

## Effects of treatment with vermicompost on the some morphological and physiological characteristics of scots pine (*Pinus sylvestris* L.)

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### Abstract

In the present study, effects of vermicompost treatment tried to be determined on some morphological and physiological seedling quality characteristics of one – year Scots pines. Experiments were set according to random plots experimental design including 14 trials with 3 repetitions. With this aim, seeds obtained from seven different sites (origins) of mixed Scots pine stands which naturally grow in the Western Black Sea Region of Turkey were used in the experiments. At the end of the vegetation period, important physiologic and morphologic parameters of seedlings in the plots, SH, RCD, TDW, RDW and total N rate were detected. After that, RI, V and QI rates, each of which is also important rational indicator in seedlings for height, root collar diameter and weight balance, were calculated with the help of morphological data. Effects of VC treatment were found to be statistically significant on all the development parameters measured in seedlings in seven origin groups. It was determined in all morphologic parameters that the best development rate was observed in Goktepe originating seedlings in both VC treatment and control groups while Geyikgolu originating seedlings showed the least development performance. It was observed from the correlation analysis that there is a positive relation between morphologic and physiologic quality criteria. It was also determined according to the results of multivariable regression analysis that elevation of the sites where seedlings were picked up was more effective on the development of seedling development than the aspect of the sites. Results of the study were found to be convenient with the related literature and showed that VC treatment contributed positively to the development of Scots pine seedlings taken from seven different origins.

**Keywords:** Vermicompost, Scots pine, seedling height, root collar diameter, ANOVA, Kruskal-Wallis H test, nursery.

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### Introduction

Rapid and consistent increase in petrol price also increases the input cost in several items of agriculture sector as it does in other sectors. One of the most effective ways of reducing production cost without ignoring crop yield and quality is the preference of concentrated basic and additive materials throughout the

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production process. Therefore, in agriculture sector, effectively functional and light materials occupying less place have gained importance. Use of such materials in farms may decrease production costs resulting from both storage and transportation depending on the size and capacity of farms.

Sustainable agriculture can be defined as the entire of the agricultural production activities performed in order to carry on meeting human needs and the balance between organisms and their environment to protect natural resources. Manure fertilizers are the alternative to synthetic (mineral) fertilizers for better plant development, crop yield and quality in the frame of aforementioned use and protection balance as they are environment friendly natural materials. Such fertilizers as manure based ones are considerably valuable sources for farms since they are cost effective, natural and rich in macro and micro elements. Fertilizing is among the most important and prevalent operations in today's farms in order to ensure increased crop yield and quality. However, manure based fertilizers began to be abandoned due to their increased transportation cost and environmental pollution caused by its distorted and untimely use (Tilman et al., 2002; Hutchison et al., 2005).

Vermicompost fertilizers are increasingly used in more and more agricultural fields and for a consistently increasing number of plant species due to its easiness to apply and concentrated structure on the contrary to the high cost manure due to its difficult storage and transportation conditions resulting from its large volume and weigh in a farm.

Composting can be applied to waste materials, removing their hazards or risks to soil (Lazcano et al., 2008). Processed materials can also be transported more easily than their wet, raw form (Lazcano and Dominguez, 2011). Composted materials have begun to be accepted as important organic fertilisers for sustainable agriculture practices because of both their benefits to soil structure and their low cost. Because these materials can offer several advantages, there have been a number of studies related to their structures and their effects on soil properties and plant growth (Atik, 2013a).

An important component of sustainable organic agriculture is vermicompost fertilisers, which have been used in many countries. Vermicompost is rich in plant nutrients. Vermicompost is a microbiologically active organic material formed from the interactions between earthworms and microorganisms during the decomposition of organic material (Domínguez, 2004).

Vermicompost and compost fertilisers have very different fungal and bacterial compositions, causing different effects on the morphological and physiological development parameters of different species on different growth media. Although several studies have reported favourable effects of vermicompost on plant growth and crop yield, various studies have found that these effects are not stable and that they may even cause plant losses in addition to decreasing plant growth and crop yield (Roberts et al., 2007; Lazcano et al., 2010a). Different earthworm species and feedstock types can cause differences in production date, technique, and physical, chemical and biological properties (Rodda et al., 2006; Roberts et al., 2007; Warman and AngLopez, 2010; Lazcano and Dominguez, 2011).

Edwards et al. (2004) and Lazcano et al. (2009) reported that vermicompost showed positive effects on root and shoot development and leaf area in vegetative propagation. There have been studies on the effects of vermicompost on the seed germination and development of ornamental plants, such as amaranthus (Dönmez and Allahverdi, 2007), petunias (Arancon et al., 2008), and chrysanthemum (Hidalgo and Harkess, 2002), and of park and forest plants, such as Anatolian black pine (Atik et al., 2009), oriental beech (Atik, 2013a), maritime pine (Lazcano et al., 2010b, 2010c), acacia and eucalyptus (Donald and Visser, 1989).

To scientifically define vermicompost fertiliser as an alternative type of organic fertiliser, more studies related to its favourable effects on plant growth, crop yield, and quality are needed, although there are already a considerable number of studies on the effects of compost fertilisers on plant growth and soil (Vivas et al., 2009; Lazcano et al., 2008; Lazcano and Domínguez, 2011).

In the present study, the effects of vermicompost fertilizer were investigated on some morphological and physiological quality characteristics in the production of forest tree seedlings. With this aim, experiment plots were designed in nursery using Scotch pine seeds from 7 different origins.

Scotch pine is the geographically most prevalent pine species all over the world. It has a wide distribution range through Europe to Asia. It survives with less than 100 frost-free days yr<sup>-1</sup> and 300 mm yr<sup>-1</sup> average rainfall at the boreal forest limit (Matyas et al., 2004). The southernmost prevalence edge of this species as natural stand in the world is Pınarbaşı zone, Turkey (38° 34' N; Kurt and Işık, 2012). About 60 million yr<sup>-1</sup>

Scotch pine seedlings are produced in forest nurseries in various parts of Turkey ([General Directorate of Forestry, 2013](#)).

The aims of this article can be summarized in the following points:

1. The effects of vermicompost fertilizer on some morphological and physiological quality characteristics of scotch pine (*Pinus sylvestris* L.) seedlings.
2. The effects of origins on the quality characteristics mentioned above.
3. The degree of the relationship between morphological and physiological quality characteristics of seedlings.

