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ALLELOPATHIC INTERACTIONS BETWEEN TWO BARLEY CULTIVARS (*Hordeum vulgare* L.) ON WEED ANNUAL RYEGRASS (*Lolium multiflorum* L.) AND CHARLOCK (*Sinapis arvensis* L.) USING THE EQUAL-COMPARTMENT-AGAR-METHOD

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ABSTRACT

The use of cultivars with allelopathic potential is one of weed management techniques. Factorial experiment in a completely randomized design with two factors including the density and culture on two barley cultivars namely Valfajer and Reyhaneh were used in laboratory, Fasa branch, Islamic Azad University, Fasa 2013. Actors examined included various densities of barley (0, 8, 16, 24, 32) and culture time (simultaneous and delayed). Ryegrass and Charlock seedling growth features were under investigation. The results showed that increasing barley plant density in both samples, compared to control, leads to the significant decrease in the seedling, radical and plumule lengths, wet and dry seedling weights of both weeds. Compared with plumule length, the radicle lengths of both weeds were more affected by a density which shows its high sensitivity. Considering the culture time of barley revealed that delayed planting has significant effect on the measured characteristics of weeds than simultaneous planting. Compared to simultaneous culture, cut-off interaction of similar densities at both culture times, showed the noticeable influence of delayed culture on weeds of ryegrass and charlock growth features, and also the most effective treatment combination was on the growth habits of weeds density of 32 and delayed culture of both barleys. Also, in order to compare the allelopathic potential of Valfajer and Reyhaneh barleys, using the linear regression model, the changes in the radicle length and plumule length of ryegrass and charlock were determined regarding applied densities. Comparison of model's parameters based on the confidence interval revealed the same reduction of both cultivars on the mentioned features and there was no noticeable significant difference between them.

Keywords: barley, weeds, allelopathic components, varieties, culture time, density, Equal-Compartment-Agar method.

INTRODUCTION

The growing human population requires the need for greater agriculture productivity. Pest management of insects, diseases and weeds is integral part of production and the weed management has an important position as one of the major limiting factors. Although, using herbicides to control weeds showed some success, unsatisfactory controls and concerns such as resistance and environmental pollution are the main challenges that make unclear the future use of chemical control methods for weeds. Due to the diverse nature and high adaptability of weeds, weed management programs require the use of diverse methods. Investigation showed that as the concentration of aqueous extract of Bersim clover increased, radicle length sometimes increased and sometimes decreased, so that in the full concentration the Rye radicle length was zero as well as the charlock radicle length in the 0.5 and complete concentration. In response to concentrations of 0.25 of the aqueous extract, the radicle lengths of Ivy, Amaranth, Rye, and Charlock respectively 76and73 percent decreased, 1/4 increased, and 67% of the control decreased [1]. An investigation, done on the allelopathic potential of barley on germination and growth of Charlock and Foxtail revealed the significant effect of barleys and used concentrations on the germination and radicle and plumule lengths of weeds seeds; increased concentration leads to the decrease in germination percentage, and radicle and plumule lengths of both weeds. Germination and growth of broad leaf weed seedlings were more sensitive than the narrow leaf weed [2]. The wheat seedlings that were planted a week earlier than ryegrass had a more significant inhibition of ryegrass radicle length than simultaneous planting and controller but there was no considerable difference between simultaneous planting and controller. It indicates that in the case of simultaneous planting of wheat and ryegrass seeds, the accumulated allelochemicals at ryegrass planting time are not available.

Both simultaneous and delayed plantings had significant effect on radicle growth of ryegrass. However, simultaneous planting showed more inhibitory effect on radicle growth of ryegrass. There was no significant effect of delayed planting on the radicle growth of ryegrass [3].

In studied the allelopathic potential of 453 types of wheat from 50 countries on the growth of annual ryegrass and found significant difference in the inhibition of ryegrass radicle growth from 9/7 to 90/9. Allelopathic potential of wheat seedlings from various countries has a noticeable difference which indicates the involvement of some genes for showing the allelopathic features. Among 453 types, 63 types showed strong allelopathic effects whith more than 81% inhibitory effect on ryegrass radical growth, while 21 types had weak allelopathic effects and less than 45% inhibitory effect on the ryegrass. The allelopathy of wheat seedlings is significantly in association with the country of origin. Some types, from Afghanistan, Canada, and Poland, showed allelopathic weak effects while Germany, Mexico and South Africa

types had strong allelopathic effects [4]. According to Huang, ryegrass radical growth has a significant decrease in the presence of wheat and the inhibitory level dependents on the number of wheat seedlings. In this experiment, increasing number of wheat seedlings leads to the increasing inhibition. Although the highest densities of wheat were able to inhibit the growth of ryegrass radicle, it had no effect on the growth of ryegrass shoot [4].

