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Studies on antimicrobial activity of pyramidal black pine (*Pinus nigra* ssp. *pallasiana* var. *pyramidata*): An endemic plant close to become extinct

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Abstract: *Turkey is one of the richest areas at middle latitudes in terms of plant diversity. One of Turkey's (Anatolia) endemic plants is pyramidal black pine (Pinus nigra ssp. pallasiana var. pyramidata). But, it was observed that the pyramidal black pine, which is part of Turkey's important biological richness, came close to become extinct. This study was carried out to investigate the drying of the pyramidal black pine in the specific Vakifkoy area having semi arid to humid climatic conditions on the one hand, and the effects of different thermal power plants on drying of the pyramidal black pine on the other hand. In addition, the study was designed to examine the effects of mistletoe on drying this plant and even the antimicrobial activities of this plant. As a result, we can conclude that the negative effects of semi arid climatic conditions, climatic variables, drought, mistletoe, silver factory and thermal power plants are still not clear on the pyramidal black pine trees. However, when the results of this study were compared with ampicillin (10 mcg) and nystatin (30 mcg) standards, it was found that extracts of pyramidal black pine were particularly found to possess stronger antimicrobial activity.*

Key words: *Pinus nigra ssp. Endemic plant, Climatic change, Biodiversity, Mistletoe, Antimicrobial activity*
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Introduction

Global climate change affects our physical and biological environment, both directly or though interaction with other stressors, and these effects will amplify as projected changes establish by now. Biodiversity, on which human society relies, undergoes alternates at different integration levels, from populations to ecosystems (Belgian Biodiversity Platform, 2007). According to the intergovernmental panel on climate change (IPCC, 2001), in its latest evaluation, concluded that approximately 20-30% of plant and animal species assessed are likely to be at increased risk of extinction if increases in global average temperature exceed 1.5-2.5°C (Belgian Biodiversity Platform, 2007). Global climate is speedly changing, with consequent geographic rearrangement of species and final climate-related extinctions (Hannah *et al*., 2007). Climate change is a dangerous and uncertain play for biodiversity. We know that the present ranges and the present degree of protection of many species will rapidly gnaw as a result of climate change (Hannah *et al*., 2005). Global climate is expected to warm 1.4 to 5.8 °C over this century, with the greatest increases expected at northern latitutes (IPCC, 2001; Estiarte *et al*., 2008).

Biodiversity conservation is one of the global environment issues of Turkey. Therefore, this issue is given rapidly much emphasis. Conserving biodiversity as climate changes is a twopronged challenge, requiring both adaptation – developed conservation strategies – and mitigation – stableness of greenhouse gases in the atmosphere (IPCC, 2001; Hannah *et al*., 2002).

The flora of Turkey is rich and diverse with well over 11,000 flowering taxa recorded in the 9-volume set of Prof. Davis's monumental work and its two supplements. The main reasons for this are climate varieties (namely, Mediterranean, continental, and oceanic), geomorphologic and soil diversities as well as the position of the area at the junction of three flora regions (Euro-Siberian, Mediterranean and Irano-Turanian). When all these factors are combined, many properties are provided for the plants to grow up and become diverse (Baser, 2002). The flora of Turkey is relatively rich (about 12,000 species) and still a great number of new species is being described. In Turkey, the rate of endemism is relatively high compared to other European countries. However, this diversity and endemic species are under considerable threat. The habitat in mountainous regions and coastal dunes are under threat. That is the reason why it is really significant to protect the diversity of plants (Avci, 2005).