## Material and Methods

### The study area

Scots pine (*Pinus sylvestris* L.) grown in the Kozcağız Temporary Nursery were used in the present study. Kozcağız Temporary Forest Nursery is in the town centre of Kozcağız (X:445335.91, Y:4592076.62), which is in the Western Black Sea region of Turkey. The nursery is located 20 km from the Bartın city centre and is at an elevation of 75 m. It has a western general aspect (Figure 1).

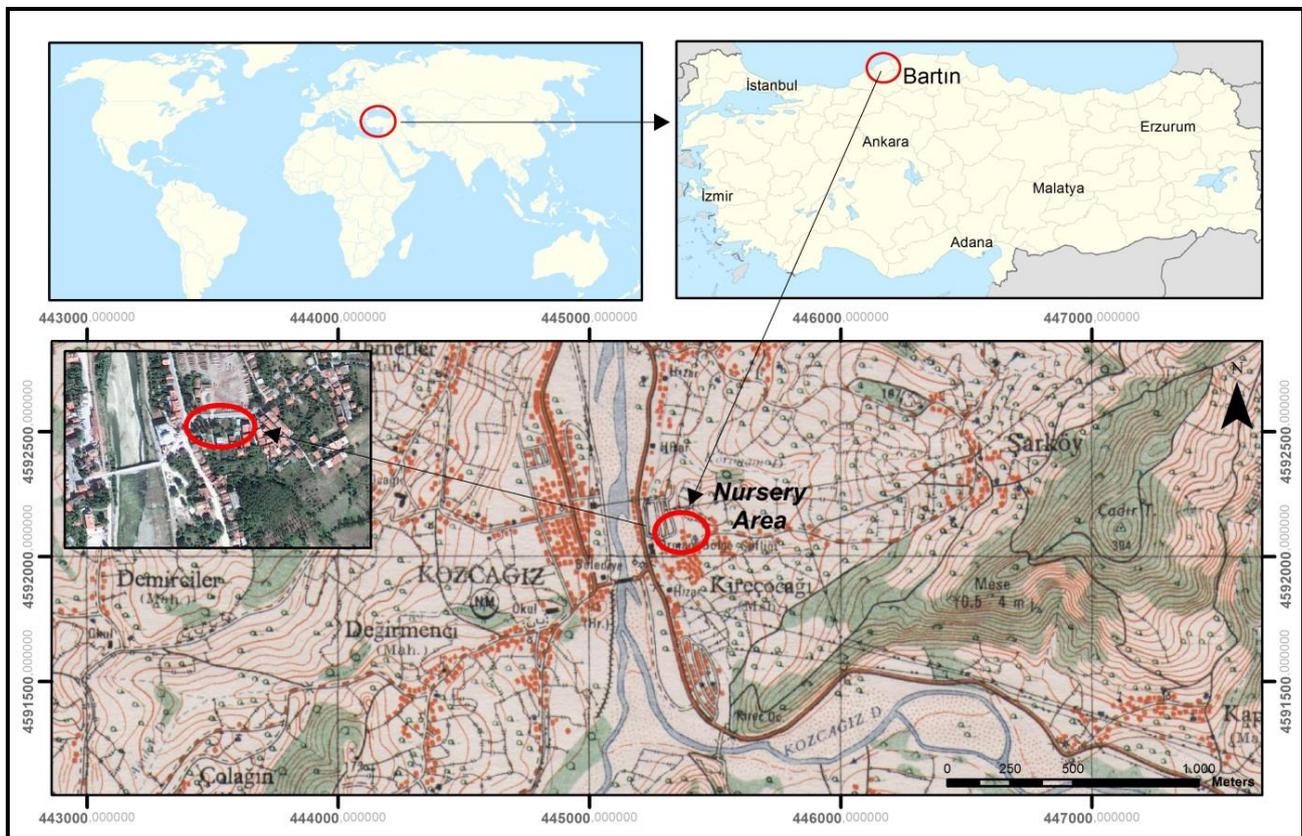


Figure 1. Location of Kozcağız Temporary Forest Nursery

According to climate data measured by the Bartın Meteorology Station, which is at 33 m from mean sea level, the mean annual, minimum and maximum temperatures are 12.6°C, 0.3°C and 18.8°C, respectively, and the annual rainfall is 1035 mm ([Turkish State Meteorological Service, 2011](#)). The vegetation period in the area is between April (11.1°C) and October (13.6°C; seven months total). The precipitation during the vegetation period is 527 mm. According to the Thorntwaite method, the area has a B<sub>2</sub>B<sub>1</sub>'rb4' climate, which means that it is similar to an oceanic climate and is mesothermal with few or no water shortages ([Atik, 2013b](#)).

The nursery was established on a plain, and the soil is a medium heavy texture, such as sandy clay, with a pale brown dark colour. The nursery's total sand level is 70.6%, while the clay level is 12.5% and the dust level is 16.9%. The soil pH is 8.0, and it is semi-alkali. The lime rate in the soil is 4.18%; therefore, it is not rich in lime. The organic substance rate of the soil is 5.05%.

### Properties of seed origin used in the study

Seeds of Scotch pine used in the study were harvested one year before the seeding and geographical location and characteristics of the harvesting areas are presented in Table 1 and Figure 2. Seeds from 7 different origins are used in the study. Seeds originating from Kurucasile and Geyikgolu were harvested from the least and the most elevated areas in the study at 490 and 1620 m, respectively, which makes an elevation difference of 1130 m.