The purposes of experiment: using a new method for allelopathic studies in barley and weeds, using the equal-compartment-agar-methods a way to isolate the effects of competition and allelopathy.

Investigating the allelopathic effect of ryegrass and charlock on Valfajer and Reyhaneh barleys. Investigating the effect of factors such as plant density, and simultaneous or delayed planting on the allelopathic potential of barley and weeds cultivars.

MATERIAL AND METHOD

The experiment site: This study was done in Islamic Azad University of Fasa in 2012-2013.

a) **Seeds collection:** Two varieties of barley called VALFAJER and REIHANE were used in this study. The seeds samples were prepared from Fasa Cooperative Companies. Moreover the ryegrass and wild mustard weeds were collected from farms in Fasa one year ago and their germinations were tested before starting the main experiment.

b) **Procedure:** The experiment was done in two parts, laboratory and greenhouse, in factorial design through Completely- Randomized- Design (CRD) in four replications in Fasa Islamic Azad University laboratory. In this study, the allelopothic effects of two already mentioned varieties of barely on two already mentioned varieties of weeds were investigated.

c) **Barely seeds sterilization method:** the seeds of barley and weeds were disinfected by three minutes treatment in ethanol and, afterward, washed four times by sterile distilled water and for fifteen minutes by sodium hypochlorite twenty five percent and five times washing by sterile distilled water. The sterile seeds of varieties of barley and weeds absorbed water for twenty four hours in distilled water and five hundred lux light in twenty five degree centigrade. Thereafter, in order to achieve seedling, the swollen seeds were cultured in Petri dishes with filter paper and were placed in germinator with twenty five centigrade temperatures for twenty eight hours.

d) **The laboratory treatment (laboratory section):** In this experiment five density of seedlings of different varieties of barley consisting of 0, 8, 16, 24, 32 seedlings in each first half of beaker were used and the sixteen seedlings of weeds were cultured in the second half of beaker. In this part, culture of weeds seedling were done in two forms of concurrent and dilatory. The results of the study are reported under laboratory conditions.

e) **The equal-compartment-agar-method:** the equal-compartment-agar- metod (E.C.A.M) was used in order to investigate the allelopathic effects and to isolate the effects of competition and allelopathy. Agar is a gelatinous substance containing linear polysaccharides obtained from the cell walls of some red algae and seaweeds.

f) **Statistical methods:** The data is analyzed with SAS software and Duncan's method is used for means comparison. Sigma plot is used to draw graphs and also regression and correlation techniques along with three-parameter logistic model are used to explain the data.

RESULTS AND DISCUSSIONS

Effects of VALFAJER barley on ryegrass weeds: Different densities of VALFAJER barley caused one percent reduction of all other characteristic of weed significantly (Table-1).The different densities of 8, 16, 24, 34, by order, reduced the length of seedling, compared to the sample. The length of rootlet and caulicle were reduced. As the density increased, the already mentioned densities caused these two factors reduction compared to the treatment of sample. The more reduction of rootlet toward caulicle might indicate that the elongation of cellules might be affected by the prevention from Gibberellins and in dole acetic acid actions by allelopothic factors.

Effects of VALFAJER barley on wild mustard weeds: The results of analysis of variance table show one percent impact of different densities of VALFAJER barley on growth characteristic of wild mustard weeds seedling (Table-2). The results of the seedling length comparison showed that densities of 8, 16, 24 and 32, compared with the treatment control, respectively reduced the seedling length. Interaction process of radicle length to the different densities of Valfajer barley was the same as seedling length. The difference between treatments showed statistically significant difference. The mentioned densities respectively lead to the reduction in the radicle length. Brown and Morra and Labafi and et al. also reported the same findings [2].

