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A Survey on methods of Control of Egyptian Broomrape: control methods tested on Cellular metabolites of cucumbers before and after the connection

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ABSTRACT: *Egyptian Broomrape Orobanch is one of the parasitic weeds which clings to the roots of most dicotyledonous and absorbs its water and food requirements with haustorium from the host .The present study is on the effects of Orobanche control methods on cellular metabolites of cucumber plant . The experimental design was factorial arranged in basic completely randomized plot design with four replications carried out in the greenhouse of Azad university of Mahabad in 2014. The first factor was Orobanche control methods (1-vitamin c tablets; 2-clove extract; 3-Urea fertilizer; and 4- drought stresses) and the second was Orobanche clinging time to the host (before and after clinging).*

The results showed Orobanche clinging time and control methods had significant effect ($p \leq 0.01$) on a *and* b *chlorophylls. while interaction of clinging time and control methods on carotenoids has was significant at $p \leq 0.01$ and compared to the average interaction unlike other methods it has a rising trend after connecting to carotenoids by vitamin c methods . Comparing average of a and b chlorophylls in different methods showed the significance of urea fertilizer ($p \leq 0.05$) and it was found that urea fertilizer among other control methods has a significant impact on controlling Orobanch eaegyptiaca . Urea concentration by dry stem and root weight of cucumber and the number of Orobanche plants has was significant at $p \leq 0.01$.*

Keywords : *Orobanch egyptiace, cucumber, cellular metabolites, control methods.*

INTRODUCTION

Egyptian Broomrape or Orobanch aegyotiaca is an obligate holoparasite weed which does serious damages to the crops such as sunflower, potato, cucumber and tomato each year in Iran, especially in Western Azerbaijan. Since this weed has no leaf and chlorophyll, connecting to the roots of the host, the parasite is then able to draw out water and nutrients from the xylem and phloem of its host which results in loss of function, wilting of the host. Orobanche parasites the cucurbit plants such as cucumbers and tomatoes and it causes their complete destruction in certain years. (Herdhenhorn *et al*, 2009; Barker *et al*, 1996). So far, many studies have been done to control the Orobanche, and efforts made to make proprietary herbicides to control this parasite; though, the results show that almost all the efforts have been unsuccessful. Nandula (1998) reported inhibitory effects of nitrogen on the growth of Orobanche when farmers used fertilizer compost in the nineteenth century. Jain and Foyo (1999) reported that urea fertilizer decreases the root length and germination percentage in Orobanche plants. Abu- Irmaileh (1994) reported that increasing the concentration of ammonium nitrate from zero to 100ppm directly reduced the germination and root length in Orobanche host plants (flax, lentils, peppers and tomatoes).

The use of allopathic properties of plants to control weeds has risen among farmers in recent years due to environmental pollution of herbicides. On the other hand, chemical control of this weed has not brought about satisfactory results because of using selective herbicides (Forouzesh *et al*, 2007) .

Thuring *et al*., (1996) had taken root the first natural stimulant for Orobanche seed germination from cotton secretions (Dhnapal *et al*., 1996). High concentration of cotton extract prevents seeds from germinating, but it serves as a stimulant in lower concentrations. One prominent feature of Orobanche seed germination is the stimulation by the host root extract. The Long-term viability of Orobanche seeds

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in the soil (Seed Bank) is the secret of this plant's strength and competence. The impact of Orobanche's connections to cell metabolite content such as chlorophyll, carotenoids and soluble sugars, which are affected by the photosynthesis phenomenon in plants, have been reported. Thus, this research aims at studying the reactions of a cucumber plant, which is a viable crop to Orobanche because of metabolite-sensitive cell, during applying some control methods against Orobanche infection risk.

METHODS AND MATERIALS

To gather Orobanche seeds and weeds from the infected fields, infected farms in the summer of 2013 at Mahabad city were identified to collect Orobanche weeds and seeds. Identifying the most infected farm, grown plants of Orobanche were harvested from the roots, transferred to the seed testing and certification laboratory and seeds were separated under laboratory conditions. Applying germination tests on seeds, it a was found that seeds have almost 98% of viability or Germination Percent (Linke *et al.,* 1992)

Soil Providing

Testing soil was provided from a pristine and unspoiled meadow and disinfected with oven in 105 ° C with low organic matter (01/0>), nitrogen (0.1%>) which P.K was less than 159, 8.32, ppm respectively.