Table 1. Origin, location, type of treatment and label of group.

| No | Origin     | Geographic location | Elevation (m) | Aspect    | Type of treatment | Label of group |
|----|------------|---------------------|---------------|-----------|-------------------|----------------|
| 1  | Ardıc      | X: 470074           | 1320          | South     | Control           | ARDCTRL        |
|    |            | Y: 4581648          |               |           | Vermicompost      | ARDVC          |
| 2  | Caldere    | X: 397783           | 1220          | Northwest | Control           | CALCTRL        |
|    |            | Y: 4529677          |               |           | Vermicompost      | CALVC          |
| 3  | Geyikgolu  | X: 439351           | 1620          | Northeast | Control           | GEYCTRL        |
|    |            | Y: 4525892          |               |           | Vermicompost      | GEYVC          |
| 4  | Goktepe    | X: 428015           | 820           | East      | Control           | GOKCTRL        |
|    |            | Y: 4561950          |               |           | Vermicompost      | GOKVC          |
| 5  | Kumluca    | X: 456924           | 1180          | North     | Control           | KUMCTRL        |
|    |            | Y: 4579890          |               |           | Vermicompost      | KUMVC          |
| 6  | Kurucasile | X: 479064           | 490           | West      | Control           | KURCTRL        |
|    |            | Y: 4625490          |               |           | Vermicompost      | KURVC          |
| 7  | Soku       | X: 464027           | 1200          | Northwest | Control           | SOKCTRL        |
|    |            | Y: 4581309          |               |           | Vermicompost      | SOKVC          |

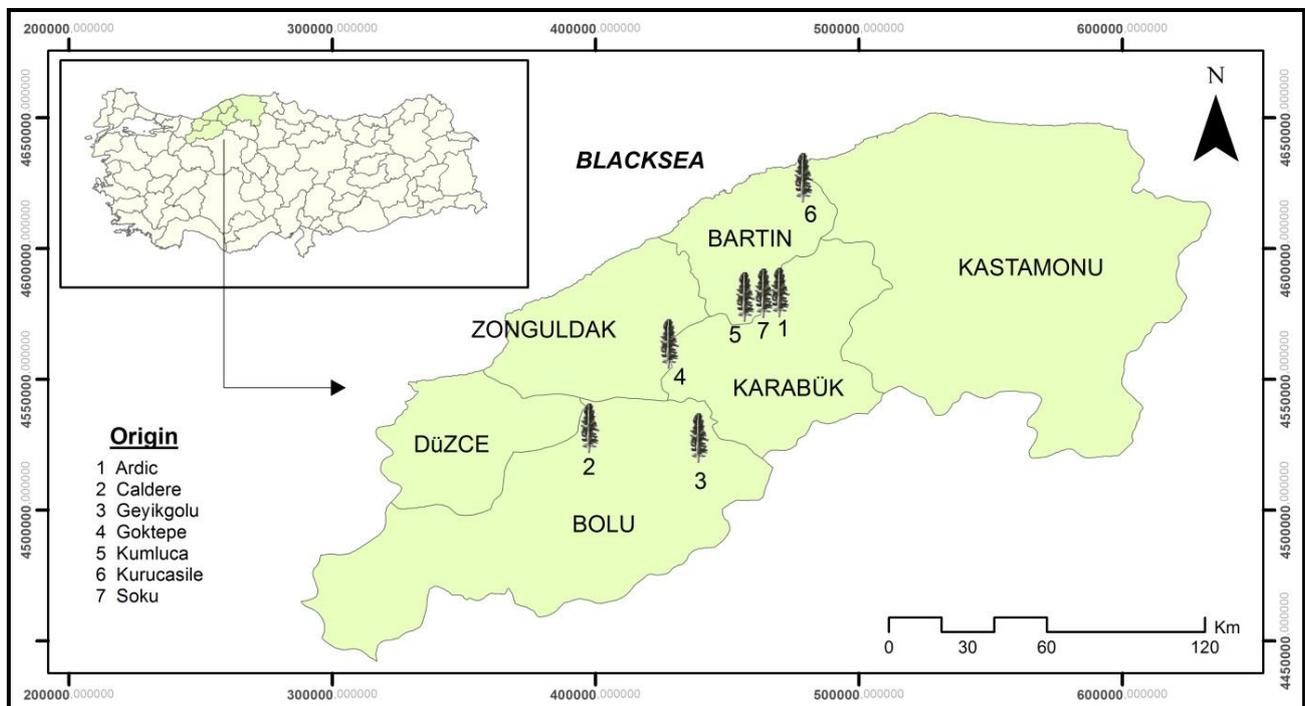


Figure 2. Location of seed harvesting areas.

### Properties of vermicompost used in the study

The employed vermicompost is called “Biyohumus®”, which is trademarked and developed by the Russian Agriculture Academy Agrochemistry Research Institution.

This vermicompost is a result of the bioprocessing of livestock and poultry farming waste by the red Californian worm (*Eisenia fetida*) (Ammosova et al., 1996; Shapoval, 2003). The average chemical composition of the vermicompost is given in Table 2.

Table 2. Average chemical composition of the vermicompost (Biohumus®).

| Component   | Quantity  |
|---|---|
| Humic acids   | 6-18%   |
| pH  | 6.5-7.2%  |
| Total nitrogen (A)  | 0.9-3%  |
| Phosphor (P)  | 0.9-2.5%  |
| Potassium (K)   | 0.6-2.5%  |
| Calcium (Ca)  | 4.5-8%  |
| Magnesium (Mg)  | 0.5-2.3%  |
| Iron (Fe)   | 0.5-2.5%  |
| Copper (Cu)   | 3.5-5.1 mg kg <sup>-1</sup>                       |
| Manganese (Mn)  | 60-80 mg kg <sup>-1</sup>                         |
| Zinc (Zn)   | 28-35 mg kg <sup>-1</sup>                         |
| Bacterial flora   | up to 20,000 billion colonies per 1 g of biohumus |
| Moisture  | 30-50%  |
| Dry organic substances (close to soil humus by the composition) | 30-70%  |

### Seeding and pre-seeding processes

Seeds were stored in a solution containing 40% sodium hypochlorite for 30 minutes, superficially sterilised, and washed using purified water to remove remnants of sodium hypochlorite. The seeds were incubated in the dark at 22°C in an aqueous solution of vermicompost (100 ml in 10 l of water - 10 ml l<sup>-1</sup>) for 18 h. The control seeds were incubated in distilled water for the same period of time, and all of the seeds were then planted in seedbeds. Two extended seeding plots were prepared by creating a 50-cm deep ditch between the treatment and control groups. Seeds were sown at a 2 cm gaps in the beginning of March. The experimental design consisted of random plots with three replicates. The seeds were covered with a mixture of saw dust, wood chips and silt and pressed using a sowing cylinder.

Plots that were selected to be in the treatment group were irrigated in the middle of each month during the vegetation season from April to October by spraying an aqueous solution of vermicompost (10 ml l<sup>-1</sup>), which was prepared using distilled water. Each seedling received 10 ml of solution. Weed control and normal irrigation were performed following routine nursery practices and in a balanced manner among plots.

