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# Effects of the Pursuit herbicide on relationships of morphological and physiological traits of two species of *Trifolium pretense* L. and *Trifolium alexandrinum* L.

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**Abstract**

To study the physiological and morphological relationships of two clover species affected by herbicides, Pursuit experiment in a factorial based on randomized completely design with 3 replications at the Damavand in 2012. The first factor consisted of two species of *Trifolium pretense* L. and *Trifolium alexandrinum* L**.** and the second factor consisted of three levels of herbicide Pursuit (0, 0.5, 1 and 1.5 litr per hectares). The traits in the study included chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, anthocyanin, flavonoids, ratio chlorophyll a to b, ratio carotenoids to total chlorophyll, dry matter, protein content and weeds destruction. Results showed that maximum response to herbicide on the percentage of protein in the leaves was recorded under 0.5 li/ha herbicide Pursuit treatment**.** The results showed that the correlation between the Chlorophyll a with chlorophyll b, total chlorophyll, carotenoids, and chlorophyll b whit total chlorophyll, carotenoids and total chlorophyll with carotenoids and carotenoids with the ratio of carotenoids to total chlorophyll, and dry matter with flavonoids are significantly related. The results of step-wise regression analysis showed that chlorophyll b and flavonoids had positive and the effect of protein content was reduced by dry matter, anthocyanin and ratio chlorophyll b to chlorophyll a.

**Key words:** Herbicide Pursuit, Protein, Response curves , *Trifolium pretense* L. and *Trifolium alexandrinum* L.

## Introduction

Nowadays, the pollution of the environment and health damarge is produced by the consumption of herbicide and has made the plant in order to help feed the plants. Types of chemical herbicides, despite their known toxicity, are widely used in Iran and developing counties. In this regard, Naji et al, (2011) noted they reported that concentration of 100 and 1000 µg/l of atrazine caused a significant increase ($P < 0.05$) in atretic oocyte in the tissue sections. Therefore, the use of herbicides found to be the

most appropriate value. Mortezapour et al (2010) reported between doses of herbicide imazethapeyr (0, 25, 50, 75 and100 g a. i. ha-1) on soybean dose 75 g a. i. ha-1 was appropriate. Understanding the relationships among common morphological and physiological traits on clover is critical to utilize these relationships effectively and to develop desirable varieties.

Tavasoli et al, (2009) in an examination of dual purpose herbicides on some physiological indexes of wheat (*Triticum aestivum L.*) in different stages of phenology reported Calculating the correlation coefficient of tested characteristics indicated that seed yield had a positive and significant correlation with dry weight and number of tillering, weight of 1000 grain, the number of seed in each spike, the length of spike in 1 showed the most correlation coefficient with height. Tonekaboni (2011) maintained that in applying herbicides on rice grain yield with biological yield had a positive correlation. The purpose of this experiment is to determine the most appropriate dose of herbicide Pursuit. Therefore, to minimize errors and to increase accuracy figures for values were applied different herbicides. However, due to herbicide damage to the crop enters and plant traits, attributes repair the damage. Therefore, these attributes must be identified and corrected to physiological or morphological characteristics of the reinforced plant. The first step in any breeding project to evaluate the desired trait variation in the population should be evaluated. Therefore, in order to assess the diversity and identifying characteristics using conventional statistical methods, the experiment was considered.

## MATERIALS AND METHODS

The experiment was planted as a randomized complete block design with factorial arrangement and 3 replications in Damavan region near Tehran, Iran (35°43N, 52°34'E) in 2012. The first factor consisted of two species of *Trifolium pretense* L. and *Trifolium alexandrinum* L. and the second factor consisted of three levels of herbicide Pursuit (0, 0.5, 1 and 1.5 litr per hectares). The traits in the study included chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, anthocyanin, flavonoids, ratio chlorophyll a to b, ratio carotenoids to total chlorophyll, dry matter, protein content and weeds destruction. In the experiment, the relationship between these traits and their effects on each other were examined in the study.

Correlation analysis and regression analysis were utilized in the study. Correlation analysis shows the possible relationships between the characteristics under study.

Regression analysis on the other hand, determines the relative effect of the remaining characteristics in the model on percentage of protein. The analyses were performed using the SPSS software version 17.