One of Turkey's (Anatolia) endemic plants is pyramidal black pine (*Pinus nigra* ssp. *pallasiana* var. *pyramidata*) which is a variety of the anatolian black pine. In Turkey, five pine species (*Pinus nigra, P. sylvestris, P. brutia, P. pinae* and *P. halepensis*) are indigenous. Of these, *Pinus nigra* ssp. *pallasiana* is the pine with the biggest surface of expansion, which is 3,313,706 ha (Karadag, 1999). There are four varieties of *Pinus nigra* ssp. *pallasiana* in Turkey, which are anatolian black pine (*Pinus nigra* ssp. *pallasiana* var. *pallasiana*), ebe black pine (*P. nigra* ssp. *pallasiana* var. *seneriana*), big fruited black pine (*P. nigra* ssp. *pallasiana* var. *yaltirikiana*) and ehrami black pine or pyramidal black pine (*P. nigra* ssp. *pallasiana* var. *pyramidata*).

Pyramidal black pine (*P. nigra* ssp. *pallasiana* var. *pyramidata*), which has a column-like and pyramidal shape, is able to grow as tall as 20 m, whose laterals grow by drawing acute angles from the body upwards; its body cannot get thick beyond a certain extent, it does not shoot forth as it cuts and grows slowly. Its appearance looks like Mediterranean pyramidal service (*Cupressus sempervirens* var. *sempervirens*), which helps us easily distinguish it from other varieties of pines. Pyramidal black pine is also named "*selvi cami, uzun cam, mihrap cami, ardic cami*" locally (Unaldi, 2004).

Different parts of these species are used for the same purpose regardless of the species. Due to their tannin contents, the dried barks have been used as a tanning agent and as an infusion to produce constipation (Bagci and Digrak, 1996; Toroglu, 2007). An essence obtained from the leaves of pines contains compounds such as pinene, cadinene, terpineol and bornyl acetate. The leaves can also be used as a mucus remover and as an antiseptic. *Pinus nigra* and *P. brutia* contain compounds such as alfa-pinene, beta-pinene, beta-caryophyllene and gemacrene (Sezik *et al*., 1997). Many plant genera suh as *Betula, Fagus, Fraxinus, Juniperus, Picea, Pinus etc.*, are among the medicinal plants recorded in some Codex and Pharmacopeias (Ceylan, 1995).

The subject of our study, pyramidal black pine was firstly discovered to be indigenous around Vakifkoy of Kutahya by Acatay and was named "*Pinus nigra* ssp. *pallasiana* var. *pyramidata* (Acatay)" as a new variety of black pine (Acatay, 1956). Pyramidal black pine variety, as an important endemic plant in Turkey, spreads across an area of 292,5 ha in 8 separate sites in Kutahya province (Yucel, 1995). Vakifkoy and its surroundings is the site with the highest number of the variety.

Vakifkoy of Tavþanli, Kutahya (Western Anatolia) is the most natural and widespread field of the variety on earth. Yet there are some problems in the field. For instance, it was understood as a result of the regional research in 1999 that the field was covered with mistletoe. In the research alone in 2002, although it was observed that the mistletoe was cleared, there were die-outs and fail-downs here and there. Therefore, it is essential that the pyramidal black pine, which is part of the prominent biological richness of Turkey, should be protected and necessary precautions taken for the survival of the species.

Due to rapid ascending population, developing technology and needs more than enough of the human beings, natural sources and biological richness are under considerable threat. Being one of the endemic tree species of the naturally distributed in limited area, and the disappering risk handling because of drought the *P. nigra* ssp. *pallasiana* var. *pyramidata* was selected as research material. The aims of the present study are (1) to discuss about biodiversity which is an important issue in the global warming or climatic changes, (2) to examine whether the climatic changes is important to help pyramidal black pine survive or not, (3) to investigate the relationship between climatic changes and drying out the pyramidal black pine, (4) to discuss the possible effects of drought on drying out this plant on semi arid to humid, (5) to research the effects of different thermal power plants on drying out this plant, (6) to examine the effects of mistletoe on drying out this plant, (7) and to detect the antimicrobial activities of this plant.

Materials and Methods

Study area: Study area is located at 39°29'40"-39°31'42" N latitude and 29°35'18"-29°38'36" E longitude. Climate type is semi arid to humid.

Three (3) field studies were carried out on the research site in 1999, 2002 and 2006 in order to identify natural growing conditions for the pyramidal black pine. Particular emphasis was placed on current situation and ecological facts of the pyramidal black pine.

