and M.A. Botella. 2004. Polyamines and ethylene changes during germination of different plant species under salinity. *Plant Sci.,* 167: 781-788.

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