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Modeling the Effect of Different Pre-soaking Treatments on Seed Germination in Stinging Nettle (*Urtica diocia* L.)

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ABSTRACT

The aim of this study was to determine the effect of different pre-soaking applications on stinging nettle (*Urtica diocia* L.) germination *in vitro* with mathematical modeling. Pre-soaking applications were control, hot water (HW), gibberellic acid (GA) and sulfuric acid (H_2SO_4). Seed from stinging nettles were investigated by mathematical models based on temperature. For this reason a model $D = a-(b \times T)+(c \times T^2)$ produced for predicting the time to emergence in relation to temperature for stinging nettle was utilized. Optimum temperature for seed germination was calculated by using the coefficients $T_0 = [-b / (2 \times c)]$ obtained from the regression models of the days to germination $GR = (12.983) - (0.458 \times T) + (0.009 \times T^2)$, which was found to be 26°C. The regression coefficient was $R^2 = 0.99$ for germination percentage. Depending on the days to germination the effect of H_2SO_4 changes on germination was $SA = (-19.309)+(5.701 \times D)-(31.576 \times SAR)$ where D is number of days and SAR is H_2SO_4 rate. Depending on the days to germination the effect of GA changes on germination is $GA = (29.567)+(0.165 \times D)-(0.001 \times GAR^2)$ where D is day and GAR is GA_3 rate. The effect of HW application is $HW = (50.699)-(2.858 \times D)+(0.624 \times T)$ where D is number of days and T is temperature. Regression coefficients were $R^2 = 0.95$ for H_2SO_4 application, $R^2 = 0.89$ for GA application and $R^2 = 0.69$ for HW application.

Keywords: mathematical modeling, medical plant, stinging nettle, temperature, textile industry
Abbreviations: GA, gibberellic acid; **HW**, hot water; **H_2SO_4**, sulfuric acid

INTRODUCTION

Medicinal plants are widely used in pharmaceuticals as well as in the cosmetic, foodstuff and textile industries, including stinging nettle (*Urtica dioica* L.), which is one of the plants that has herbal and nutritious properties. Stinging nettle leaves cooked as a potherb are added to soups and can also be dried for winter use. Stinging nettle is a very nutritious food that is easily digested and is high in minerals (especially iron), vitamin C and pro-vitamin A (Hojnik *et al.* 2007).

Germination is a critical stage in the life cycle of all plants. It often controls population dynamics with major practical implications and depends on temperature (Kevseroglu and Caliskan 1995; Keller and Kollmann 1999). All growth processes within seeds are chemical reactions activated by the addition of water, temperature and the presence of oxygen. Therefore, the germination process requires moisture, oxygen and temperature ranges which are specific to particular crops. The higher the temperature, the faster the rate of chemical reactions. But, there are biological limitations as to how much temperature can be raised, which varies with plant species (Uzun 1996; Flores and Briones 2001). Germination rate usually increases until the temperature reaches 30–35°C. However, the imbibed seeds of some plant species exhibit thermodormancy at higher temperatures (Kevseroglu *et al.* 2000; Villalobos *et al*. 2001).

On the other hand, it is literally impossible to describe a particular optimum temperature value for germination (Meyer *et al*. 1965). Optimum temperature for germination is the temperature value which results in the highest germination rate in the shortest period of time (Sagsoz 1990; Hakansson *et al*. 2002). Moreover, optimum germination temperatures are not the same for all seed species. Generally, optimum temperatures are preferred for both seed germination and plant growth. Therefore, it will be useful to know minimum, optimum and maximum temperatures required for plant growth and development (Meyer *et al.* 1965; Kevseroglu 1997; Arechiga *et al.* 2000; Odabas *et al*. 2007a, 2007b).

In many medicinal plants several studies have been carried out under field conditions to examine seed germination and emergence. In this study, we aimed to examine the germination performance and germination duration of the seeds of stinging nettle *in vitro* by adapting a mathematical modeling.

MATERIALS AND METHODS

Plant material

Stinging nettle seeds were collected from plants growing in the wild in Samsun province, Turkey (41° 35′ N lat., 35° 56′ E long., and 50 m asl) during the summer of 2007 and stored at 4 ± 1°C in sealed plastic bags until use germination tests.