### Results

Results showed that herbicide Pursuit nonlinear regression effect on the percentage of protein in the leaves *Trifolium alexandrinum* L. (Egyptian clover) and on Persian clover (*Trifolium pretense* L*)* is not significant (Table 1). The highest percentage protein of leaves is in the *Trifolium alexandrinum* L. (Egyptian clover) in intake herbicide Pursuit 0.5 li/ha respectively (Figure 1). The results showed that the correlation between the Chlorophyll a with chlorophyll b, total chlorophyll,

carotenoids, and chlorophyll b whit total chlorophyll, carotenoids and total chlorophyll with carotenoids and carotenoids with the ratio of carotenoids to total chlorophyll, and dry matter with flavonoids are significantly related. (Table 2).

The results of step-wise regression analysis are presented in Table 3. Based on these results, there was a significant regression relationship between remaining traits of model and biomass ($P \leq 0.05$). The results of step-wise regression analysis showed that chlorophyll b and flavonoids had positive and the effect of protein content was reduced by dry matter, anthocyanin and ratio chlorophyll b to chlorophyll a.Comparison of the data histogram (Figure 2) shows that the obtained data had a suitable correspondence with normal curve and Figure 3 shows that fitting the data to the regression line was acceptable and therefore regression model is correct (Rezaei & Soltani, 2003).

**Discussion**

High doses of herbicides Pursuit can be harmful (Pasha, 2013) and herbicides Pursuit in large quantities for the plant is dangerous (Hoseiny-Rad & Jagannath, 2011). In this study, the protein content of the leaves was decreased with increasing concentration of the herbicide. Mortezapour et al (2010) stated that the report among herbicides imazethapeyr values (0, 25 50, 75 and 100 grams of material per acre) at 75 grams of material per acre of soybeans was appropriate. Plant pigments are associated with each other. These pigments are dry matter, and have a direct effect on the protein. Chlorophyll b and flavonoid had a positive effect on the protein content. Chlorophyll and flavonoids have a relationship with a variety of proteins and are found in the plant. On the other hand, dry matter, ratio chlorophyll b to chlorophyll a and anthocyanins are reduced in the protein content. Many herbicides are interfered with photosynthesis through blocking electron transport system and pigments in the chloroplast membranes are destroyed and plant face the loss of photosynthetic pigments (Balke, 1985; Velini et al., 2010).

It reduces photosynthesis and dry matter accumulation and thus the protein is too low. Also, anthocyanins have reduced the protein. Herbicides can prevent electron transfer in plant pigments that cause oxidative stress (Zhu et al., 2009; Wagner, 1979). The result show that a decent amount of herbicide is helpful for human health and the environment and the traits that can be used in times of herbicides to avoid crop stress should be strengthened.

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Table1. Analytical response of application of herbicide Pursuit values in the variable under study

| Trait | Clover Cultivar | Mean-square | | | C.V. (%) |
|---|---|---|---|---|---|
| | | Linear Regression | Regression Grade 2 | Regression Grade 3 | |
| percentage of protein in the leaves | Persian | 0.22ns | 0.06ns | 6.14ns | 36.61 |
| | Egyptian | 0.64ns | 0.001ns | 17.43* | 21.67 |
| **, *, and ns indicate significance at 1% and 5%, and no significant difference, respectively. | | | | | |

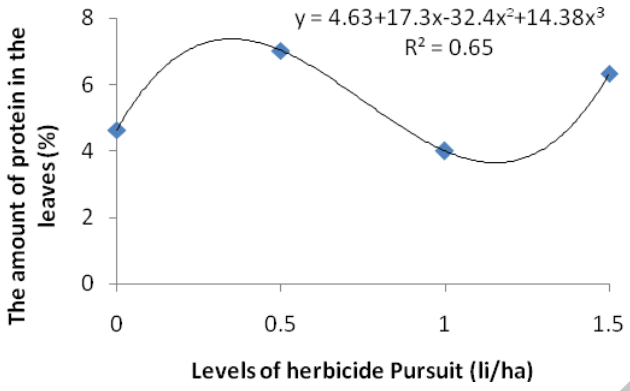


Figure1: Effect of herbicide Pursuit dose on percentage protein of leaves of Egyptian clover (*T. alexandrinum*).