Meteorological station's report: Records of meteorology stations provided by the Turkish State Meteorological Service placed on the site, and earth reports provided by the Ministry of Agriculture and Rural Affairs also contributed extensively.

Climatic change: Drought indexes were calculated according to De Martonne (De Martonne, 1942) and Erinc method (Erinc, 1965).

$$\frac{\frac{P}{T+10} + \frac{12p}{t+10}}{2}$$

P: Annual average precipitation amount (mm)

p: The precipitation in the driest month (multiplied by 12, number of months in a year)
T: Annual average temperature (°C)
t: The temperature in the driest month
10: Constant coefficient used to avoid negative values

$$I_m = \frac{P}{T_{om}}$$

I_m : Precipitation efficiency indices
P : Annual average precipitation amount (mm)
T_{om} : Annual average maximum temperature (°C)

Elemental measurements: Aseptically prepared powder of the several parts (Leaves, cones, barks) of the pyramidal black pine used in this elemental analyser. Analysis of elements such as carbon, hydrogen, nitrogen and sulphur (C, H, N and S) were performed using a LECO CHNS 932 (Inonu University, Malatya, Turkey) elemental analyser.

The several parts (leaves, cones, barks) of pyramidal black pine used in this study were collected from the region of Vakifkoy of Tavsanli, Kutahya (Western Anatolia) in winter season. The species collected were authenticated according to the conventional method (Davies, 1965). The collected parts were ground using a blender. The solvents selected (ethyl alcohol, methanol, ethyl acetate, acetone) were purchased from Merck, Darmstadt and they were used without further purification (Harmala *et al*., 1992; Mitrokotsa *et al*., 1993).

The microorganisms tested in this study were provided from the culture collections of the Microbiology Laboratory of the Science and Art Faculty of the University of Kahramanmaras Sutcu Imam, in Kahramanmaras - Turkey.

Escherichia coli DM, *Micrococcus luteus* LA 2971, *Staphylococcus aureus* Cowan1, *Mycobacterium smegmatis* CCM 2067, *Pseudomonas aeruginosa* ATCC 9027, *Enterococcus faecalis* ATCC 15753, *Bacillus megaterium* NRS, *Streptococcus faecalis* DC 74, *Bacillus brevis* FMC 3 and *Bacillus subtilus* ATCC 6633 bacteria, and *Kluyveromyces fragilis* DC 98 and *Candida albicans* ATCC 10239 fungi were used in the study.

Screening plant extracts for their antimicrobial activity was conducted using the disc assay described by Bauer *et al.* (1966).

The collected parts of the species mentioned above were identified and broken into pieces, and aliquots (20 g) were separately extracted with ethyl alcohol, methanol, ethyl acetate, acetone solvents (150 ml) for 24 hr using soxhlet apparatus (Khan *et al*., 1988).

Most of the solvents of ethyl alcohol, methanol, ethyl acetate or acetone extracts were evaporated in vacuum at 30°C using a rotary evaporator until 1 ml. All the extracts thus obtained were injected into empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher and Schüll No: 2668, Germany) in amounts of 50 µl. Discs injected with pure ethyl alcohol, methanol, ethyl acetate, or acetone served as negative controls (Bradshaw, 1992; Collins *et al*., 1989). Standard antibiotic discs such as ampicillin (10 mcg) and nystatin (30 mcg) used for comparison and as positive controls were provided by the Microbiology Division of Medicine Faculty of Kahramanmaras Sutcu Imam University, in Kahramanmaras-Turkey. Spread plates were then kept at 4°C temperature for 2 hr to allow diffusion of extracts prior to incubation.