### Data collection and analyses

At the end of the vegetation period in the Kozcağız Temporary Nursery, two 1 aged Scots pine seedlings were extracted from each of the five lines in a plot for a total of 30 seedlings. After extraction, seedling roots were pruned to a length of 15 cm and washed to remove soil remnants. Morphological measurements were conducted over 30 seedlings for every treatment type while physiological ones were performed using 3 seedlings in different repetition plots for each treatment type.

The morphological characteristics measured were the seedling height (SH), root collar diameter (RCD), trunk dry weight (TDW), and root dry weight (RDW). These characteristics are widely used to assess seedling quality (Zatylny and St-Pierre, 2006; Güner et al. 2008, Gülcü & Uysal 2010, Deligöz, 2012, Atik, 2013a; b). The robustness index (RI=SH/RCD), volume (V=TDW /RDW) and quality index (QI= SDW /RI+V) were calculated using the values obtained from the abovementioned parameters.

Seedling height was measured from the root collar to where the terminal bud connects to the bole at ±1 mm, and the root collar diameter was measured to ±0.01 mm. The root and trunk dry weights were measured by drying and storing seedlings at 105°C for 24 hours by weighing to ±0.001 g. As a growth limiting nutrient, N is important in both agricultural and wild ecosystems. The most of the terrestrial plant species have been shown to produce more biomass greater quantities of nitrogen, when growing on one N source compared with another, i.e. they appear to display a preference. (Tilman, 1985; Vitousek and Howarth, 1991; Britto and Kronzucker, 2013),

Nitrogen plays vitally important roles in growth and developments of plants since it takes place in the structures of proteins. Nitrogen, the constituent of plant proteins, amino acids, nucleic acids, enzymes, ATP, ADP and chlorophyll, is also required for the formation of new cells to form (Bozcuk, 1986). Therefore, total rate of nitrogen in plant was taken into account as physiological characterised seedling quality criteria. Kjeldahl Method (Bremner, 1965) was used for the determination of total nitrogen rate in plant material and this rate, calculated to be in the unit of mg g<sup>-1</sup> in dry matter, was considered to be the percentage of dry weigh. First, relative value parameters not showing normal distribution were log transformed (log<sub>10</sub>(x)), and numeric value parameters were square root transformed ( $\sqrt{x}$ ). Then, the effects of vermicompost treatment on the measured parametrically parameters in the origin groups were determined using a Student T test. The effects of vermicompost treatment on the measured parametrically parameters among the origin groups were determined using an analysis of variance (ANOVA). In cases of significant differences in the ANOVA, related groupings were determined using the Duncan test.

The effects of vermicompost treatment on the measured non parametric parameter (total nitrogen content) in the origin groups were determined using a Mann-Whitney U test. The effects of vermicompost treatment on the measured non parametric parameter among the origin groups were determined using a Kruskal-Wallis H test. In cases of significant differences in the Kruskal Wallis H test, related groupings were determined using the Dunnett T3 test (Wilcox, 1987). The significance level and direction of the relationship between morphological and physiological parameters were determined using correlation analysis. Relationship between elevation and aspect, one of the geographical characteristics of the harvest areas, and seedling growth was determined using multi-regression analysis. The ArcGIS 10, MS Office and SPSS Inc. (2003) Statistics 18.0 software packages were used to process, transfer and statistically analyse the data.

## Results

Table 3 represents some morphologic and physiologic quality characteristics of one- year Scots pine seedlings and the results of statistical assessment for VC treatments in origin groups and the groups, code, unit and mean values of treatment types Table 3.

Table 3. Groups, average values of data and results of Student t and Mann-Whitney U test.

| Origin     | Group   | Morphological Parameters |                   |                   |                   |                             |     | Physiological Parameter |                   |
|------------|---------|--------------------------|-------------------|-------------------|-------------------|-----------------------------|-----|-------------------------|-------------------|
|            |         | Based on the measurement |                   |                   |                   | On developing computational |     | Total N (%)             |                   |
|            |         | SH (cm)                  | RCD (mm)          | TDW (g)           | RDW (g)           | RI                          | V   |                         |                   |
| Ardic      | ARDCTRL | 13.2 <sup>a</sup>        | 4.2 <sup>b</sup>  | 1.9 <sup>c</sup>  | 0.7 <sup>d</sup>  | 3.1                         | 2.7 | 0.4                     | 0.83 <sup>a</sup> |
|            | ARDVC   | 15.6 <sup>a</sup>        | 5.2 <sup>b</sup>  | 2.4 <sup>c</sup>  | 0.9 <sup>d</sup>  | 3.0                         | 2.7 | 0.6                     | 1.32 <sup>a</sup> |
| Caldere    | CALCTRL | 9.5 <sup>e</sup>         | 3.0 <sup>f</sup>  | 1.4 <sup>g</sup>  | 0.5 <sup>h</sup>  | 3.2                         | 2.8 | 0.3                     | 0.82 <sup>b</sup> |
|            | CALVC   | 14.6 <sup>e</sup>        | 4.9 <sup>f</sup>  | 2.3 <sup>g</sup>  | 0.8 <sup>h</sup>  | 3.0                         | 2.9 | 0.5                     | 1.29 <sup>b</sup> |
| Geyikgolu  | GEYCTRL | 8.0 <sup>i</sup>         | 2.5 <sup>j</sup>  | 1.2 <sup>k</sup>  | 0.4 <sup>l</sup>  | 3.2                         | 3.0 | 0.3                     | 0.76 <sup>a</sup> |
|            | GEYVC   | 9.9 <sup>i</sup>         | 3.4 <sup>j</sup>  | 1.6 <sup>k</sup>  | 0.6 <sup>l</sup>  | 2.9                         | 2.7 | 0.4                     | 1.17 <sup>a</sup> |
| Goktepe    | GOKCTRL | 12.3 <sup>m</sup>        | 3.9 <sup>n</sup>  | 1.8 <sup>o</sup>  | 0.6 <sup>p</sup>  | 3.2                         | 3.0 | 0.4                     | 0.91 <sup>a</sup> |
|            | GOKVC   | 16.8 <sup>m</sup>        | 5.6 <sup>n</sup>  | 2.6 <sup>o</sup>  | 1.0 <sup>p</sup>  | 3.0                         | 2.6 | 0.6                     | 1.39 <sup>a</sup> |
| Kumluca    | KUMCTRL | 12.2 <sup>r</sup>        | 3.9 <sup>s</sup>  | 1.7 <sup>t</sup>  | 0.6 <sup>u</sup>  | 3.1                         | 2.8 | 0.4                     | 0.86 <sup>a</sup> |
|            | KUMVC   | 16.5 <sup>r</sup>        | 5.5 <sup>s</sup>  | 2.6 <sup>t</sup>  | 0.9 <sup>u</sup>  | 3.0                         | 2.9 | 0.6                     | 1.37 <sup>a</sup> |
| Kurucasile | KURCTRL | 12.1 <sup>v</sup>        | 3.9 <sup>y</sup>  | 1.7 <sup>z</sup>  | 0.6 <sup>aa</sup> | 3.1                         | 2.8 | 0.4                     | 0.85 <sup>b</sup> |
|            | KURVC   | 16.3 <sup>v</sup>        | 5.5 <sup>y</sup>  | 2.5 <sup>z</sup>  | 0.9 <sup>aa</sup> | 3.0                         | 2.8 | 0.6                     | 1.34 <sup>b</sup> |
| Soku       | SOKCTRL | 12.2 <sup>ab</sup>       | 3.9 <sup>ac</sup> | 1.8 <sup>ad</sup> | 0.6 <sup>ae</sup> | 3.1                         | 3.0 | 0.4                     | 0.90 <sup>a</sup> |
|            | SOKVC   | 16.6 <sup>ab</sup>       | 5.5 <sup>ac</sup> | 2.6 <sup>ad</sup> | 0.9 <sup>ae</sup> | 3.0                         | 2.9 | 0.6                     | 1.40 <sup>a</sup> |

