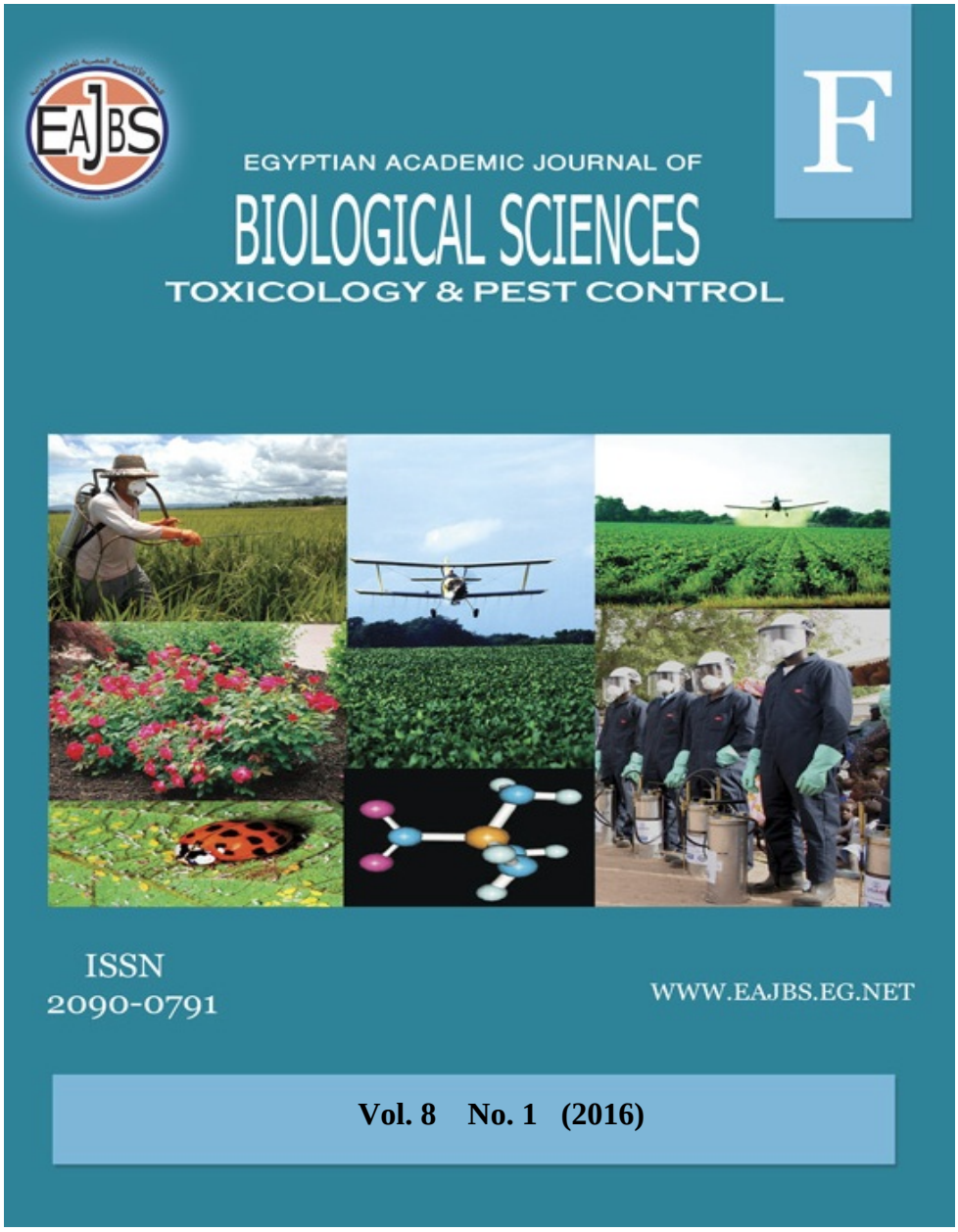

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Effect of Sodium Chloride on Germination of Dodder (*Cuscuta trifolii Bab*) and Clover (*Trifolium alexandrinum* L.) Seeds

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ABSTRACT

Different concentrates of sodium chloride(100, 200, 300,400 and 500 mM) were applied against the seeds of dodder(*Cuscuta trifolii Bab*), at the faculty of agriculture (Saba Basha), Alexandria University during the years of 2014 and 2015. Different morphological and biochemical characters were assessed. The effects of different concentrates of NaCl on seed germination and protein of *Cuscuta trifolii Bab* and *Trifolium alexandrinum* seeds cleared that *Trifolium alexandrinum* is more tolerant to salinity than *Cuscuta trifolii* Bab. So, NaCl can be used as a pre emergency agent to control the seeds of *Cuscuta trifolii* Bab to avoid the harmful herbicides that affect the environment and contaminate the foliage. At the Same time it will be more safe in animal feeding.