Experimental procedures

The pre-soaking applications used in the study were different doses of gibberellic acid (GA), hot water (HW) and sulphuric acid (H_2SO_4). All chemicals were purchased from SIGMA Chemical Co. Before placing the seeds in Petri dishes, they were soaked in 50, 100 or 150 ppm GA solution, 40, 50 and 60°C hot water, or 1, 2 and 3% H_2SO_4 solutions for 30 min and controls. To evaluate the effect of light on germination, the study was performed in growth chambers under both continuous illumination (14.4 $\mu molm^2s^{-1}$ light intensity) and darkness. Temperature was set at 26°C. The experimental design was a factorial randomized block design with three replications with 50 seeds in each. Germination was measured as a percentage, 20 days after seeds germinated at the level of 99% and the experiment was initiated.

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Short Communication

Methods

The seeds were placed on moisturized filter paper (Watman No: 1) kept in each Petri dish. For each replication (3 replications per application), 50 stinging nettle seeds were placed in each Petri dish. The data were analyzed using ANOVA and differences among treatments were tested using Duncan's Multiple Range Test (level of significance was $P<0.01$).

Seed germination was observed daily and germinated seeds were counted in different applications at 26°C. A multi regression model was produced $D = a-(b \times T)+(c \times T^2)$ to predict the time elapsing from seed sowing to emergence by carrying out multi regression analysis.

In the above model; D represents the time elapsing from seed sowing to germination as days, T represents temperature (°C) and a, b, c represent coefficiencies.

The rate of variation in seedling emergence can be obtained from a derivative of the above equation $Dd/dt = (-b)+(2 \times c \times T)$. Another equation determining optimum temperature (T_0) for emergence is $T_0 = (-b / 2 \times c)$.

Depending on the days the changes of pre-soaking applications were determined using mathematical modeling and were shown by 3-D graphics.

By taking into consideration these stages of the model, optimum germination temperatures were determined and standard equations predicting germination percentage for stinging nettle were produced.

RESULTS AND DISCUSSION

Germination is a continuous process involving various physiological activities within seeds; it is typically considered from a practical point of view. That is, germination is represented by the first visual appearance of the radicle from the outer most structure enveloping the embryo. Seed germination is perhaps the most thoroughly examined aspect of plant development. It has been studied extensively in controlled environments.

Nevertheless, the primary factors governing seed germination in arable soils are temperature, water potential, and air quality (Roberts 1988; Roberts and Ellis 1989).

Light intensity has been recognized since the mid-19th century as a germination-controlling factor and it is frequently found to be a requirement for wild plant species rather than domesticated ones (Baskin 2004). Light has a regulatory effect on germination and endogenous seed dormancy is usually related to the absence of light (Arechiga *et al*. 1997; Cirak *et a.* 2007).

In the present study, nettle seeds could germinate effectively only in the presence of light and no pre-soaking treatment induced germination. Since light intensity in the wild is extremely high in Turkey, plants could germinate at a high level in response to exposure to increased light intensities in the laboratory.

According to the results of variance analysis, the presoaking treatments tested had a significant ($P<0.01$) effect on germination rates. Untreated control seeds under light produced the highest germination rate observed in the present study (83%) followed by light-mediated 30 and 40°C HW treatments (45 and 39%, respectively). Light intensity was found to be the most effective factor in promoting germination. This negative effect was more pronounced in H_2SO_4 treatments.

Analysis was carried out until the lowest standard errors of independent variables values (namely T and T^2), and the highest R^2 values (regression coefficients) of the equations (mathematical models) were obtained.

Optimum germination temperature was found 26°C. The equation below was obtained in order to find the ideal germination temperature (**Fig 1**). GR is germination (%) and T is temperature (°C).