The effect of Reyhaneh barley on ryegrass: various densities of Reyhaneh lead to the significant decrease of one percent in all measured features of the weed (Table-3). Densities of 8, 16, 24, and 32, respectively reduced seedling length more than the control. With the increasing density of barely, the radicle length will decrease so that the mentioned densities, compared with the control, respectively showed a significant reduction. Based on the findings of some researches various densities of barely create a competition

for factors such as dissolved food and minerals. The increase of control density leads to the competition between the seedlings of the same cultivar [2].

The effect of Reyhaneh barley on the charlock: results of the analysis of variance table showed the one percent effect of different densities of Reyhanehon charlock seedling growth features (Table-4). The comparison results of the seedling length showed that densities 8.16, 24, and 32, respectively create a more significant reduction than the treatment control. Interaction process of radicle length to the different densities of Reyhaneh barley was the same as seedling length and statistically there was significant difference between different treatments. Compared with the control, the mentioned densities lead to the reduction. Wu investigation also revealed the same results [5].

Investigation of the change process of length of ryegrass and wild mustard weeds rootlet and caulicle through regression equation: regarding the importance of regression studies in better interpretation of results and determination of the transmittal process of aforesaid characteristic to the densities of VALFAJER and REIHANE barley, length of rootlet and caulicle was justifiable through linear simple regression for determining the significant diversity between different varieties, the parameter of linear regression model were compared through confidence interval. Length of ryegrass rootlet and caulicle: The rootlet and caulicle reaction to VALFAJER and REIHANE barley densities obey the linear simple regression equation (Figure-1 and 2). This function could justify the rootlet and caulicle changes and all their model's parameters were significant (figure1, 2). The comparison of the fitted model in varieties of VALFAJER and REIHANE barley, according to confidence interval showed that the mentioned characteristic in terms of intercept (a) (intersection of regression lines) and model slopes (b) have no significant differences and model slopes between barley varieties have decreasing (negative) process. In fact, the negative process model slope shows the length of measured characteristic decrease by density increase. The achieved results reveal that ryegrass susceptibility to applied densities and its growth characteristic were affected by secreted substances from barley varieties but the differences between different varieties was not significant.

Charlock radicle and plumule lengths: Changes in charlock radicle and plumule lengths with different densities of Valfajer and Reyhaneh barleys was justified by a simple linear regression So that the coefficient model for the mentioned features was noticeable (Figures-3 and 4). Fitting the regression model to the data revealed that the reaction of radicle and plumule lengths features to the different densities of barleys follows a significant reduction. although the Valfajer and Reyhaneh barleys affect the charlock radicle and plumule lengths, comparing the parameters of the linear regression between two cultivars showed no significant difference in the intercept and slope of the regression line base on confidence interval; then, it can be concluded that there is no remarkable difference between barley cultivars in terms of the influence on the mentioned features. In the case of radicle length, nonlinear regression model justified this reduction with increasing density of barely planting, so that the density of 0-8 showed a sharp devaluation, 8-24 a slow devaluation, and again the density of 24-32 showed a sharp devaluation in the radicle length reduction (Figure-3 and 4).

DISCUSSIONS

The findings showed that there is a relationship between the density of barley seedlings and its inhibitory activity on weeds, so that the maximum density of seedlings leads to the maximum inhibition. Weed species differs in terms of their sensitivity to different barley cultivars. Barleys show noticeable differences considering the inhibition on weed species growth. The efficiency of the equal-compartment-agar-method in the separation of competition and allelopathic depends on the considered organ. For example, wu suggest that toxicity of plants' extracts varies among cultivars [5]. Among 39 studied cultivars, 15 types of wheat significantly decreased more than 50% of the radicle length of ryegrass; and among them just 6 types lead to more than 90% reduction in radicle length of ryegrass. Kalalak, kurung, and sunberry had a strong inhibition on ryegrass radicle growth with lengths of 0/2, 0/7, and 0/1 cm. the average inhibition of radicle growth was 52/4 and for the germination it was 31/2. Obviously, the inhabitation was more effective on the radicle growth than germination[6].Simultaneous and delayed planting also showed positive effect on the measured features and weeds; in this case, various studies proved this issue for example, according to the Bais studies, the wheat seedlings that were planted a week earlier than ryegrass had a significant inhabitation on ryegrass radicle length compared with control and simultaneous planting, however, no significant differences were found between the control and simultaneous planting. It indicates that, in the case of simultaneous planting of wheat and ryegrass seed, the accumulated allelochemicals at ryegrass planting time are unavailable. Both simultaneous and delayed planting showed remarkable effect on the ryegrass radicle growth. However, the simultaneous planting had inhibitory effect on the ryegrass radicle growth [1]. Delayed planting showed no remarkable effect on the ryegrass plumule growth. Moreover, growth of different organs of weed differ from each other due to the allelopathic effects of crop and also different cultivars of the same plant have various effect on weed which has been proved by several investigations [4]. Allelopathic potential of 453 types of wheat from 50 countries were studied on the growth of annual ryegrass and significant differences in ryegrass radicle growth inhibition, from 9/7 to 90/9, were reported. The allelopathic potential of wheat seedling from different countries revealed notable difference which indicates the interference of several genes for the expression of allelopathic features. Among 453 cultivars, 63 types had strong allelopathic effects with more than 81% inhibitory effect on the ryegrass radicle growth, while 21 type shad weak allelopathic effects with the less than 45% inhibitory