INTRODUCTION

Clover (*Trifolium alexandrinum* L.) is widely grown for forage in several countries. Mean while, weeds area major problem in agriculture. Clover is an important leguminous forage crop used as cattle feed, and is introduced in different ways. It is strongly suppressed by weed infestation especially with the parasitic weeds dodders (*Cuscuta sp*) considered as serious parasitic flowering plants (Altekretee *et al*., 1981). Dodder seeds have the ability to remain viable for many years (Karapetyan 1972 and Ashton, 1976).As, weeds cause economic losses in crop growing areas but, we have to keep their level below the economic threshold level.

Chemical methods for controlling weeds are used routinely to control weeds all over the world (Giudice, 1981).Today, we have not clear strategies for controlling the dodder weed. Farmers usually apply only traditional methods such as solarization, spraying herbicides, cultivating resistant varieties, trap and catch crops or cultural methods .The shortage of chemicals and their high costs increase the difficulties of using the herbicide in the fields. Dodder can be controlled, after attaching clover plants, by means of chemicals but this sort of treatment may have a negative effect on clover forage. More than 900 million hectares of land world-wide, approx. 20 % of the total agricultural land, are affected by salt, accounting for more than 6% of the world's total land area. NaCl is the predominant salt causing salinization, and it is unsurprising plants have evolved mechanisms to regulate its accumulation (Munns and Tester, 2008).

Therefore, the purpose of this study was to determine (a) whither the germination of *Cuscuta trifolii* Bab and *Trifolium alexandrinum* was inhibited by an osmotic effect or a specific ion effect, (b) the effect of osmotic stress on the percentage of seed germination, (c) the ability of seeds to germinate after being treated with different concentrates of the salt, and (d) The effect of salt concentrations on protein content for both species based on SDS-page**.**

MATERIALS AND METHODS:

The present experiment was carried out at the faculty of agriculture (Saba Basha) Alexandria University during the years of 2014-2015. Completely randomized design with three replication and five salinity levels of NaCl (100, 200, 300,400 and 500mM)were used. Each experimental unit included three Petri dishes (100×150 mm) dimensions in which15 healthy and homogenous seeds were put on the No.1 Watman filter paper. Seeds of *Cuscuta trifolii* Bab and *Trifolium alexandrinum* were obtained from agricultural research station in Alexandria .First, to disinfect the seeds; they were dipped in 10% Hypochlorite Sodium solvent then washed three times by distilled water. Each Petri dish was supplied with a dose of six ml of the considered concentrate. Eventually, their lids were closed by parafilm and had been located in growth room. The temperature adjusted in 25o C. This experiment took 7 days.

Inspected measurements:

1- **Germination Percentage (G. P.):** was calculated daily at a specific time beginning from the second day, for *Trifolium alexandrinum* and *Cuscuta trifolii* Bab. Germination was considered when the seed has a root of more than three mm length. The experiment continued till we could find more germinated seeds. Germination percentage. GP: Ni / N × 100, where, Ni: number of germinated seed till i^{th} day and N= total number of seeds.

2- **Germination Race (GR):**was calculated beginning from the second day to the5^{th}day ,24 hours intervals, germinated seeds were counted, and their race was determined by Maguire equation (Maguire, 1962)

$$GR = \sum_{i=1}^{n} \frac{St}{Dt}$$

G. R.: Germination Race (number of germinated seed in each day), Si: number of germinated seeds in each numeration, Di: number of days till n^{th} numeration and n: number of numeration times.