**Student t test for SH, RCD, TDW and RDW :** <sup>a</sup>:t=-11.769, p<0.05; <sup>b</sup>:t=-13.097, p<0.05; <sup>c</sup>:t=-12.324, p<0.05; <sup>d</sup>:t=-13.353, p<0.05; <sup>e</sup>:t=-17.456, p<0.05; <sup>f</sup>:t=-19.962, p<0.05; <sup>g</sup>:t=-20.669, p<0.05; <sup>h</sup>:t=-21.077, p<0.05; <sup>i</sup>:t=-6.908, p<0.05; <sup>j</sup>:t=-8.890, p<0.05; <sup>k</sup>:t=-10.503, p<0.05; <sup>l</sup>:t=-10.066, p<0.05; <sup>m</sup>:t=-20.211, p<0.05; <sup>n</sup>:t=-22.037, p<0.05; <sup>o</sup>:t=-21.284, p<0.05; <sup>p</sup>:t=-18.174, p<0.05; <sup>r</sup>:t=-33.749, p<0.05; <sup>s</sup>:t=-25.646, p<0.05; <sup>t</sup>:t=-29.454, p<0.05; <sup>u</sup>:t=-25.987, p<0.05; <sup>v</sup>:t=-35.375, p<0.05; <sup>y</sup>:t=-29.130, p<0.05; <sup>z</sup>:t=-27.581, p<0.05; <sup>aa</sup>:t=-23.512, p<0.05; <sup>ab</sup>:t=-22.623, p<0.05; <sup>ac</sup>:t=-22.407, p<0.05; <sup>ad</sup>:t=-20.678, p<0.05; <sup>ae</sup>:t=-22.815, p<0.05;