$GR = (12.983) - (0.458 \times T) + (0.009 \times T^2)$

SE=0.105*** 0.01*** 2.07E^{-4}***

$R^2=0.9956$

$T_{opt.}=[-b/2 \times c]=26°C$

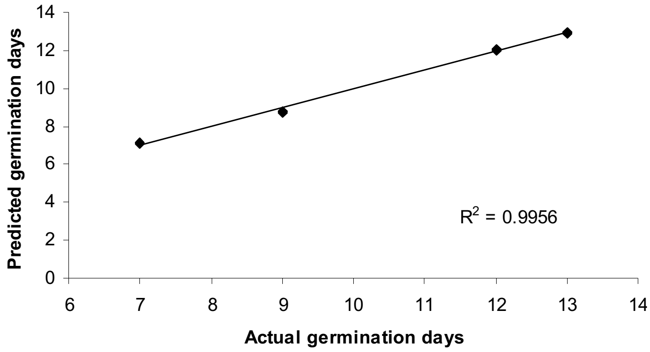

Fig. 1 Relationship between actual and predicted germination days for stinging nettle.

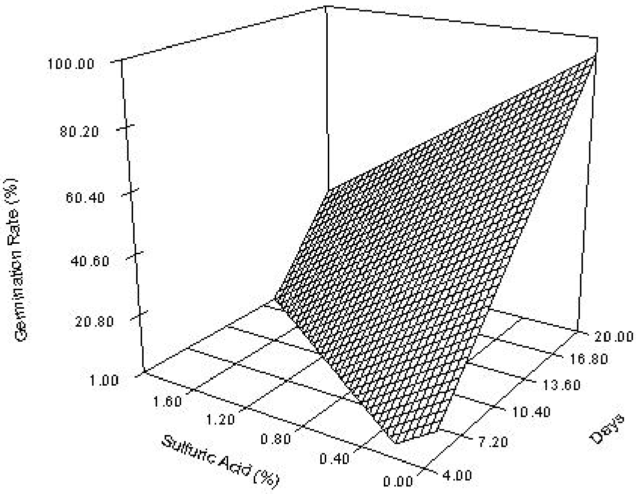

Fig. 2 Changes in germination percentage of stinging nettle with days and sulfuric acid percentage (%).

H_2SO_4 doses were applied both in the dark and under 14.4 µmolm²s^{-1} light intensity. At the end of the application the percentage germination in the dark was very low, and the seeds that germinated were weak. The percentage germination in the light was higher than in the dark, but compared to the control group it was lower. When H_2SO_4 was applied, the equation below was obtained in order to find the ideal germination day. In the equal obtained D symbolizes the number of days for germination, AR symbolizes the acid rate, SE is the standard error and R^2 is the regression coefficient. In the graph drawn with the help of this equation the changes in germination rate, which depends on the days and acid rate, are shown (**Fig. 2**).

$GR(SA) = (-19.310) + (5.701 \times D) - (31.576 \times AR)$

SE=9.344*** 0.614*** 4.622***

$R^2= 0.9571$

The effect of GA application on the germination percentage depends on the number of days. When the effect of GA on the germination percentage was examined, the seeds in the dark germinated at a lower rate than those under the light. In the GA doses applied and under light the germination percentage was lower than the control group. Depending on the days when we expressed the effect of GA application on germination percentage in a mathematical way, the equation below was found (**Fig. 3**) in which D symbolizes the number of days for germination, GAR symbolizes the GA rate, SE is the standard errors and R^2 is the regression coefficient.

$GAR = (29.567) + (0.165 \times D^2) - [0.001 \times (GA^2)]$

SE= 5.68*** 0.025*** 0.003***

$R^2= 0.8922$

The graph drawn with the help of this equation showed that the increase in GAR affected the germination percentage negatively (**Fig. 4**).

It was observed that pre-soaking application before ger-

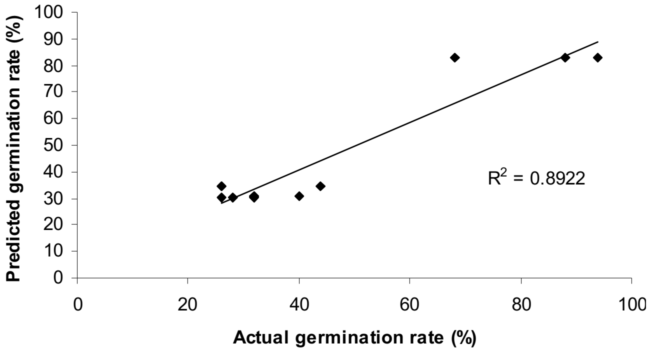

Fig. 3 Relationship between actual and predicted germination days for stinging nettle by effect of gibberellic acid application.

