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RESEARCH ARTICLE

Modeling of the Effects of Nitrogen Doses on Agronomic Characteristics and Leaf Area of *Hypericum pruinatum L.*

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Abstract: The effects of nitrogen treatments on leaf area and some agronomical characteristics of *Hypericum pruinatum L*. under greenhouse conditions were investigated in this research. The randomized block design with three replications was used as statistical analysis. According to the results, fresh and dry yields, plant height, flowering shoot number per plant increased significantly with elevating nitrogen doses. The prediction model was formulized as DP = a + (b x D). Where DP is dependent parameter (leaf width, leaf length, plant length, number of shoot, dry yield, and fresh yield) and D is nitrogen dose. According to prediction model, the leaf area model was found as LA= a + (b x W) + (c x L) where LA is leaf area, W is leaf width, L is leaf length and a, b, and c are coefficients. According to model equations the highest R^2 values was 99.6% in fresh yield and the lowest R^2 value was 86.8% in leaf length and they were found significant at the P<0.001 level.
Keywords: *Hypericum*, nitrogen fertilizer, modeling, agronomy

INTRODUCTION

The extraction of *Hypericum perforatum L*. are commonly used in EU countries as a drug and food additive for the treatment of mild to moderate depression (Fiebich *et al.* 2011). *Hypericum* consists of 484 species in forms of small trees, shrubs and herbs (Crockett and Robson 2011). Turkey is one of the important country for the genus *Hypericum* and there are 46 endemic *Hypericum* species (Guner *et al.* 2012).

One of the endemic and perennial plant of Anatolian flora is *Hypericum pruinatum* Boiss (Camas *et al.* 2013), hyperforin, adhyperforin, chlorogenic acid, neochlorogenic acid, caffeic acid and 2,4-dihydroxybenzoic acid (Cirak *et al.* 2015), amentoflavone, hyperoside, isoquercitrin, quercitrin, quercetin, avicularin, rutin, (+)-catechin and (-)-epicatechin (Cirak et al. 2014). *Hypericum* extracts which are especially hypericins and hyperforins has antidepressant activities (Guedes and Eriksson 2005; Du *et al.* 2006). The pharmacological effects of *Hypericum* extracts have made also an important contribution to the antimicrobial (Zhao *et al.* 2010) and antidepressant (Butterweck *et al.* 2000) activities. This similarity of its chemical composition could be considered as potential cultivated plant being used as an alternative crop to *H. pruinatum* instead of *H. perforatum*.

Macro and micronutrients have proven effects on plant growth and development as well as substrate content and enzymes activity, thereby, chemical compound accumulations and finally on plant/plant derived product quality (Montoya-Garcia *et al.* 2018). Thereby, timely and sufficient supply of nutrients is the first practice of agricultural affecting both biomass production and quality of drug in medicinal plants (Odabas *et al.* 2016; Barroso *et al.* 2018). Considering the specific importance of edaphic and physiological factors with regards to plant production and key role of nitrogen availability in plant development and chemistry, we aimed to evaluate the effects of nitrogen, applied in different doses on growth and chemical accumulation levels of greenhouse-grown *H. pruinatum* plants in the current study.

Leaf is very important part of plants. Thus, leaf area has important role for researches where some physiological phenomenon such as light, photosynthesis, and respiration etc (Centritto *et al.* 2000). Also, leaf area is important for cultural practices. The estimation of leaf area that goal to calculate non-destructively of leaf area. It can be useful tool for researches with many advantages in agricultural experiments. Furthermore, such mathematical models reduce experimental variability by allowing researchers to make leaf area measurements on the same plants throughout a study (Oner *et al.* 2011).

2. MATERIAL AND METHODS

Seeds of *Hypericum pruinatum* L. were germinated in small viols and emerged seedlings were transplanted into pots. The pods were filled with the commercial peat. The seedlings were moved to greenhouse. The greenhouse temperature was 24 °C relative humidity 75% i and 400 µmol m^{-2} s^{-1} PAR (Parabolic Anodized Reflector). The pods were watered daily until they reached maturity, then three times a week. They were fertilized with five levels of nitrogen including 33% pure nitrogen as NH_4 (0, 3, 6, 9 and 12 kg da^{-1}) after plants reached average 20 cm length in the greenhouse. Experimental design was randomized block design with three replications. At the end of 56 days cultivation period, the tops 2 / 3 of plants were harvested, dried at 21°C.