3- **Seed vigor (S. V.):** this index was determined by the following formula according to Abdul-baki and Anderson, (1970) method: Strong seed index = {germination percentage × means of seedling length (radical + plumule)mm}/ 100

4- **Dry weight(gm.):** 10 selected plants from each Petri dish separated their radicle, plumule and measure each plat's radicle and plumule length separately. Plants dried in oven with a temperature of 75^{o}C for 24 hours.

Protein Analysis

SDS–polyacrylamide Gel Electrophoresis (SDS-page) of Seed Storage Proteins: Seeds of *Cuscuta trifolii* Bab and *Trifolium alexandrinum* which were treated with different levels of salinity were squashed. Then, both water soluble and water-non soluble protein were sequentially separated in 1.5 ml Eppendorf tubes according to Laemmli (1970). Separating gel (12.5%) was made by solubilizing 20 µg protein with sample buffer (62.5 m MTris-HCl, pH 6.8, 20% (w/v) glycerol, 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol and 0.01% (w/v) bromophenol blue). Electrophoresis was performed at 80 V

for two and half hours by using Bio-Radelectrophoresis system. Coomassie Brilliant (0.25%) Blue R-250 was used for staining for 2 hours and then destained with 50% methanol and 10% acetic acid. The molecular weight of protein was analyzed by comparing sample bands to the standard protein molecular weight marker bands (116.0 – 14.4kDa, New England).

Statistical analysis:

Statistical analysis was run to check the significance of differences between mean treatments (Goulden, 1952). The least significant differences (L.S.D) were determined according to Duncan (1955). Protein gels were scored as 0/1 for absence/presence of the bands, respectively and the resulting 35 protein bands were analyzed using the NTSYS-pc2.0 software (Rohlf, 1998). Phylogenetic dendrograms were constructed using the UPGMA method (Unweighted Pair-Group Method with arithmetical algorithms Averages; Sneath and Sokal, (1973)

RESULTS AND DISCUSSIONS

Effect of different NaCl concentrates on seed germination of *Trifolium alexandrinum* and seedling growth characteristics.

1- Germination percentage and race:

The obtained results in Table (1) and Figure 1 cleared the effect of salinity stress level on germination percentage and race of *Trifolium alexandrinum*. There were high significant variations between all salt concentrations in relation to both characters. The maximum germination percentage was 93.33% at the salt concentrations of 100 and 200 mM, respectively. Germination percentage was 46.67%at the salt concentrations of 300mM. While, the minimum germination percentage was zero% at the salt concentrations of400 and 500 mM.The germination race was affected by the different salt concentrations. The maximum germination race occurred at the concentration of 100 and 200 mM recording(14%), while, the lowest germination race value was zero at 400 and 500 mM.

2- Radicle and plumule length:

The obtained results in Table (1) showed significant differences in radicle and plumule length of *Trifolium alexandrinum* under different salt concentrations. Results showed that the more the salinity level increases the more the seedlings radicle and plumule length is decrease. The maximum reduction in radicle length and plumule related occurred at 400 and 500 mM by zero. The maximum radical length occurred by the concentration of 100Mm by 1.5 cm. Also, the highest plumule length occurred by the concentration of 100 Mm by 3.87 cm.

3- Seed vigor:

The results presented in Table (1) and Figure 2 showed the effect of different salinity levels on seed vigor index of *Trifolium alexandrinum*. The utmost reduction of seed vigor index took place under 400and 500mM which recorded a value of zero. Whereas, the least reduction of seed vigor index was 5.028 under 100mM.

4- Wet and dry weight:

Impact of salinity stress concentrations on wet and dry weights of *Trifolium alexandrinum* was shown in Table (1). Wet and dry weights had been decreased with the increase of salinity levels. Increasing salinity levels, seedlings wet weight amounts decrease extremely with 300mM(0.042g). The utmost wet weight was achieved by 100mMby (0.094g) while the utmost dry weight was achieved by 100mMby (0.011g). On the other hand, the lowest dry wet weight under300mM by 0.006g.