effect on the ryegrass radicle growth. The allelopathy of wheat seedlings was significantly associated with the country of origin. Some types from Afghanistan, Canada, and Poland showed weak allelopathic effects while, some types from Germany, Mexico and South Africa had strong allelopathic effects. Huang suggested that in the presence wheat cultivars ryegrass radicle growth significantly decreased, and the inhibitory level depends on the number of wheat seedlings during this experiment. Increase the number of wheat seedlings leads to the maximum inhabitation. Although the maximum density of wheat was able to inhibit the growth of ryegrass radicle, it had no effect on the growth of ryegrass plumule. Also, wheat seedlings planting, a few days before weeds planting, can be useful in allelochemicals realization and the inhibition of some weeds [4]. Allelopathic potential of wheat depends on ryegrass planting and number of wheat seedlings. There was a significant difference, between 23/98 -90/91, among the wheat cultivars at the seedling stage in terms of the effect of allelopathic potential on radicle growth of annual ryegrass. Interactions between crop and weed in the early stages of germinations is critical and if a weed species can be inhabited by crops allelopathic during the period of planting, later on the crop will be superior to the weeds.

Table-1. Analysis of variance of Valfajer Barley variety affect on seedling characteristic of ryegrass weed.

Dry weight of seedling	Wet weight of seedling	Caulicle length	Rootlet length	Mean square seedling length	Degree of freedom	S.O.V
0.001^{ns}	1.32^{**}	8.72^{ns}	3.94^{ns}	8.18^{ns}	3	Replication
0.009^{**}	0.90^{**}	25.02^{ns}	9.50^{**}	41.00^{**}	1	Culture time
0.014^{**}	0.83^{**}	38.90^{**}	23.12^{**}	112.68^{**}	4	Density
0.04^{**}	2.12^{**}	21.02^{**}	8.14^{**}	17.24^{**}	4	Density× Culture time
0.001	0.07	6.32	2.97	1.18	30	Error
10.21	9.09	8.70	10.16	9.09		CV%

Ns, *, ** - Non significant and significant at the 5%, 1% level of probability.

Table-2. Analysis of variance of Valfajer Barley variety affect on seedling characteristic of wild mustard weed.

Dry weight of seedling	Wet weight of seedling	Caulicle length	Rootlet length	Mean square seedling length	Degree of freedom	S.O.V
0.0005^{**}	0.54^{**}	4.42^{ns}	0.70^{ns}	5.82^{ns}	3	Replication
0.003**	2.07^{**}	21.02^{**}	15.62^{*}	72.90^{**}	1	Culture time
0.021^{**}	2.10**	79.22**	$39\text{-}81^{**}$	222.66^{**}	4	Density
0.003^{**}	0.22**	20.27**	16.25^{*}	13.96**	4	Density× Culture time
0.0007	0.11	2.38	2.88	1.49	30	Error
9.55	11.57	9.32	9.42	8.36		CV%

Ns, *, ** - Non significant and significant at the 5%, 1% level of probability.

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Table-3. Analysis of variance of REIHANE Barley variety affect on seedling characteristic of ryegrass weed.