Preparation of micro-organism culture: All the bacteria mentioned above were incubated in Nutrient Broth (NB) (Difco) at 37±0.1°C for 24 hr, and the yeasts were incubated in sabouraud dextrose broth (SDB) (Difco) at 25±0.1°C for 24 hr. The bacteria and yeasts (prepared as above) were injected into petri dishes (9 cm) in amount of 0.01 ml (10^5-10^6 ml^{-1} for the bacteria and 10^5 ml^{-1} for the fungi) (NCCLS, 1997), 15 ml of mueller hinton agar (MHA, Oxoid) and sabouraud dextrose agar (SDA) (sterilized in a flask and cooled to 45-50°C) were homogenously distributed onto the sterilized petri dishes (Collins *et al*., 1989). Sterilized blank paper discs of 6 mm in diameter were saturated with extracts by micropipette per disc, then placed onto the agar plates which had previously been inoculated with the above organisms. The petri dishes were left at 4°C for 2 hr, and then the injected plates with bacteria were incubated at 37±0.1°C for 24 hr, plates inoculated with fungi were incubated at 25±0.1°C for 48 hr (Bradshaw, 1992; Collins *et al*.,1989). At the end of the period, inhibition zones were measured with a transparent ruler in millimeters (mm). These studies were performed in triplicate.

Table - 1: Element analysis results of barks, leaves, cones of pyramidal black pine in Vakifkoy

Samples	Element analysis results (%)			
	C	H	N	S
Barks	49.21	6.524	0.302	0.062
Leaves	51.18	6.906	0.836	0.117
Cones	52.24	6.463	0.601	0.038

C = Carbon, H = Hydrogen, N = Nitrogen, S = Sulphur

Results and Discussion

Being the subject of the research, the pyramidal black pine was described as a variety of the black pine in 1956 by Acatay. Pyramidal black pine, a variety of anatolian black pine, is one of the endemic plants in Turkey. Its scarce natural distribution area is limited to the province of Kutahya in the west of Turkey (Acatay,1956; Yaltirik,1986; Yucel,1995; Unaldi, 2004).

Neogene old limestone constitutes the rock bottom in natural distribution areas, and the plant expands on fractured calcareous rocks and stony brown forest soil and rendzina soil composed on these. Rich calcium carbonate content of the soil in the distribution areas is outstanding.

While anatolian black pine (*Pinus nigra* ssp. *pallasiana* var. *pallasiana*), and *Quercus cerris* as well as ebe black pine *(Pinus nigra* ssp. *pallasiana* var. *seneriana*), another endemic variety are seen predominantly on the site after 1,000 m altitude, the variety can be seen individually. The variety becomes more intense at a height between 1,100-1, 250 m, diminishing after 1,250 m height to disappear after 1,300 meters. Within this area, dominant species in forests are *Juniperus oxycedrus* and *J. communis*. Akkemik and Aras (2005) specified that black pine trees at lower altitudes in the Mediterranean region in Turkey have significantly positive response to precipitation. Turna *et al*. (2006) stated that anatolian black pine is attitudinal distribution generally ranges from 250 to 1550 m.

Data recorded by Tavsanli Meteorology Station show that annual average temperature in natural distribution site of the variety is 11°C. While average temperature in this place in the coldest month (January) is 0.9°C, the average for the hottest month is 20.8 °C. Annual temperature amplitude on the site is 20°C with continental transition thermal regime. Saatcioglu (1976), and Mayer and Aksoy