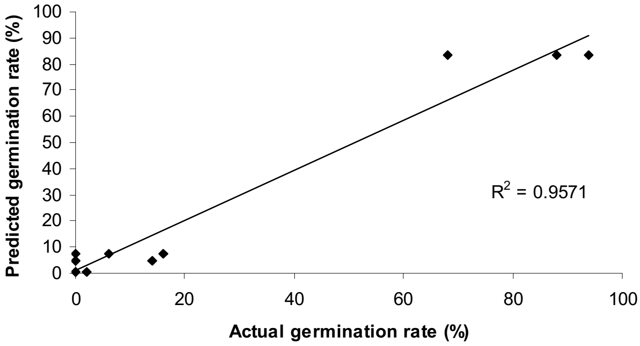

Fig. 5 Relationship between actual and predicted germination days for stinging nettle by the effect of hot water application.

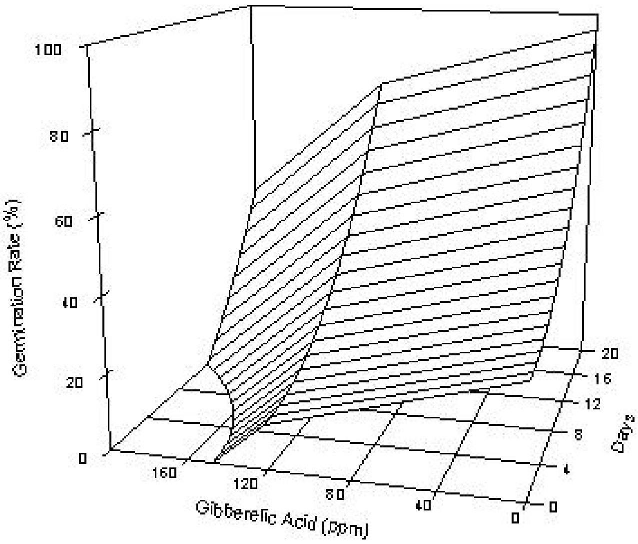

Fig. 4 Changes in germination percentage of stinging nettle with days and gibberellic acid rate (ppm).

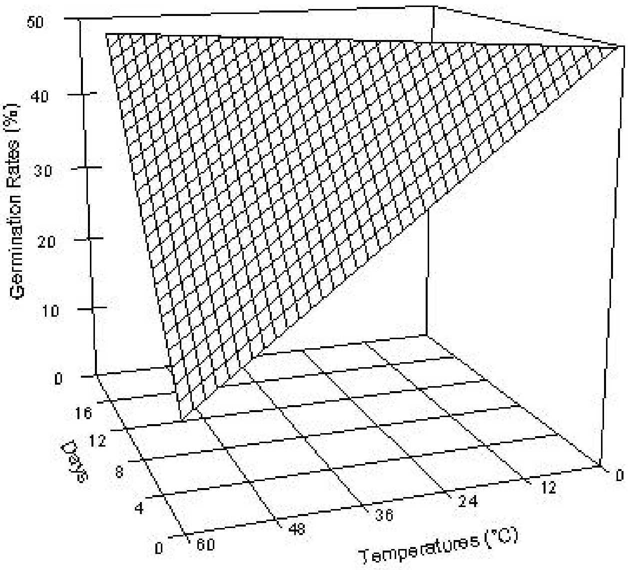

Fig. 6 Changes in germination percentage of stinging nettle with days and temperature (°C) by the effect of hot water application.

mination affected the germination negatively as in H_2SO_4 and GA applications. Depending on the days when we expressed the effect of HW application on germination percentage in a mathematical way, the equation below was found (**Fig. 5**).

$$GR(HW) = (50.699) - (2.858 \times D) + [0.624 \times (HW)]$$

SE= 4.39*** 0.65*** 0.16***

R^2= 0.6915

where D is the number of days for germination. In the graph drawn with the help of this equation it was observed that HW application affected seed germination negatively. This negative effect increased as the HW application increased (**Fig. 6**).

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