EFFECTS OF COLD STRATIFICATION AND H2SO4 PRE-TREATMENTS ON GERMINATION OF SEA BUCKTHORN (HIPPOPHAE RHAMNOIDES L.) SEEDS UNDER OPEN FIELD AND GREENHOUSE CONDITIONS

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ABSTRACT

This study was carried out to determine the effects of some pre-treatments including soaking in concentrate (96%) H_2SO_4 for 1, 2, 3 and 4 minutes and cold stratification for 10, 20, 30, 40 and 50 days on seed germination and to investigate how to overcome dormancy of *Hippophae rhamnoides* L. seeds. The seeds were sown in the greenhouse and under open field conditions. The statistical approach was a randomized complete block design with three replications. Germinated seeds were observed periodically during 35 days to determine germination percentages and germination rates. The highest germination percentages (44.4% and 75.5%) were obtained from the seeds soaked in H_2SO_4 for 2 minute and sown both open field conditions and in the greenhouse, respectively. The lowest germination percentages were obtained from the control seeds which were sown under both two conditions (20.7% and 39.2%). While the best germination rate (11 days) was determined from 3-minutes H_2SO_4 pre-treatments in the greenhouse, 10-day cold stratification gave the best germination rate (9 days) under open field conditions.

KEYWORDS:
Hippophae rhamnoides, germination, stratification, H_2SO_4

INTRODUCTION

Vegetation cover is one of the most important factors in preventing and controlling soil erosion. It gives long-term soil surface protection by providing leaf cover that reduces rain-drop effects. In addition, it helps better soil structure development through establishing a root system, thereby increasing infiltration and soil stability [1, 2]. *Hippophae rhamnoides* is native to northwestern Europe through central Asia to Altai Mountains, western and northern China, and the northern Himalayas [3, 4, 5, 6]. In addition, *H. rhamnoides* is also a native plant species distributed in different regions in Turkey [7, 8].

H. rhamnoides is a drought-tolerant plant occurring in sandy and salty landscapes and is known as an important species in preventing soil erosion [4, 5, 9, 10]. The species is also considered as fine vegetation in improving soil fertility and restoring degraded sites in high hills [5, 11, 12]. Sea buckthorn can withstand temperatures from -43°C to +40°C. It is considered to be drought resistant however, most natural populations of sea buckthorn grow in areas receiving 400-600 mm of annual precipitation. The plant tolerates high soil pH up to 8.0 and salt from sea water around the costal regions [5]. The most important step in the bio-preventive measures for checking soil erosion is the selection of suitable stabilizing drought-tolerant plants in semi-arid and arid areas [13, 14, 15, 16]. The aim of silvicultural interventions in these areas should be increase the quality of the forests and vegetation cover [17].

A very hardy deciduous shrub or a small tree, *H. rhamnoides* is used primarily for ornamental purposes. In Europe and Asia, it is used to form hedges and because of its nitrogen-fixing symbionts, serves to enrich and protect soils [18, 19]. The plant has also a variety of medicinal uses. It has been used medicinally in China for at least 12 centuries, and sea buckthorn oil is used clinically in hospitals in Russia and China [4, 20, 21]. The berries of the species are source of vitamins, and use in preparations of various products including beverages and marmalades [5, 21, 22, 23, 24, 25].

Seeds of many woody plant species cannot germinate even if they are sown under optimal moisture, oxygen and soil conditions [26, 27]. This problem is called dormancy and its causes are a hard and impermeable seed coat, immature or dormant embryo, absence of endosperm, or thick, fleshy seed cover. There is great deal of variation in germination ability of seeds even within the same species [28, 29, 30, 31, 32, 33]. Poulsen [34] and Landis [26] reported that dormancy among and within seed lots of the same species varies with provenance, crop year and individual trees.

TABLE 1
Results of Statistical Analyses Showing the Relationship of the GP and GR with Different Treatments in the Greenhouse

Pre-treatments	F-Ratio	GP (%)	F-Ratio	GR (days)
Control		39.2a		14.2f
Cold stratification for 10 days		46.6ab		12.3c
Cold stratification for 20 days		53.3b		12.6d
Cold stratification for 30 days	25.733*	62.1c	747.711*	14.4g
Cold stratification for 40 days		71.0d		13.6e
Cold stratification for 50 days		71.0d		11.8b
Soaking in H_2SO_4 for 3 minutes		71.0d		11.0a
Soaking in H_2SO_4 for 1 minute		73.3d		14.8h
Soaking in H_2SO_4 for 4 minutes		74.0d		11.2a
Soaking in H_2SO_4 for 2 minutes		75.5d		14.3fg

* : Pre-treatments, significantly different at α= 0.05

TABLE 2
Results of Statistical Analyses Showing the Relationship of the GP and GR with Different Treatments under Open Field Conditions

Pre-treatments	F-Ratio	GP (%)	F-Ratio	GR (days)
Control		20.7a		16.9i
Cold stratification for 10 days		22.2ab		8.7a
Cold stratification for 20 days		26.6b		15.4h
Cold stratification for 30 days	21.931*	33.3c	3074.751*	10.4c
Cold stratification for 40 days		34.7cd		11.3d
Cold stratification for 50 days		37.0cde		10.2b
Soaking in H_2SO_4 for 3 minutes		37.7cde		11.6e
Soaking in H_2SO_4 for 1 minute		39.9def		14.8f
Soaking in H_2SO_4 for 4 minutes		41.4ef		11.3d
Soaking in H_2SO_4 for 2 minutes		44.4f		15.2g

* : Pre-treatments, significantly different at α= 0.05

There are various germination obstacles in *Hippophae* sp. seeds resulting in propagation difficulties [6, 11, 32, 35, 36]. Generally pre-treatments such as submersion in hot water, mechanical or chemical scarification, and hot aeration are used for seed coat dormancy while the cold and warm stratifications are usually applied to dormancy caused by restrictions at the embryo level [26]. Among these methods and techniques, especially cold stratification for 15 to 90 days at 2 to 5°C, submersion in concentrate H_2SO_4 and soaking in different concentration of KNO_3, GA_3, Thiourea pretreatments are well-known and used to increase germination percentage of *Hippophae* seeds [5, 6, 11, 37]. In addition, stratification for 15 days is sufficient when seeds are sown in the fall [38]. The optimum temperature for the germination of *H. rhamnoides* seed is the variable temperature of 20-30°C and germination tests may be run in 40 days on stratified seeds in sand flats [6, 39].