Dry weight of seedling	Wet weight of seedling	Caulicle length	Rootlet length	Mean square seedling length	Degree of freedom	S.O.V
0.007*	0.70*	12.02*	10.75**	43.38**	3	Replication
0.096**	7.22**	16.10**	22.50**	67.60**	1	Culture time
0.11**	9.80**	135.30**	82.50**	410.04**	4	Density
0.009**	1.10**	18.91**	8.18**	26.53**	4	Density× Culture time
0.001	0.20	3.95	1.09	7.95	30	Error
8.78	10.51	10.54	11.80	12.21		CV%

Ns, *, ** - Non significant and significant at the 5%, 1% level of probability.

Table-4. Analysis of variance of REIHANE Barley variety affect on seedling characteristic of wild mustard weed.

Dry weight of seedling	Wet weight of seedling	Caulicle length	Rootlet length	Mean square seedling length	Degree of freedom	S.O.V
0.0009ns	1.19**	3.06ns	0.46ns	4.66ns	3	Replication
0.14**	16.64**	35.91**	37.83*	147.45**	1	Culture time
0.12**	15.40**	114.98**	128.75**	465.02**	4	Density
0.09**	3.69**	41.08**	38.02*	54.14**	4	Density× Culture time
0.002	0.13	2.68	2.19	9.25	30	Error
11.17	8.10	13.20	12.74	10.89		CV%

Ns, *, ** - Non significant and significant at the 5%, 1% level of probability.

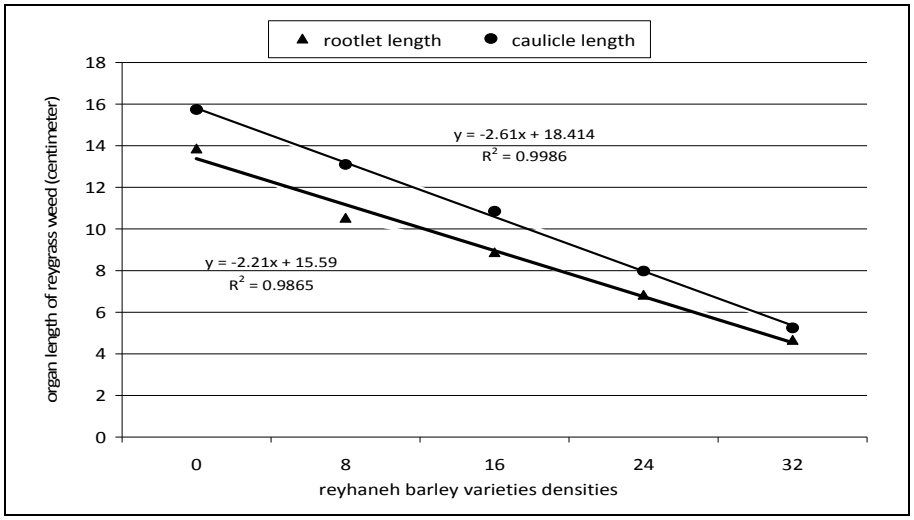

Figure-1. The effects of Reihane barley varieties densities on organ length of ryegrass weeds.

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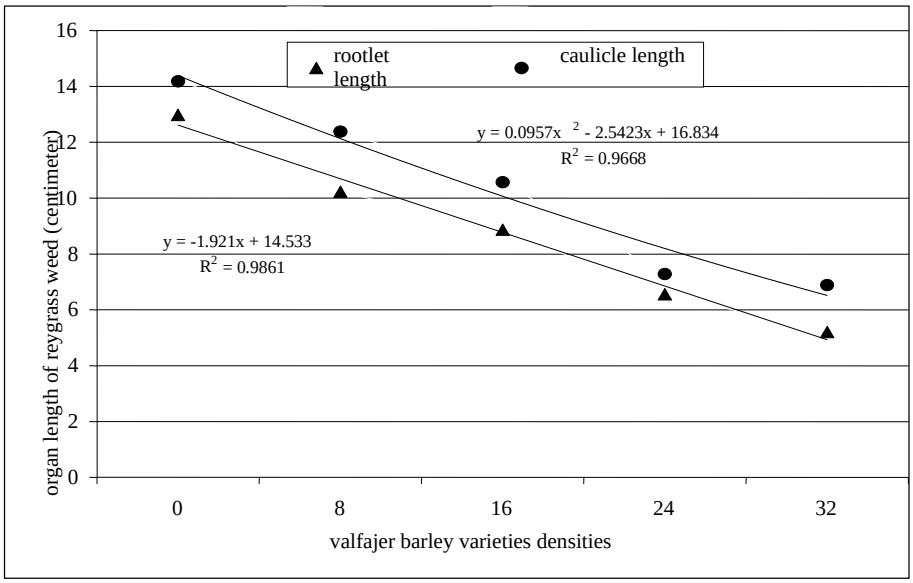

Figure-2. The effects of Valfajer barley varieties densities on organ length of ryegrass weeds.