**Mann-Whitney U test for Total N :** <sup>a</sup>:Z=-1.964, p<0.05; <sup>b</sup>:Z=-1.993, p<0.05

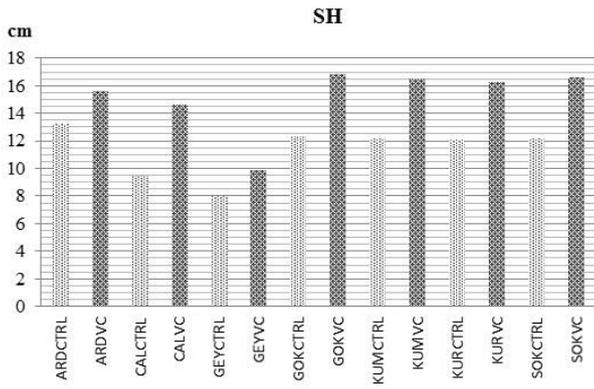


Figure 3. Mean SH values for treatment types

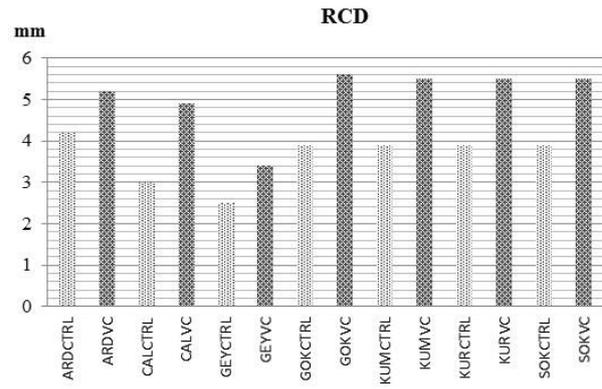


Figure 4. Mean RCD values for treatment types

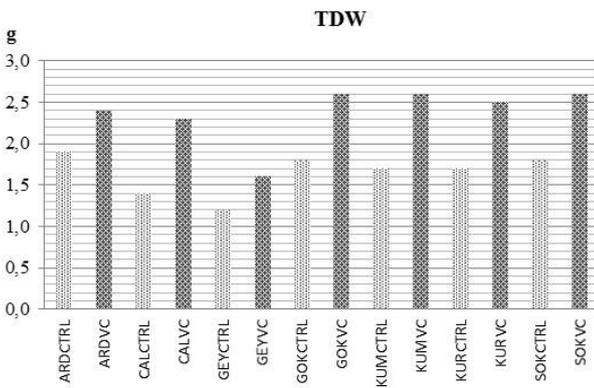


Figure 5. Mean TDW values for treatment types

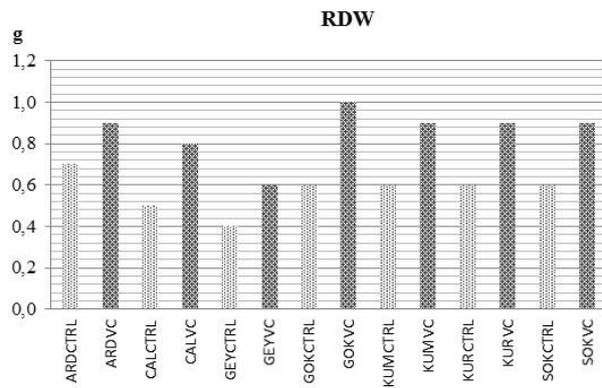


Figure 6. Mean RDW values for treatment types

According to Table 3, for all 7 origins, differences between VC treatment types and control are statistically significant for SH, RCD, TDW and RDW values. From this result, VC was found to be effective on the development of seedling in one – year Scotch pine seedlings growing under outdoor conditions due to its effects on morphologic parameters mentioned above. It may be stated that such effect of VC is valid for all of 7 different origins. It was also found that VC treatment was effective on the total rate of nitrogen as it is in morphological parameters.

RI ratio is important for it can represent the ratio of height to diameter of plant. It has been reported that seedlings with a SH/RCD ratio are more resistant to the stresses resulting from snow and living cover and that their development rate is greater after planting (Burdett, 1983; Burdett et al., 1983). Aldhous (1975) stated that this ratio should be lower than 6.0. Keskin (1992) determined that planting density can affect the root collar diameter, seedling dry weight, the number of secondary branches and secondary roots, but not the seedling height and root to trunk ratio in red pine (*Pinus brutia* Ten.). According to data obtained from SH and RCD measurements, RI ratio ranged for each treatment type from 2.9 to 3.2. Scots pine seedlings in temporary forest nursery of Kozcağız were found to be balanced in terms of SH and RCD rates in 14 treatment types including 7 different seed origins. The V rate is especially effective at expressing the impact of the water stress on the seedling; in other words, it expresses the physiological state of the seedling. When seedlings have a maximum V rate of 3, their tolerance to aridity is higher because they can regain water lost through transpiration. Therefore, the use of seedlings with a maximum V rate of 3 is suggested for plantings in arid regions (Eyüboğlu, 1979). Seedlings with extended root systems have a greater percentage of live weight because they have a larger number of roots. In addition, seedlings with hairy roots can quickly produce new roots immediately after planting, which can increase the adaptation capability of seedlings (South and Mitchell, 1999). V rates were found to be higher in VC treated groups in a study by Atik (2013a), where the effects of VC treatment and seeding density were investigated on the morphological characteristics of oriental beech seedlings, however, this rate was observed to reduce with increasing growth density.

It was found from the results obtained from TDW and RDW measurements that V rate changed in all treatment types from 2.6 to 3.0. It may be stated from this finding that VC treatment and origin variety do not have any effects on V rate in Scots pine seedlings. It was stated in a seedling quality classification study by Özpay and Tosun (1993) using oriental beeches in different ages that QI increased with seedling age while Aslan (1986) reported that seedling quality increased with QI closing to 1. In the present study, QI ranged between 0.3 and 0.6 in all treatment types, generally in the same origin and in the VC treated groups this rate was found to be higher. Therefore, VC treatment contributed positively to QI value. In the same way, Atik (2013a) reported that VC treatment in one – year oriental beech seedlings increased QI while seeding density had negative impact on QI. Table 4 represents the results of ANOVA, Kruskal-Wallis H, Duncan and Dunnett T3 statistical tests related to the effects of VC treatment on seedling growth characteristics considering 14 different treatment types including 7 origins.

Table 4. Results of statistical analysis of the effects of VC treatment on seedling growth characteristics.

| Group   | Homogeneous groups |          |          |          | Total N           |
|---------|--------------------|----------|----------|----------|-------------------|
|         | SH                 | RCD      | TDW      | RDW      |                   |
| ARDCTRL | E                  | D        | C        | E        | b                 |
| ARDVC   | C                  | B        | B        | C        | a                 |
| CALCTRL | H                  | G        | F        | H        | b                 |
| CALVC   | D                  | C        | B        | D        | a                 |
| GEYCTRL | I                  | H        | G        | I        | b                 |
| GEYVC   | G                  | F        | E        | G        | a                 |
| GOKCTRL | F                  | D        | D        | FG       | b                 |
| GOKVC   | A                  | A        | A        | A        | a                 |
| KUMCTRL | F                  | D        | D        | FG       | b                 |
| KUMVC   | AB                 | A        | A        | AB       | a                 |
| KURCTRL | F                  | D        | D        | FG       | b                 |
| KURVC   | B                  | A        | A        | B        | a                 |
| SOKCTRL | F                  | D        | D        | EF       | b                 |
| SOKVC   | AB                 | A        | A        | AB       | a                 |
|         | <b>F ratio</b>     |          |          |          | <b>Chi Square</b> |
|         | 357.810*           | 341.702* | 326.724* | 274.258* | 39.850**          |

ANOVA \* :  $p=0.000$ ;  $p<0.05$

Duncan test (A-I) : Same letters in each column show homogenous groups ( $p<0.05$ )

Kruskal-Wallis H test \*\* :  $p=0.000$ ;  $p<0.05$

Dunnett T3 test (a-b) : Same letters in each column show homogenous groups ( $p<0.05$ )

Morphological and physiological development differences in Scotch pine seedlings as the result of VC treatment between treatment types are statistically significant for all parameters. According to the result of Duncan test, totally 9 homogenous groups were categorised in SH. KUMVC and SOKVC treatment types constituted Group A by combining with GOKVC and Group B by combining with KURVC. ARDVC and CALVC constituted Group C and D alone. The first four groups including Group D are among the VC treated groups while this rule was not valid for only GEYVC treatment which constituted Group G alone. In addition, seedlings in GEYCTRL treatment type were included in Group I by representing the lowest level of increase in height and the ninth and the last group. GEYVC and GEYCTRL treatment types were constituted using seeds harvested from the area at 1620 m. Elevation difference between Kozcağız Temporary Nursery and origin groups is the largest for Geyikgolu origin group. This large elevation difference is thought to be effective on the fact that seedlings in GEYCTRL and GEYVC treatment types show a slower development trend compared to those in other treatment types according to SH assessment at the end of a vegetation period. It is Geyikgolu origin group where vegetation season begins the latest and lasts the earliest (the shortest growing season) due to its highest elevation among origin groups. Growth is thought to start later and end earlier by causing less height increases depending on growth season time scale taking place in genetic codes of the seedlings in this group. Seedlings in GOKVC treatment type represent the largest RCD development as in height. Treatment types were categorised into 8 homogenous groups according to mean RCD values in seedlings. The types of GOKVC, KUMVC, KURVC and SOKVC, all of which were treated with VC

constituted Group A and ARDVC, CALVC and ARDCTRL formed alone B, C and D groups while KURCTRL, KUMCTRL, SOKCTRL and GOKCTRL altogether formed Group E and GEYVC, CALCTRL and GEYCTRL types alone formed F, G and H groups alone.