Table 1: Effect of different NaCl concentration on seed germination of *Trifolium alexandrinum* and growth of seedling characteristics

Concentrations (Mm)	Germination percentage (%)	Germination race	Plumule length (mm)	Plumule length (mm)	Seed vigor	Wet weight (g)	Dry weight (g)
100	93.33[a]	14[a]	1.5[b]	3.87[b]	5.028[b]	0.094[a]	0.011[a]
200	93.33[a]	14[a]	1.47[b]	2.1[c]	3.317[c]	0.06[b]	0.008[b]
300	46.67 [b]	7[b]	0.57[c]	0.6 [d]	0.553[d]	0.042[c]	0.006[c]
400	0.0[c]	0.0[c]	0.0[d]	0.0[d]	0.0[d]	0.0[d]	0.0[d]
500	0.0[c]	0.0[c]	0.0[d]	0.0[d]	0.0[d]	0.0[d]	0.0[d]
Control	100[a]	15[a]	2.83[a]	5.33[a]	8.166[a]	0.1[a]	0.011[a]
LSD=0.05	8.386	1.258	0.487	0.757	0.840	0.015	0.001
***Similar letters in each column hadn't any significant statistical difference**							

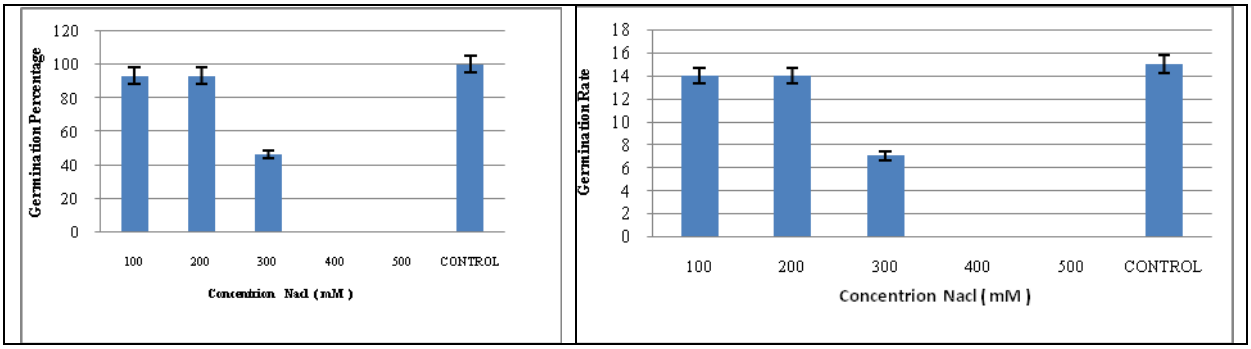

Fig.1: Effect of different concentrations of NaCl on germination percentage (left) and rate(right) of *Trifolium alexandrinum*

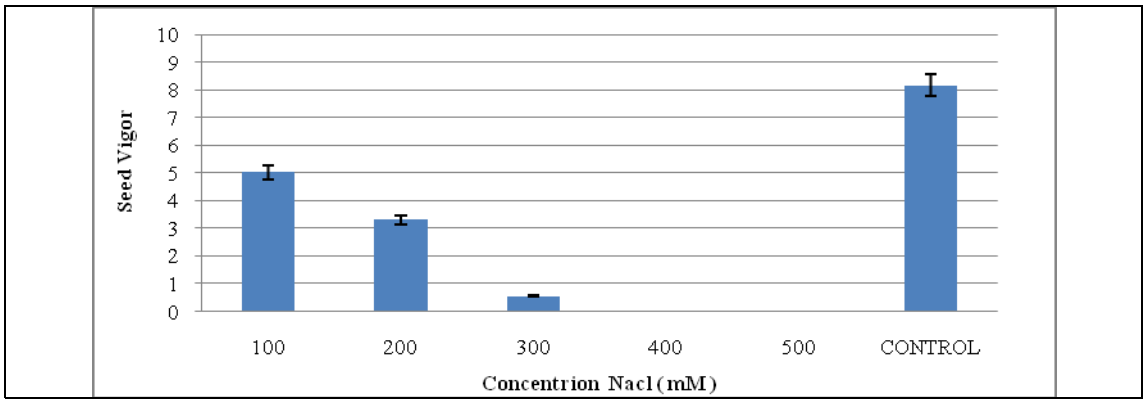

Fig. 2: Effect of different concentrations of NaCl on seed vigor of *Trifolium alexandrinum*

Effect of different NaCl concentrations on seed germination of *Cuscuta trifolii* Bab and the growth characteristics of the seedlings.