Table - 2: Antimicrobial activities of the extracts of pyramidal black pine

Microorganisms	Diameter of inhibition zone (mm)														
	Leaf				Cone				Bark				Standards		Con.
	a	b	c	d	a	b	c	d	a	b	c	d	Amp	Nst	a,b,c,d
Escherichia coli	10	10	10	15	13	16	13	16	15	16	14	18	10	NT	0
Micrococcus luteus	12	11	11	15	14	15	9	14	15	18	13	10	10	NT	0
Staphylococcus aureus	11	9	8	11	13	14	10	13	13	15	9	10	12	NT	0
Mycobacterium smegmatis	12	10	11	15	15	16	12	15	14	18	11	12	19	NT	0
Pseudomonas aeruginosa	15	7	7	7	16	17	15	15	15	17	12	10	10	NT	0
Enterococcus faecalis	12	11	11	14	15	14	8	13	14	15	11	10	16	NT	0
Bacillus megaterium	11	10	10	11	7	7	0	7	15	11	11	11	20	NT	0
Streptococcus faecalis	11	11	10	14	15	14	10	15	15	16	11	11	20	NT	0
Bacillus brevis	11	10	10	14	15	14	9	13	14	20	14	15	17	NT	0
Bacillus subtilus	11	11	11	16	15	13	8	12	13	14	15	12	17	NT	0
Kluyveromyces fragilis	0	0	0	0	7	7	0	0	0	0	0	0	NT	13	0
Candida albicans	16	20	20	21	21	20	19	20	18	20	18	19	NT	18	0

a = ethyl alcohol, b = methanol, c = ethyl acetate, d = acetone, Con. = Control, Amp = Ampicillin 10 mcg, Nst = Nystatin 30 mcg, Standards = Standard antibiotics, NT = Not tested

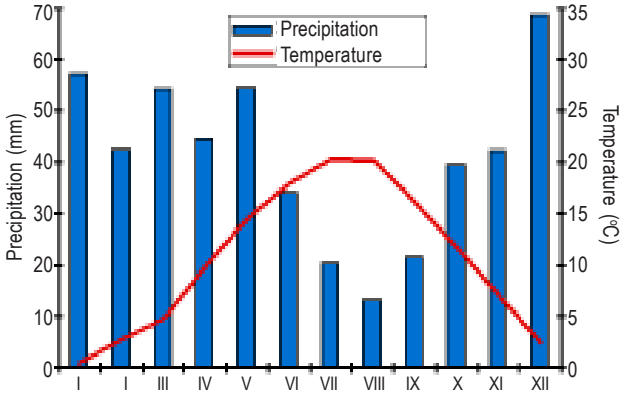

Fig. 1: Precipition and temperature of the study area

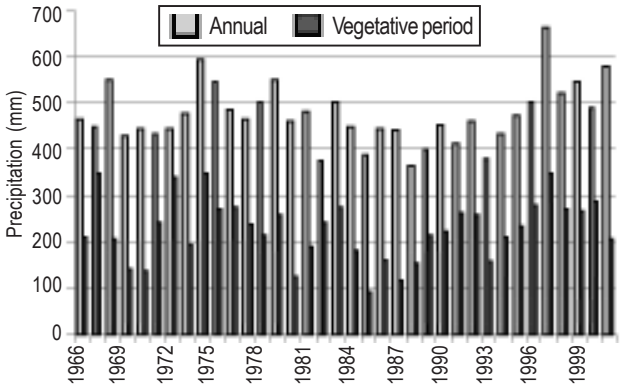

Fig. 2: Amounts of precipitation for the whole year and vegetative period in Tavsanli

(1998) stated that *Pinus nigra* is resistant to climatic extremes in the areas from very humid sites in high mountainous regions to semi dry sites in central Anatolia (Fig. 1).

Annual average precipitation on the site is 491 mm. There is an acute dry period of 4 months through the yearly precipitation graphic. Precipitation regime is under "Mediterranean – Central Anatolia Transition Type". Therefore fall starts to increase in October and is maximized in December and January; while it is relatively decreased in March. Fall increases again in May to a certain extent, and a considerable diminishing is recorded after June.

The site with indices value of 13.4 is located between semi arid and humid places according to De Martonne annual drought indices formula; and its flora is in pure steppe category. As for the Erinc method; the site is classified as semi-humid climate type with indices value of 26.5 and the flora is dry forest in park appearance.

Classified under Mediterranean phytogeographic region in terms of plant geography, this place was arranged as "nature preservation area" by Ministry of Forestry in 1993. Though Charitable Forest Nature Preservation Area has a surface of 685 hectares, the surface of the area with intense presence of the pyramidal black pine is only 292.5 hectares.