The aim of this study was to examine the influence of cold stratification (10, 20, 30, 40 and 50 days) and soaking in concentrate H_2SO_4 (1, 2, 3 and 4 minutes) pre-treatments on dormancy of *H. rhamnoides* seeds.

MATERIALS AND METHODS

Ripe fruits were collected from *H. rhamnoides* individuals in September 2010, in Sarikum-Sinop located in the north western part of Turkey (Altitude: 100 m). The seeds were separated from the fruit material, rinsed with tap water, dried in the shade and stored at 4±1°C in plastic bags.

The following pre-treatment applications were used to determine their effects on germination percentage (GP), and germination rate (GR) of *H. rhamnoides* seeds;

- Cold stratification (CS) for 10, 20, 30, 40 and 50 days,
- Submersion in concentrated (98%) sulphuric acid for 1, 2, 3 and 4 minutes,
- Control (no treatment).

The seeds were stratified at 4±1°C by putting layers of moistened sand and seeds on top of each other. Since there was a risk for some of the seeds to be mixed with the sand because of their small size, linen cloth was placed between the sand and the seeds. The moisture of the sand and the seeds was checked regularly so that the seeds would not get mouldy.

The seeds were sown in polyethylene pots under open field conditions and in the greenhouse in the spring (4th of April) 2011. The pots were filled with growing medium composed forest soil, creek sand and manure (1:1:1). The experimental design was a randomized complete block with three replications for each treatment and 45 seeds were sown in each replication. The number of germinated seeds (evaluation done according to ISTA Rules (1993)) were recorded on the 4th, 7th, 10th, 14th and in every week (7 days) after the 14th-day counting. The below formula was used when determining germination rate [40];

$$GR = \frac{(n1 \times t1) + (n2 \times t2) + (n3 \times t3) + ... + (ni \times ti)}{T}$$

Where;
GR: Germination rate
n: Number of days for each counting of germinated seeds
t: Number of germinated seeds at each counting day
T: Total number of germinated seeds

The whole experiment lasted for 35 days when it was observed that the seeds stopped germinating. Data from the treatments were analyzed by the SPSS statistical software after arc-sinus transformation was applied to GP values to meet ANOVA assumptions. The ANOVA and Duncan tests were used to compare treatment groups to find out whether they showed any statistically significant differences with significance level (α) set at 0.05.

RESULTS AND DISCUSSION

Statistical analyses showed that the highest germination percentage (75.5%) among all treatments was obtained from the seeds soaked in H_2SO_4 for 2 minutes in the greenhouse conditions. The lowest germination percentage (20.7%) was obtained from the control seeds under open field conditions (Table 1 and Table 2). According to Busing and Slabaugh [6], Airi et al. [11] and Li and Schroeder [35] and cold stratification for 15 to 90 days at 2°C to 5°C, submersion in concentrate H_2SO_4 and soaking in different concentration of KNO_3, GA_3 and Thiourea pre-treatments are used to increase germination percentage of *Hippophae* sp. seeds. On the other hand, according to Airi et al. [11]'s results the pre-treatments on seeds before sowing were significantly increased the germination percentages compared to control (24-30%). They obtained 63-71% germination percentages from cold stratification for 30 days. Busing and Slabaugh [6] reported that germination of untreated sea buckthorn seeds ranged from 6% to 60% after 60 days. Olmez [37] implied that the germination percentage were 100% in seeds soaked in H_2SO_4 for 1 minute and 98.8% in seeds cold stratified for 30 days in the laboratory conditions. Ozdemir [32] also determined the germination percentages between 11 and 80% from untreated seeds in the laboratory conditions for different populations at the end of 28th day.

In addition, better germination results were determined from H_2SO_4 pre-treatments than cold stratification both in the greenhouse and under open field conditions (Table 1 and Table 2).

Cold stratification treatments gave better results than control sowings. The best germination percentages (37.0% and 71.0%) for open field and greenhouse conditions were obtained from 50-day cold stratification among the cold stratification durations, respectively (Table 1 and Table 2). Grover [38] stated that untreated seeds should be sown in the fall but stratification treatment for 15 days was required before sowing the seeds. Gultekin [41] and Genc [42] also reported that cold stratification for 12-14 weeks was necessary before sowing the seeds in the spring.

The best germination rate (11 days) in the greenhouse was determined from H_2SO_4 for 3 minutes and 4 minutes pre-treatments. On the other hand, the best germination rate under open field conditions was 9 days in seeds cold stratified for 10 days (Table 1 and Table 2).

Consequently, among all the pre-treatments applied to the *H. rhamnoides* seeds, soaking in sulphuric acid for 2 minutes resulted in the highest germination percentage (75.5%) in the greenhouse. It can be said that 40 or 50-days cold stratification pre-treatments can use instead of H_2SO_4 scarification to overcome dormancy of *H. rhamnoides* seeds (Table 1 and Table 2). The results indicate that the pre-treatments by submersion in H_2SO_4 and sowing in the greenhouse gave the better results than using cold stratification and sowing under open field conditions.

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