Table2. Correlation analysis on morphological and physiological traits of two species of *Trifolium pratense* and *T. alexandrinum*

| | Chlorophyll a | Chlorophyll b | Chlorophyll total | Carotenoids | Ratio of chlorophyll b to chlorophyll a. | Ratio of carotenoids to chlorophyll total. | Percentage destruction of weeds | Percentage dry matter | Flavonoids | Anthocyani$^{ns}$ | Percentage protein |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Chlorophyll a | 1 | 0.80$^{**}$ | 0.98$^{**}$ | 0.85$^{**}$ | 0.3$^{ns}$ | 0.17 $^{ns}$ | 0.09 $^{ns}$ | 0.27 $^{ns}$ | 0.18 $^{ns}$ | 0.25 $^{ns}$ | 0.38 $^{ns}$ |
| Chlorophyll b | | 1 | 0.91$^{**}$ | 0.70$^{**}$ | 0.3 $^{ns}$ | 0.04 $^{ns}$ | 0.13 $^{ns}$ | 0.28 $^{ns}$ | 0.21 $^{ns}$ | 0.2 $^{ns}$ | 0.16 $^{ns}$ |
| Chlorophyll total | | | 1 | 0.84$^{**}$ | 0.11 $^{ns}$ | 0.1 $^{ns}$ | 0.1 $^{ns}$ | 0.28 $^{ns}$ | 0.19 $^{ns}$ | 0.24 $^{ns}$ | 0.3 $^{ns}$ |
| Carotenoids | | | | 1 | 0.27 $^{ns}$ | 0.62$^{**}$ | 0.02 $^{ns}$ | 0.36 $^{ns}$ | 0.2 $^{ns}$ | 0.19 $^{ns}$ | 0.3 $^{ns}$ |
| Ratio of chlorophyll b to chlorophyll a. | | | | | 1 | 0.36 $^{ns}$ | 0.17 $^{ns}$ | 0.06 $^{ns}$ | 0.03 $^{ns}$ | 0.06 $^{ns}$ | 0.34 $^{ns}$ |
| Ratio of carotenoids to chlorophyll total. | | | | | | 1 | 0.23 $^{ns}$ | 0.22 $^{ns}$ | 0.2 $^{ns}$ | 0.08 $^{ns}$ | 0.16 $^{ns}$ |
| Percentage destruction of weeds | | | | | | | 1 | 0.16 $^{ns}$ | 0.09 $^{ns}$ | 0.25 $^{ns}$ | 0.12 $^{ns}$ |
| Percentage dry matter | | | | | | | | 1 | 0.94$^{**}$ | 0.16 $^{ns}$ | 0.09 $^{ns}$ |
| Flavonoids | | | | | | | | | 1 | 0.17 $^{ns}$ | 0.18 $^{ns}$ |
| Anthocyani$^{ns}$ | | | | | | | | | | 1 | 0.21 $^{ns}$ |
| Percentage protein | | | | | | | | | | | 1 |

**, *, and ns indicate significance at 1% and 5%, and no significant difference, respectively.

Table 3
Results of step-wise regression analysis on morphological and physiological traits of two species of *Trifolium pratense* and *T. alexandrinum*

| S.O.V. | df | MS | $R^2$ |
|---|---|---|---|
| Regress | 5 | 11.16* | 52% |
| Error | 18 | 0.29 | |
| Total | 23 | | |

**, *, and ns indicate significant at P≤0.01, P≤0.05, and not significant, respectively.

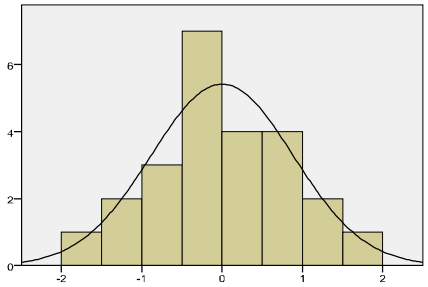


Figure 2**:** Comparing the histogram data of the normal curve (on morphological and physiological traits of two species of *Trifolium pratense* and *Trifolium alexandrinum*

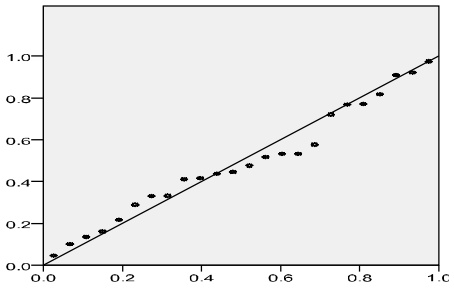


Figure 3: The data fit the regression line (on morphological and physiological traits of two species of *Trifolium. pratense* and *Trifolium alexandrinum*