Pilot projects and the location of implementation

The experiment was conducted as a pot research in natural conditions in the greenhouse at the Crop Research Greenhouse at Islamic Azad University of Mahabad in 2014 on the basis of completely randomized factorial design with four replications. The first factor was cucurbit or kitchen garden control methods, including vitamin C pills, extracts of cloves, urea fertilizer, drought and the second factor was the connection of Orobanche to the host, including before and after the connection. Three preliminary tests were conducted to determine the appropriate amount of the dose or premium amount of the treatment. Accordingly, the concentrations of the treatment does were calculated and applied respectively in the experiment as follows: Vitamin c tablets (8 tablets of net vitamin C (Ascorbic Acid) in one liter of distilled water) extracts of cloves (8 grams in a liter of distilled water) urea fertilizer in three experiences during the growth period (11 ± 5 / 0 g / each pot = 150 kg / ha). Pilot or experimental pots were filled with three kilograms contaminated soil and a 0.001gram Orobanche seed embracing 98% GP (Germination Power) or viability was planted in the pots at the depth of 5 cm. Planting operations are carried out by wet planting method and the viability of the local cucumber seed was 99%. Ten cucumber seeds were planted in each pot and watered regularly with civil tap water. Greening and growing the plants, treatment was applied to the four remaining plants in the 4-leaves. Determining the soil fertility capacity to apply the drought treatment, soil moisture content in the period of 50 % crop fertility compared with the control group for 12 days during the test. The following measurements were made after the treatment:

1- Traits related to the cellular metabolites containing photosynthetic pigments, including chlorophyll a, b and carotenoids.

Chlorophyll and Carotenoids' Measurement

5.0 grams of fresh leaf tissue gradually eroded in the mortar with 80% volume acetone to measure chlorophyll and carotenoids. The eroding was continued till chlorophyll entered acetone and the amount of the liquid in the balloon reached 10cc. The resulting solution was centrifuged at 3000 rpm for 10 minutes, then the absorption of the filtrated solution was read in waves of 470,645, 663 nm by spectrophotometer 2100-Vis. Next, the flowing relation was used to analyze the results (Irigoyen *et all,* 1992).

$$\text{Chlorophlla} = (19/3\times A663 - 0/86\times A645)/V\ 100W$$

$$\text{Chlorophlla} = (19/3\times A645 - 3/6\times A663)\ /\ V\ 100W$$

$$\text{Carotenoides} = 100\ (A470) - 3/27\ (\text{mg ch.a}) - 104\ (\text{mg chl. b})/227$$

Measuring the sugars of the solution

Measuring the sugar of the solution, 0.5 g of dried leaves is combined with 5 ml of 95% volume ethanol in a mortar and leaves are crushed. Next, the transparent and clear part of the extract is isolated and it is kept in a caped test tube. Sediments are crushed with 70% volume ethanol again and isolation action is repeated once more. Extracts centrifuged at 3500 rpm for 10 minutes at 10°C and then they are placed in a boiling bath with 100° C a temperature for 10 minutes after adding anthrone to the extract. After cooling, the amount of light absorbed by the samples is read by spectrophotometer at 625 nm wavelength (Irigoyen et al., 1992). Following equation is used to provide standards for pure glucose and total amount of the sugars: $y = ax + b$

TRAITS RELATED TO THE MORPHOBIOLOGICAL CHARACTERISTICS OF OROBANCHE

Measuring the number of bushes and height of the Orobanche

Using the millimeter ruler, the height of Orobanche was measured, and the number of bushes was also counted.

Orobanche's dry weight of the root and pedicle

Cucumber plants were removed from the pots and Orobanches bushes were carefully separated to measure Orobanche's dry weight of the root and pedicle. Bushes were put in the paper bags and placed in the oven at 70 ° C for 48 hours. Using digital scale with 0.001 precision, dry weights of Orobanches calculated and determined.

The analysis of data

Using the SPSS 21 software, Statistical analysis of data was done by comparing the means and using Duncan's multiple range tests with probability or p-level of 1% and 0.05.

RESULTS AND DISCUSSION

Photosynthetic pigments

The results of variance analysis showed that photosynthetic pigments have been influenced by the first factor, control methods of Orobanche, and it is significant at the 1% level.