The general distribution of *P. nigra* is especially high mountainous zones of the Mediterranean basin (Specht *et al*., 1988). Its widest distribution in the world is in Turkey with approximately 2,527 and 675 hectares. Most parts of the natural distribution of this species in Turkey have a water deficiency during summer (Sevgi and Akkemik, 2007). According to the IPCC report (2001), drought risk is one of the most important issues in Europe, and the occurrence and frequency of hot days has increased during the 20th century. This situation can be considered as an important issue affecting both human health and natural ecosystems. Warming is an important issue to be considered in plantations of *P. nigra* (Sevgi and Akkemik, 2007).

It was observed during site studies held in 1998 that pyramidal black pines suffer from mistletoes (*Viscum album*), a parasite plant. Mistletoes preferentially parasitize trees and shrubs, and their greatest diversity is found in forests and woodlands (Calder, 1983; Hawksworth, 1983). They prefer disparate hosts in diverse biomes such as conifers (Hawksworth, 1983; Hawksworth and Wiens, 1996). Although mistletoes are known as parasitic plants on coniferous forest of Turkey, their control is still an important problem for the forest service (Yuksel *et al*., 2005). Mistletoes are entirely dependent on their hosts for water and nutrients and affect their hosts mainly by competing for the limited resources (Devkota, 2005). The extent to which the host is affected depends not only on how much of the resource is diverted by the parasite, but also on the overall supply available to the host (Graves, 1995). Mistletoe infection can also have a strong impact on the larger communities in which it occurs by altering forest structure and composition (Geils and Hawksworth, 2002).

As mistletoes suck inorganic nutrition elements of the plant through its absorptive stems, it weakens the plant and thus hinders its normal growth and sometimes dries it entirely. In addition, mistletoes lead to swelling of the branches and the body causing waste of these parts. Furthermore, decrease of seeds yield and lack of increase is seen on the tree. Branches start drying gradually from the tip to the stem (Yuksel *et al*., 2005). Unger (1992) points out that a severe mistletoe epidemic may lead to 32% loss of increase in forest floor. According to Dooling (1978), volume increase in trees intensely infected with mistletoes is diminished by 1/7 or 14%.

As a consequence of the manual protection carried out in 2001, mistletoes were collected with the help of workers and machinery. Although the clearing of the site from mistletoes can be considered as a positive development, partial drying was observed on the pyramidal black pines while some trees were entirely dried during site studies held in July 2002. As for this issue, although drying of the trees was explained with drought recorded in previous years by Institute of Forestry Research, it was later noted that precipitation amount for both the whole year and vegetation period (April-November) during 1966-2001 in Tavsanli was not low enough to allow drying of the trees. Moreover, falls recorded in the vegetation period within the last 35 years (25-80% of annual precipitation amount), we could observe that this amount did not drop below 35% in the last 15 years (Fig. 2-3).

Therefore, we need to explain the drying of the pyramidal black pines with other supportive ideas. The first probable reason is the ETI Silver Factory and the Seyitomer and Tuncbilek Thermal Plants, which are placed near the site. It was researched whether the mentioned factories had a drying effect on the trees of the variety. Results of the element analysis are presented in Table 1.

Carbon (C) value found as a result of the studies carried out on plants is around 43.6% and Hydrogen (H) value around

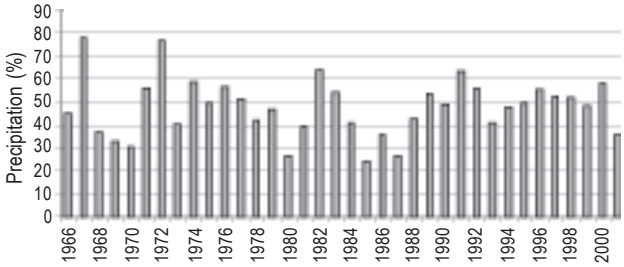

Fig. 3: Percent of precipitation in the vegetative period to annual precipitation amount in Tavsanli