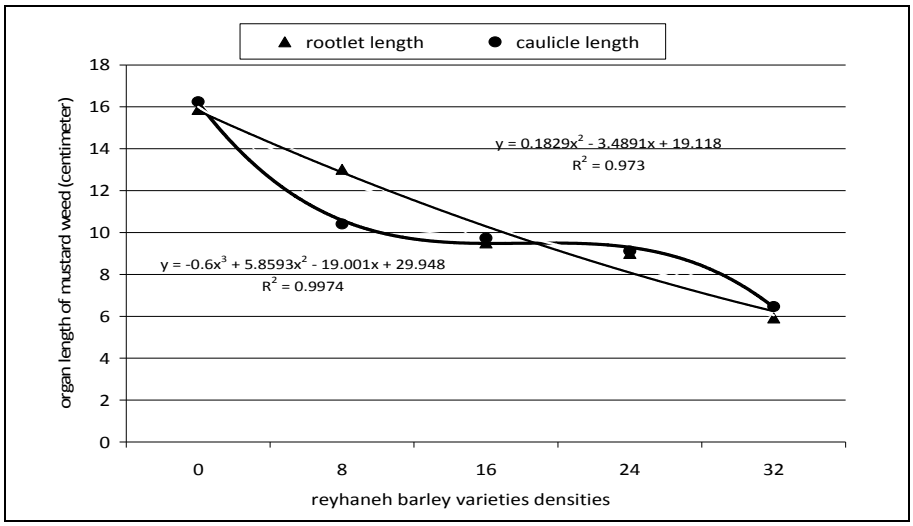

Figure-3. The effects of Reihane barley varieties densities on organ length of wild mustard weeds.

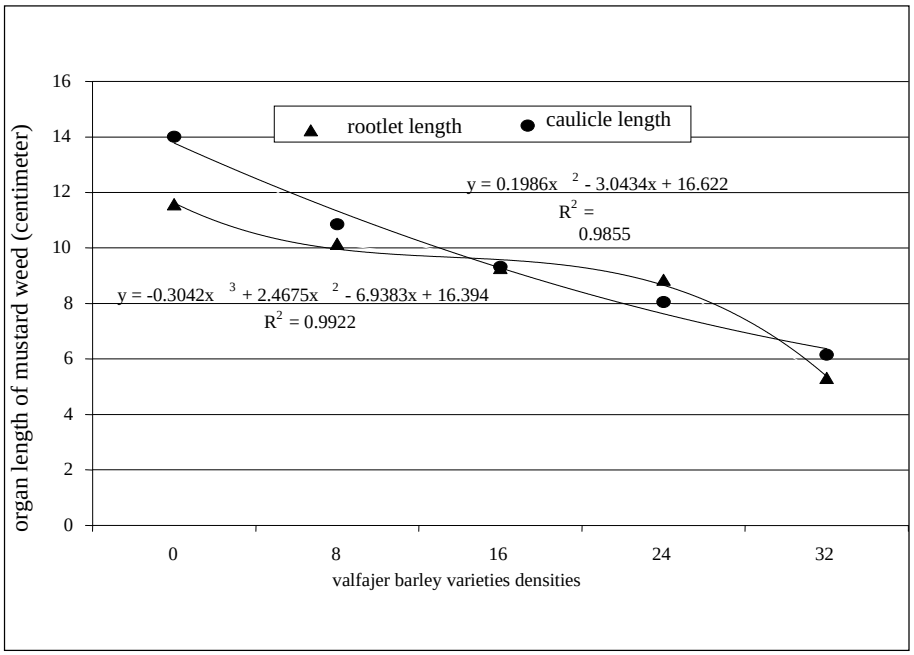

Figure-4. The effects of Valfajer barley varieties densities on organ length of wild mustard.

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