Table 1
Analysis of variance of control methods and connection time on chlorophyll a, b and carotenoids

S.O.V	*df*	*Mean-square*		
		chlorophyll a,	*chlorophyll b*	*carotenoids*
Control Methods	1	1.762**	0.354**	0.019*
Connection Time/	4	0.177**	0.117**	0.047**
Control Methods Control Methods	4	0.059ns	0.049^{ns}	0.015**
Pilot error	30	0.032	0.018	0.003
CV		20.25	17.37	13.26

Nonsignificant, significant at p value of 5% and 1% respectively= ** , *ns,

The comparison the means showed that urea fertilizer has the greatest impact on the amount of chlorophyll a, b and carotenoids. Providing positive absorb reactions in the plant, urea fertilizer increases the amount of chlorophyll a and chlorophyll b and carotenoids 54, 49 and 72 percent respectively in comparison with the control group. Nitrogen plays an essential role in formation of chlorophyll ring. Effecting on the core, urea fertilizer will provide an increase in photosynthetic motives obviously. Lack of Nitrogen yields in yellowing the corn leaves color, and inhibits the plant's growth (Malakut *et al.,* 1974). When Nitrogen deficiency increases, the plant lignifies that is the result of making excessive carbohydrates because no other factor can be used in producing amino acids and other nitrogen compounds) kaffe et al., 2002 .(Urea fertilizer has a high absorption ability and it has a great mobility after absorption. In this study, an increase in chlorophyll b and carotenoids is due to increase in the amount of chlorophyll content.

Soluble sugars

Analysis of variances showed that there is no significant difference in the percentage of soluble sugars in leaves of cucumber in methods used to control (Table 2).

Table.2
Analysis of variance of control methods for Sugar soluble ,Root dry weight ,Shoot dry weight

S.O.V	*df*	*Mean-square*		
		Sugar soluble	*Root dry weight*	*Shoot dry weight*
Control Methods	4	2.128	0.128	2.239**
Experimental error	15	0.729	0.012	0.127
CV		29.28	7.95	25.84

Non significant, significant at p value of 5% and 1% respectively= ** , *ns,

Morphological traits

Root dry weight

Analysis of variance showed that there is a significant difference at the 1% level of the root dry weight in the methods used to control Orobanche weed, (Table 2). Comparison of the data showed that the highest root dry weight in the treatment with urea is (0.607 g) and the lowest root dry weight is related to the treatment with vitamin C(0.177g); although, no significant difference was observed in other methods of treatments. Since the root of the cucumber is the first part of the plant that is invaded by Orobanche, it can be concluded that the more Orobanche invades the plant, the less the cucumber root grows because Orobanche is considered as the cucumber root parasite.

Furthermore, investigating the effect of nitrogen fertilizer on the root of the sunflower after infecting by Orobanche the results of the research done by Mesbah et al (2013) showed nitrogen fertilizer derived from urea leads to the greatest increase in root dry weight of sunflower. Also Marium and suwanketnikom (2004) showed that the greatest amount of root dry weight of tomato plant infected by Orobanche has been attained when the treatment with urea has been applied.

Stem dry weight

Results of analysis of variance for the stem dry weight showed that there was a significant difference at the 1% level between experimental treatments to control the Orobanche, (Table 2). The comparison of the means showed the highest stem dry weight in the treatment of urea is (2.245g) and the lowest is obtained in vitamin C treatment (0.385g). Urea, obviously, has a considerable influence on the growth of the root and shoot. Nitrogen increases cell division rate, although it is absorbed through the roots, urea has high mobility in plants. Beheshti (2002) reported that nitrogen usage can improve photosynthetic radiation absorption rate, PAR and RuE. These two parameters increase stem dry weight. Swaefy et al (2007) reported that urea fertilizer can have a positive and meaningful effect on increasing the Mentha piperita height .

Minbshi *et al* (2000) indicate tomatoes shoot dry weight is significantly increased when treated with nitrogenous fertilizer in the presence of Orobanche infection.

Height and number of Orobanche

Data analysis of variance shows that there is a significant difference in the level of 1% in Orobanche's control procedures in terms of the impact on the quality and number of bushes. Comparing the means, the results show that the largest number of Orobanche belongs to the treatment of the control group of plants (13cm) and the lowest amount belongs to the treatment (2cm) (Table 3).