6.2% (Bozcuk, 1986). C and H values are not low in terms of nutrition elements according to the analysis results carried out on healthy pine trees (Table 1). Nitrogen (N): N value from healthy (alive) individual trees is found between 0.30% - 0.83%. N values gained from healthy black pine trees are found between 0.9% - 1.7% (Irmak and Cepel, 1959; Irmak and Cepel, 1969; Wittich 1961). There is a significant difference between N values of pointers of healthy black pine trees. Thus it can be concluded that N values might have an effect on drying of the trees. As for the Sulfur (S); Sulfur must be addressed from the air pollution aspect. Sulfur amount up to 0.1% (1000 ppm) - 0.5% (5000 ppm) is said not to be dangerous according to various resources. Values below 0.15% are regarded as inadequate nutritive substance. Whereas there is not a significant difference between S values of healthy black pine pointers, it was found below normal values on barks and cones. It was seen that this element is not problematic for air pollution; however, it is low in nutritive substance content (Table 1).

However, it should be kept in mind that the damage was a result of a gradual process rather than an immediate one. The fact that mentioned damages were seen on the pyramidal black pines on the site supports the possibility that negative effects of Tuncbilek thermal plant for approximately 52 years (since 1956), Seyitomer Thermal Plant for 19 years (since 1989) and ETI silver plants for approximately 20 years since 1988 caused drying of the pine trees and that dry periods exacerbated this situation.

According to the Singh and Agrawal (2008), sulphur dioxide (SO_2) and oxides of nitrogen and ozone to some extent are the mainly causes of acid rain. These pollutants originate from human activities such as combustion of burnable waste, fossil fuels in thermal power plants and automobiles. These constituents interact with reactants present in the atmosphere and result into acid deposition (Singh and Agrawal, 2008).

According to the Makineci and Sevgi's study (2005), the effects of Seyitomer thermal power plant on annual ring widths of declined Austrian pine trees were investigated. This study found that mean monthly temperature and precipitation did not show significant differences in periods. However, mean annual ring widths,