The 500 leaf samples were used the validation of the estimation model. At first, they were placed on the scanner and scanned (300 dpi resolution) on A4 sheets (at 1:1 ratio). Then, the sheets were saved as jpg file. Image processing technique was used to measure actual leaf area of the image (Caliskan *et al.* 2017). The choice of leaf sizes determined for the measurement was determined according to the change in leaf features. (*e.g.*, size, shape, and symmetry). Considering these factors, maximum leaf width (W) and length (L) were selected to correlate with leaf area (Oner *et al.* 2011; Odabas *et al.* 2017).

Then, the multiple linear correlation coefficient (r) and the coefficient of determination (R^2) were calculated. For each model, the mean absolute error (MAE), the root of mean square error (RMSE), and the mean absolute percentage error (MAPE) were calculated by means of equations:

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$$MAE = \frac{1}{n}\sum_{i=1}^{n}|x_i - x| \quad (1)$$

Where n is the number of errors and $|x_i - x|$ is the absolute errors.

$$RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(y - y')^2} \quad (2)$$

Where and are the calculated and predicted values, respectively. Mean absolute percentage error (MAPE) is a measure of accuracy in a fitted series value in statistics. It was used for comparison of the predicted model performance.

$$MAPE = \frac{1}{N}\sum_{i=1}^{N}\left|\frac{A_i - P_i}{A_i}\right| x\,100 \quad (3)$$

Where, A_i is the observed value and P_i is the predicted value. The statistical analysis was performed MATLAB software.

3. RESULTS AND DISCUSSION

The multiple regression analysis was used for determination of the best fitting equation. This equation was helped for estimation of leaf area. The goodness of fit statistic describes how well it fits a set of observations. Measures of goodness of fit typically summarize the discrepancy between actual values and the predicted values under the model (Table 1). When Table 1 is examined, all R^2 and adjusted R^2 values are higher than 0.86. Although MSE, RMSE, and MAPE values vary according to parameters, they were found to be 0.321, 0.567, and 2.397 for leaf width respectively. For the other parameters were shown in Table 1.

The R^2 values show us the relationship between actual values and predicted values. For instance, the R^2 of leaf area was found 0.904. That means, the model can estimate the leaf area 90% accuracy. The highest accuracies were found dry and fresh yield values (Table 1). According to analysis of variance, leaf width, leaf length, plant length, number of shoot, dry yield, fresh yield and leaf area were found statistically important (Table 2). Pandey and Singh (2011) found for individual species, the coefficient of determination between the two sets of estimates varied between 0.933 and 0.998.

The leaf area in a canopy is an important variable affecting light interception, photosynthesis and carbohydrate production (Landsberg and Sands 2011). The leaf area is estimated by equations that correlate a measured variable with the actual leaf area and it is performed indirectly or directly on the leaves or even using digital measuring instruments. The other parameters were modeled based on the change in nitrogen dose. The analysis of the data was performed for each parameter separately (Table 3).

The coefficients that for predicting the best model were found with various subsets of the independent variables. These variables are dose (D), height (H), and width (W). The best estimating equations for the parameters were tested and formulized (Table 4). The nitrogen in the soil is associated to organic matter. That's why, this is one of the critically criteria considered in the current recommendation of nitrogen fertilization to define the amount to be applied. The amount of nitrogen may vary with species, amount of organic residue, temperature, and humidity. Nitrogen stands out among the essential nutrients for plants, depending on environmental conditions.

When the Table 4 examined, there are so close relationship between actual and predicted values. The selection of models requires a balance between predictive qualities and the including the least number of variables necessary to predict parameters. Due to the simplicity and convenience of linear equations, they have been used to estimate the models. This close relationship shows that the obtained equations make accurate predictions. The equations of leaf width, leaf length, plant length, number of shoot, dry yield, and fresh yield can calculate different nitrogen doses. But, the equation of leaf area can only calculate leaf length and leaf width.

4. CONCLUSIONS

As a result of this research, it can be concluded that the mathematical equations (prediction models) are potentially an efficient and feasible tool for predicting of the dependent parameters. This approach is much simple than adopting a high dimensional polynomial regression. The order of polynomial increases due to accuracy and the number of terms in polynomial increases exponentially according to its degree. In this study, we developed the mathematical models for predicting parameters (leaf width, leaf length, plant length, number of shoot, dry yield, fresh yield, and leaf area) for the medicinal plant namely *Hypericum pruinatum* L. Such models would also allow researchers to estimate the parameters easily and high accuracy. The models that we found in this research can be used safely.

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AUTHOR CONTRIBUTIONS

D.K. conceived and designed the experiments, conducted the experiments, and collected the data. M.S.O. did the statistics evaluations. Authors wrote and approved the final manuscript.