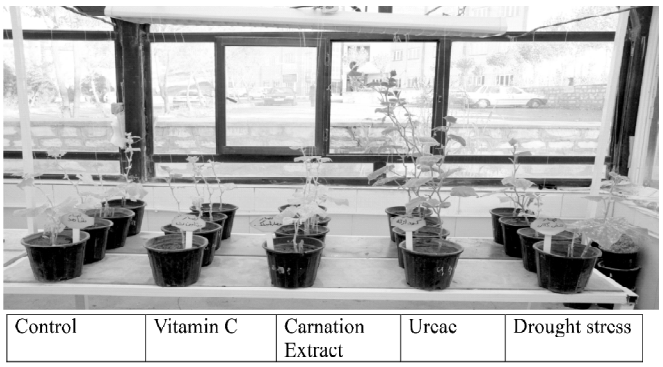
Table 3
Analysis of variance of control methods for the number and height of Orobanche

S.O.V	*df*	*Mean-square* *Number of Orobanche bushes*	*Height of Orobanche bushes*
Control methods	4	0.729**	0. 218^{ns}
Experimental Error	15	0.065	0.076
CV		29.75	32.17

Insignificant, significant at p value of 5% and 1% respectively= ** , *ns,

There are numerous reports showing nitrogen fertilizers have a positive effect on reducing the density of Orobanche weed. Nandula (1998) stated that ammonium sulfate in the presence of 14 and 28 kg nitrogen in a hectare decreases damages of Orobanche to species of broad beans, Krnata.

Picture 1: Before Egyptian Broomrape connection.