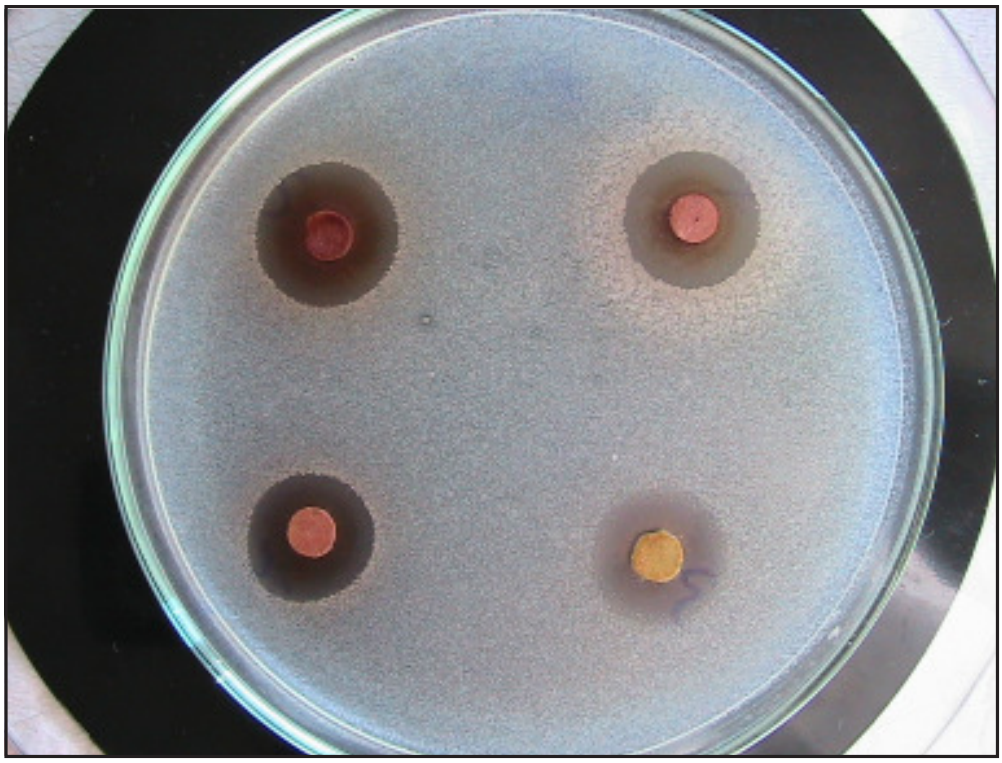

Fig. 4: The inhibition of *P. aeruginosa* growth by the ethyl alcohol, methanol, ethyl acetate and acetone extracts of the cones

and the relationship between mean annual ring widhts and climatic data in various periods were found to be significantly different. According to Yilmaz *et al*. (2004), the power plant create pollution problems and the care should be taken to minimize their negative environmental effects.

Furthermore, in this study, the microbial effects of pyramidal black pine were investigated. Table 2 depicts the antibacterial and antifungal activities of the ethyl alcohol, methanol, ethyl acetate and acetone extracts of the leaves, cones and barks of pyramidal black pine on various bacteria and fungi.

As shown in Table 2, all the extracts of the tree had antifungal effects against *Candida albicans*. But, the extracts of the parts of the tree had no antifungal effects against *Kluyveromyces fragilis*, except for ethyl alcohol and methanol extracts of cone. All the extracts of all the parts of the tree inhibited the growth of all the bacteria tested with zones of inhibition between 7-20 mm. Yet, the cone's ethyl acetate of this species did not inhibit the growth of *Bacillus megaterium*. These results are in good agreement with those found in other studies (Toroglu, 2007; Bagci and Digrak, 1996). Some differences to be observed in the results are thought to come from different chemical components in the plant species and bacteria studied. The inhibition of *Pseudomonas aeruginosa* growth by the ethyl alcohol, methanol, ethyl acetate and acetone extracts of the cones is illustrated in Fig. 4.

Global warming is one of the most important issues of the world in the near future. Bindi and Olesen (2003), Cherubini *et al.* (2003) and Korner (2003) pointed out that drought risk is the most important issue for Mediterranean basin and during the last decades, the warm air wave frequency has increased. Our study area is under the semi arid and humid conditions of the Mediterraean phytogeografic region, so the effect of global warming is not clear and probably this issue will not have an important effect on the trees around semi arid and humid regions in the near future.

However, (1) because felling age in *P. nigra* is more than 80 years in Turkey, forest planners should consider species and site selections in plantations (Sevgi and Akkemik, 2007),

(2) This situation results in narrowing of the species, even to the danger of extinction. This study agrees with the reports of Tan (1992), that the transfer of these plant genetic resources to future generations can only be possible by preserving them. In order to keep their existence it is essential to use consciously and cultivate medicinal and aromatic plants,

(3) The necessary studies for increasing the scientific studies about the mistletoe, discovering and testing alternative biological agents should be accelerated (Yuksel *et al*., 2005),

(4) *P. nigra* has rich biological resources such as antimicrobial agents. Recently, the obtaining of medicinal plants has shown a

remarkable increase in the use of drugs, spice and drink industry (Toroglu, 2007),

(5) Nature is the only source to exploit such a potential. Systematic screening of aromatic biodiversity often leads to the discovery of new leads with development potential. It is then either the work of agronomists to use them as sources of genetic material for the development of new crops or for improving existing ones, or chemists to synthesize (Baser, 2002),

(6) Turkish forests also have a global correlation. This rich and extensive resource, combined with a matchless geographical location, means that with global climate change, Turkey may have an important phytogeographical role, *e.g.* as a reservoir for species transfer both within Turkey and from Turkey to Europe. Their significance was recognised in the Helsinki Resolution (MCPFE, 1993; Colak and Rotherham, 2006).This proposes that it is important to select and protect key gene-pool reserves.

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