In general, RCD development showed similarities with SH. However, in RCD development, KUMVC, SOKVC, GOKVC and KURVC treatments were collected under only one homogenous Group A, where mean RCD development in seedlings was the largest. As can be understood from homogenous groups above, treatment type groups treated with VC constitute homogenous distribution groups as the treatment types where RCD development was the largest. Treatment types were categorised into 7 homogenous groups according to TDW rates. Group A was constituted with the combination of KURVC, SOKVC, KUMVC and GOKVC treatment types and this group is where mean TDW rate is the largest. CALVC and ARDVC treatments took place in Group B. Totally, 6 of 7 VC treated treatment types constituted the first two homogenous groups with the largest TDW rate. Only GEYVC treatment type constituted Group E by taking place behind control groups of Ardic, Goktepe, Kumluca, Kurucasile and Soku origins according to TDW rate. Treatments obtained as the result of mean RDW values at the end of 3 repetitions took place in 9 homogenous groups. GOKVC treatment type having the largest mean RDW value constituted Group A by combining with SOKVC and KUMVC treatments. Group B was formed by the combination of SOKVC, KUMVC and KURVC while ARDVC and CALVC constituted homogenous Groups C and D alone. The largest mean RDW development rate was obtained in treatment groups composed of VC treated seedlings except for GEYVC treatment type.

Nitrogen is accepted to be a vitally important element for plant growth and development since it can take place in protein structure (Bozcuk, 1986). Total N rate in plant and its differences between the treatment groups were evaluated statistically using related data and Kruskal-Wallis H test. Differences in total N rate in seedlings in treatment groups were found to be statistically significant ( $\chi^2=39.850$ ;  $p<0.05$ ). Homogenous groups categorized based on total N rate in seedlings according to Dunnett T3 test and the distribution of treatments for these groups are presented in Table 4. Seedlings were divided into two homogenous groups according to test results related to total N rate; VC treated ARDVC, CALVC, GEYVC, GOKVC, KUMVC, KURVC and SOKVC types took place in Group A while control groups ARDCTRL, CALCTRL, GEYCTRL, GOKCTRL, KUMCTRL, KURCTRL, SOKCTRL were in Group B. The results of the correlation analysis for determining the interaction relationship between physiological and morphological measurements are given in Table 5. It was determined from the results of the analysis that there was a strong positive relationship between all morphological characteristics ( $r\geq 0.90$ ). A strong positive relationship was found between Total N and TDW, while there is moderately strong and positive relationship between Total N and other morphological measurements (SH, RCD and RDW). These results are similar to those found by Dirik (1993) and Coşgun et al. (2008) in red pine seedlings, Eser (2007) in Greek juniper seedlings, Atik and Allahverdi (2007; 2008) and Atik (2013a) in oriental beech seedlings.

Table 5. The results of the correlation analysis.

| Measured Characteristics | SH | RCD     | TDW     | RDW     | Total N |
|--------------------------|----|---------|---------|---------|---------|
| SH                       | 1  | 0.980** | 0.970** | 0.969** | 0.834** |
| RCD                      |    | 1       | 0.968** | 0.968** | 0.857** |
| TDW                      |    |         | 1       | 0.966** | 0.907** |
| RDW                      |    |         |         | 1       | 0.899** |
| Total N                  |    |         |         |         | 1       |

\*\*.: correlation is significant at 0.01 level

Effects of average elevation and aspect variables in treatment groups were assessed using multi-regression models by accepting morphologic and physiologic seedling quality characteristics to be dependent variables. In the first regression model, 42.2% ( $R^2=0.422$ ) of SH is explained by elevation while the aspect variable remains weak. In the second regression model where the dependent variable was RCD, 15.4% ( $R^2=0.154$ ) of growth is expressed by the elevation variable, while the aspect variable remains weak. According to the third regression model, 13.0% ( $R^2=0.130$ ) of TDW is expressed by elevation, and aspect variable remain weak in expressing this change. According to the fourth regression model, 11.8% ( $R^2=0.118$ ) of RDW is expressed by elevation, and aspect variable remain weak in expressing this change. It was determined that Regression Model 5 could express only 2.1% of the change in total N rate in plant ( $R^2=0.021$ ) and both of the independent variables of elevation and aspect in the model were weak to express this change.

Table 6. Results of multiple regression analyses explaining variation in seedling growth.

|                | 1.Model: Dependent Variable<br>SH  |           |         | 2.Model: Dependent Variable<br>RCD     |           |         | 3.Model: Dependent Variable<br>TDW |           |         |
|----------------|------------------------------------|-----------|---------|--|-----------|---------|------------------------------------|-----------|---------|
|                | Coefficient                        | Std Error | Sig.    | Coefficient                            | Std Error | Sig.    | Coefficient                        | Std Error | Sig.    |
| (Constant)     | 17.510                             | 0.485     | 0.000** | 5.730                                  | 0.174     | 0.000** | 2.617                              | 0.086     | 0.000** |
| Elevation      | -0.004                             | 0.000     | 0.000** | -0.001                                 | 0.000     | 0.000** | -0.001                             | 0.000     | 0.000** |
| Aspect         | -0.082                             | 0.079     | 0.302*  | -0.026                                 | 0.028     | 0.366*  | -0.005                             | 0.014     | 0.737*  |
| R <sup>2</sup> | 0.422                              |           |         | 0.154                                  |           |         | 0.130                              |           |         |
| F-statistic    | 45.203                             |           |         | 37.957                                 |           |         | 31.272                             |           |         |
|                | 4.Model: Dependent Variable<br>RDW |           |         | 5.Model: Dependent Variable<br>Total N |           |         |                                    |           |         |
|                | Coefficient                        | Std Error | Sig.    | Coefficient                            | Std Error | Sig.    |                                    |           |         |
| (Constant)     | 0.944                              | 0.033     | 0.000** | 1.191                                  | 0.148     | 0.000** |                                    |           |         |
| Elevation      | 0.000                              | 0.000     | 0.000** | 0.000                                  | 0.000     | 0.370*  |                                    |           |         |
| Aspect         | -0.002                             | 0.005     | 0.741*  | 0.005                                  | 0.024     | 0.853*  |                                    |           |         |
| R <sup>2</sup> | 0.118                              |           |         | 0.021                                  |           |         |                                    |           |         |
| F-statistic    | 27.885                             |           |         | 0.412                                  |           |         |                                    |           |         |