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Table 1. Goodness of fit statistics

Source	R^2	Adjusted R^2	MSE	RMSE	MAPE
Leaf width (mm)	0.951	0.935	0.321	0.567	2.397
Leaf length (mm)	0.901	0.868	1.581	1.258	3.111
Plant length (cm)	0.956	0.942	3.144	1.773	2.896
Number of shoot	0.973	0.964	1.200	1.095	3.698
Dry yield (kg da^{-1})	0.996	0.994	9.304	3.050	4.197
Fresh yield (kg da^{-1})	0.997	0.996	50.969	7.139	3.242
Leaf area x Dose	0.898	0.869	1289.845	35.914	6.215
Leaf area (mm^2)	0.904	0.900	1469.832	38.338	6.602

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Table 2. Analysis of variance for the used parameters

Source		DF	Sum of squares	Mean squares	F	Pr > F
Leaf width (mm)	Model	1	18.769	18.769	58.470	0.005
	Error	3	0.963	0.321		
	Corrected Total	4	19.732			
Leaf length (mm)	Model	1	43.264	43.264	27.359	0.014
	Error	3	4.744	1.581		
	Corrected Total	4	48.008			
Plant length (cm)	Model	1	206.116	206.116	65.559	0.004
	Error	3	9.432	3.144		
	Corrected Total	4	215.548			
Number of shoot	Model	1	129.600	129.600	108.000	0.002
	Error	3	3.600	1.200		
	Corrected Total	4	133.200			
Dry yield (kg da^{-1})	Model	1	6568.969	6568.969	706.062	0.000
	Error	3	27.911	9.304		
	Corrected Total	4	6596.880			
Fresh yield (kg da^{-1})	Model	1	48135.844	48135.844	944.408	0.000
	Error	3	152.908	50.969		
	Corrected Total	4	48288.752			
Leaf area (mm^2)	Model	2	650825.639	325412.820	221.395	0.000
	Error	47	69082.112	1469.832		
	Corrected Total	49	719907.751			

Table 3. Model parameters

Source		Value	Standard Error	t	Pr > |t|	Lower bound (95%)	Upper bound (95%)
Leaf width (mm)	Intercept	14.120	0.439	32.174	< 0.0001	12.723	15.517
	Dose	0.457	0.060	7.647	0.005	0.267	0.647
Leaf length (mm)	Intercept	25.520	0.974	26.200	0.000	22.420	28.620
	Dose	0.693	0.133	5.231	0.014	0.271	1.115
Plant length (cm)	Intercept	38.240	1.373	27.842	0.000	33.869	42.611
	Dose	1.513	0.187	8.097	0.004	0.919	2.108
Number of shoot	Intercept	11.400	0.849	13.435	0.001	8.700	14.100
	Dose	1.200	0.115	10.392	0.002	0.833	1.567
Dry yield (kg da^{-1})	Intercept	28.140	2.363	11.910	0.001	20.621	35.659
	Dose	8.543	0.322	26.572	0.000	7.520	9.567
Fresh yield (kg da^{-1})	Intercept	86.280	5.530	15.602	0.001	68.681	103.879
	Dose	23.127	0.753	30.731	< 0.0001	20.732	25.522
Leaf area (mm^2)	Intercept	-333.28	43.976	-7.579	< 0.0001	-421.756	-244.819
	Width	17.340	3.321	5.221	< 0.0001	10.658	24.021
	Length	17.185	2.671	6.435	< 0.0001	11.812	22.557

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Table 4. Equations, actual and predicted values

Dependent parameter	Equation	Actual values	Predicted values
Leaf width (mm)	14.12 + (0.456 x D)	13.700	14.120
		15.600	15.490
		17.300	16.860
		18.700	18.230
		19.000	19.600
Leaf length (mm)	25.52 + (0.693 x D)	24.400	25.520
		29.100	27.600
		29.300	29.680
		32.500	31.760
		33.100	33.840
Plant length (cm)	38.24 + (1.513 x D)	36.400	38.240
		44.400	42.780
		48.900	47.320
		51.200	51.860
		55.700	56.400
Number of shoot	11.4 + (1.2 x D)	12.000	11.400
		15.000	15.000
		18.000	18.600
		21.000	22.200
		27.000	25.800
Dry yield (kg da^{-1})	28.14 + (8.543 x D)	31.300	28.140
		50.000	53.770
		78.100	79.400
		106.300	105.030
		131.300	130.660
Fresh yield (kg da^{-1})	86.28 + (23.126 x D)	93.800	86.280
		150.000	155.660
		218.800	225.040
		293.800	294.420
		368.800	363.800
Leaf area (mm^2)	-333.287 + (17.34 x W) + (17.19 x L)	229.610	235.511
		289.390	287.374
		350.440	356.578
		386.370	356.268
		330.080	304.559

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CONFLICTS OF INTEREST

"The authors declare no conflict of interest".