Picture 2: After Egyptian Broomrape connection

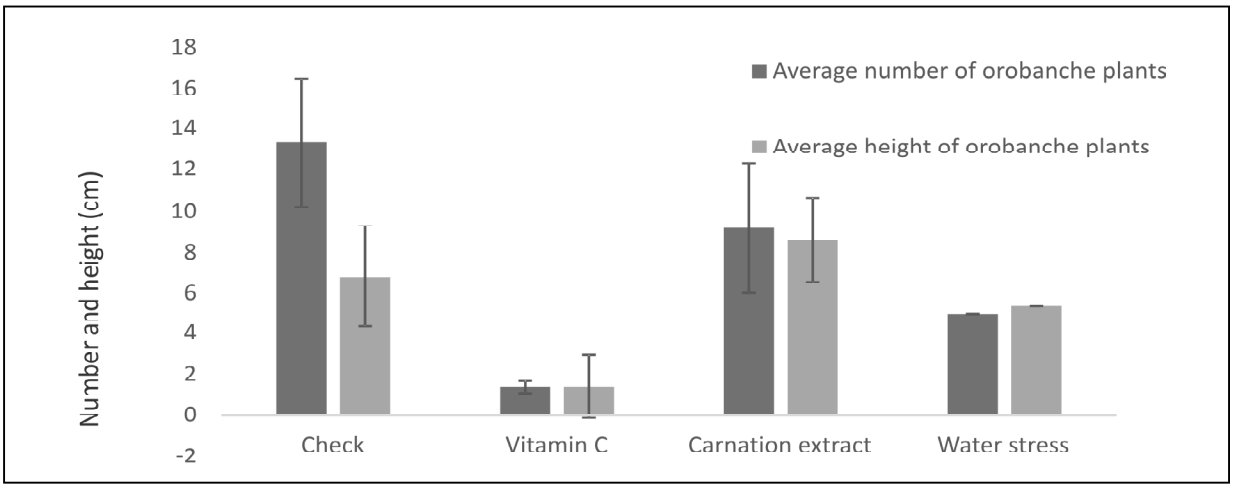


Figure 1: Average number and height of orobanche plants for different treatments

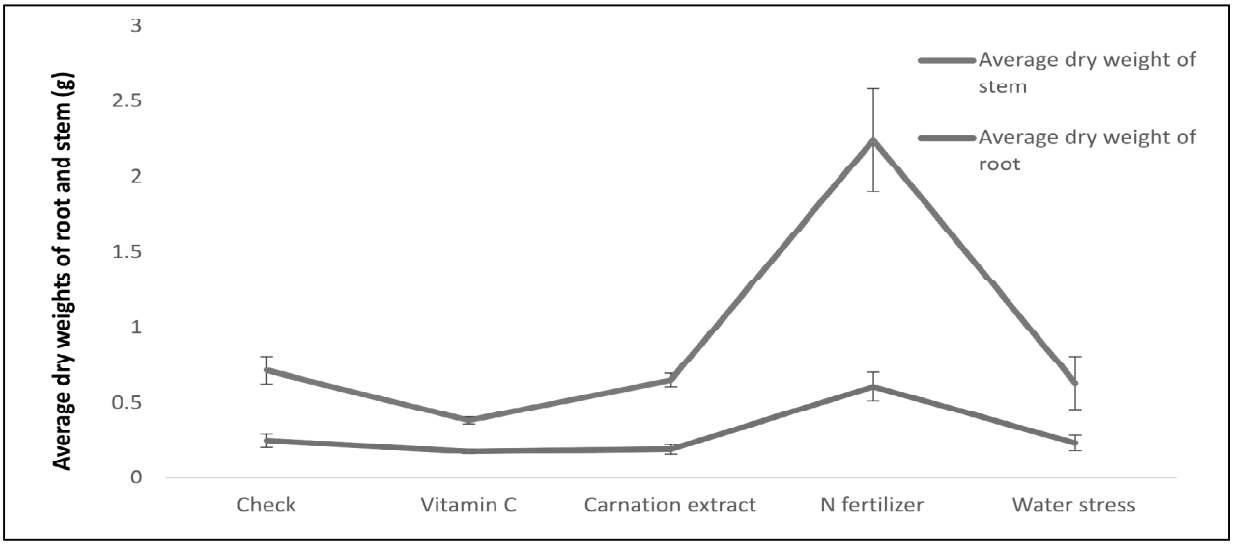


Figure 2: Average dry weights of root and stem

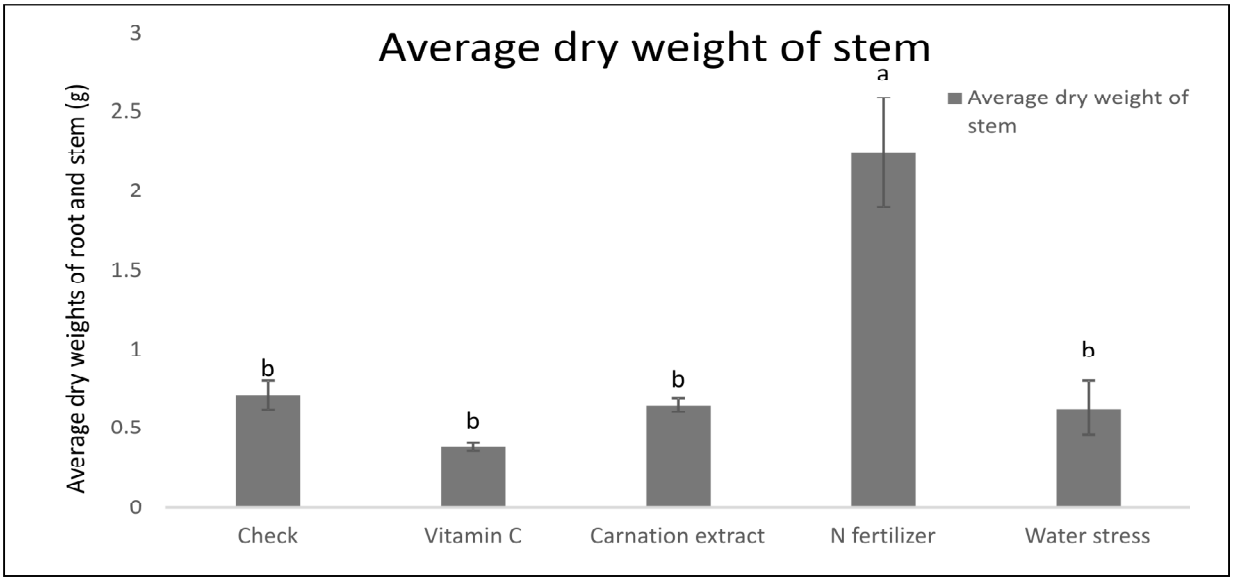


Figure 3: Average dry weights of root and stem

Hezewijk (1993), van Hezewijk *et al.* (1981) and Abu-Iemaileh (1996) in studies also show the positive effect of nitrogen fertilizer on reducing germination and parasitization of Orobanche.

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