\* Not significant ( $p > 0.05$ ).

\*\* Significant at the 0.05 level ( $p < 0.05$ ).

## Discussion

Several studies have been conducted on the production of VC fertilisers using different organic materials and techniques (Manukovsky et al., 2001; Ndegwa and Thompson, 2001; Tognetti et al., 2005). The effects of various types of VCs with different contents have been investigated on the agricultural crop yield and plant resistance of vegetables such as Indian spinach (Geodakian and Erofeeva, 1996; Ndegwa and Thompson, 2001), garlic (Argüello et al., 2006), strawberries (Arancon et al., 2004), tomatoes (Atiyeh et al., 1999, 2000, 2001; Hashemimajd et al., 2004; Gutierrez-Miceli et al., 2007), peppers (Arancon et al., 2005), corn (Lazcano et al., 2011), eggplant (Gajalakshmi and Abbasi, 2004) and fruits such as papaya and banana (Cabanas-Echevarria et al., 2005; Acevedo and Pire, 2004). In addition, there have been studies on the effects of VC on the seed germination and development of ornamental plants, such as amaranthus (Dönmez and Allahverdi, 2007), petunias (Arancon et al., 2008), and chrysanthemum (Hidalgo and Harkess, 2002), and of park and forest plants, such as Anatolian black pine (Atik et al., 2009), maritime pine (Lazcano et al., 2010a, 2010b), acacia and eucalyptus (Donald and Visser, 1989), oriental beech (Atik, 2013a).

Edwards et al. (2004) and Lazcano et al. (2009) reported that VC showed positive effects on root and shoot development and leaf area in vegetative propagation.

Positive effect of VC treatment on morphologic and physiologic seedling quality characteristics in Scotch pine seedlings is convenient with related literature. SH, RCD, TDW, RDW and total N rates were found in all seedlings from 7 origins treated with VC were larger than the controls. It was stated from statistical analysis that effect of VC on SH, RCD, TDW, RDW and total N rates, which are important morphologic and physiologic parameters in one – year Scotch pine seedlings, was statistically significant.

Treatment types were divided into 9, 8, 7 and 9 homogenous categories according to the development performance of seedlings for SH, RCD, TDW and RDW. Treatment types were also divided into two categories for total N rate in nurseries as VC treated and not treated.

It was found that Goktepe originating seedlings showed the best development in all morphological parameters in both VC treated and control groups while Geyikgolu originating ones showed the least development performance. This condition might have resulted from the fact that the elevation difference between the areas seeds were taken and seedlings grew was the largest for Geyikgolu origin.

Another point to be focused on in the study is the sign and significance of relationship between development parameters. It is possible according to the results of correlation analysis to mention a positive relationship between all parameters. Relationship between all morphologic parameters is strong while it is again strong between total N and TDW and moderate between total N and other parameters. Such significant relationship between morphologic parameters in all treatment types are in convenience with the RI, V and QI indices determined in seedlings. This condition shows that seedlings have a balanced growth performance for

height, diameter, root and trunk weigh. It may be withdrawn from positive association between morphological parameters measured in seedlings and total N that total N rate has direct effect on plant development. The most apparent relationship between elevation and seedling development performance was determined in Geyikgolu originating GEYVC and GEYCTRL treatment types harvested at 1620 m. Seedlings shooting from the seeds harvested at this elevation produced are 20 % more quality both morphologically and physiologically than those from other origins. According to the results of multivariable regression analysis, effect of elevation at which seeds used in nursery were harvested, and aspect on plant development parameters may change depending on parameter type. The largest of such effects was obtained from the first regression model where SH was accepted to be dependent variable. It was stated according to model that SH development in seedling could be associated with elevation variable by 42.2 % which was 15.4 % in the second model, where RCD was accepted to be dependent variable, 13.0% in TDW, 11.8% in RDW and 2.1 % in total N, respectively.

It may be concluded that elevation of the site where seedling is harvested is more effective on SH growth of plant than its aspect. However, it was determined when 5 models were taken into account that two independent variables are inefficient and superficial for the expression of development performance in seedling. It is thought that it may be possible to make a healthier relationship between the parameters mentioned above by adding more and multi-facets independent variables to the model including stand formation structure.

## Conclusion

Use of fertilizers is common in great majority of today's farms to ensure the crop yield and quality. As in all other sectors, global increase in oil prices compels producers to search for ways of reducing production cost by protecting the quality conditions in seedling production sector. In this respect, VC fertilizers are forefront for their concentrated structure, easiness to use and store and low transportation cost. However, the number of studies should be increased in order to determine clear and common effects of VC fertilizers using more species in various areas.

The effect of Biyohumus®, a type of VC fertilizers, was investigated on some morphological and physiological characteristics of Scots pine seedlings. It was found that VC treatment contributed positively to growth performance of Scotch pine seedlings. In the light of these results, it may be suggested that VC fertilisers can be evaluated as an alternative to manure fertilizer in the nurseries where Scotch pine seedlings are produced.

Quality and the number of related studies like present one should be increased in order to offer clearly the use of VC fertilizers in nurseries. It is thought that multidirectional effects of VC fertilizers should be determined using the experimental plots to be designated in the nurseries founded in the areas with different edaphic, climatic and physiologic conditions considering various plant species, VC and treatment types. From this point of view, concrete and clear contributions can be supplied to agriculture sector.

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