1- Germination percentage and race:

The results in Table (2) and Figure 3 showed the effect of different salinity concentrations (100, 200, 300, 400 and 500mM) on the germination percentage and germination race of *Cuscuta trifolii* Bab. The highest germination percentage and germination race occurred by the 200 mMby means 35.56 and 5.33, respectively, followed by 100 and 300mM which recorded 33.33 and 22.22 for germination percentage and (5and 3.33) for germination race, respectively. The results showed that the high salt levels effect of the germination percentage and rate for *Cuscuta trifolii* Bab In conclusion, this safe material can be used to control the *Cuscuta trifolii* Bab as an alternative and safe material.

Table 2: Effect of different NaCl concentration on seed germination of *Cuscuta trifolii* Bab and growth of seedling characteristics

Concentrations (Mm)	Germination percentage (%)	Germination rate	Plumule length (mm)	Plumule length (mm)	Seed vigor	Wet weight (g)	Dry weight (g)
100	33.33^{b}	5.0^{b}	0.57^{b}	1.83b	0.8^{b}	0.043^{b}	0.003^{b}
200	35.56^{b}	5.33^{b}	0.53^{b}	1.87^{b}	0.86^{b}	0.041^{b}	0.003^{b}
300	22.22^{c}	3.33^{c}	0.4^{b}	1.4^{b}	0.39^{b}	0.039^{b}	0.002^{b}
400	0.0^{d}	0.0^{d}	0.0^{b}	0.0^{c}	0.0^{b}	0.0^{c}	0.0^{b}
500	0.0^{d}	0.0^{d}	0.0^{b}	0.0^{c}	0.0^{b}	0.0^{c}	0.0^{b}
Control	100^{a}	15.0^{a}	2.33^{a}	3.03^{a}	5.37^{a}	0.087^{a}	0.017^{a}
LSD=0.05	9.271	1.391	0.543	0.658	1.206	0.004	0.003
***Similar letters in each column hadn't any significant statistical difference**							

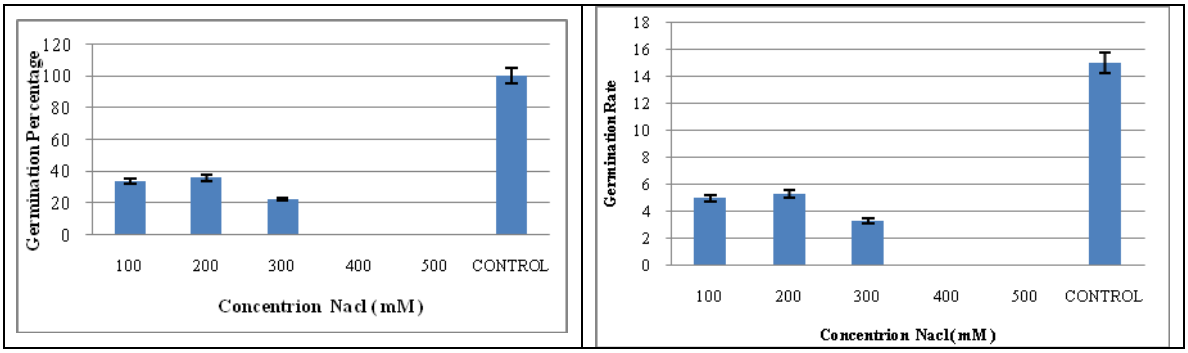

Fig. 3: Effect of different concentrations of NaCl on germination percentage and rate of *Cuscuta trifolii* Bab

2- *Radicle and plumule length:*

The effects of salinity stress on radicle and plumule length have been shown in Table (2). The obtained results declares a significant differences in radicle and plumule length affected by different salt concentrations.The results in Table (2) showed that different concentrations of NaCl have an effect on the radicle length and plumule of *Cuscuta trifolii* Bab. Comparison of radicle and plumule length of *Cuscuta trifolii* Bab at different salinity levels (100, 200, 300, 400 and 500 mM) showed that when salinity level increase, seedlings radicle and plumule length were decreased (Table 2). The most reduction in radicle length and plumule related to 300mM (0.4 and 1.4 respectively). These results are in the line with Munns and Termaat, (1986) who suggested that salinity decrease radicle and plumule growth and increasing salinity level, increases the amount of reduction. Also Salinity, declines plumule and radicle growth, and by increasing salinity the reduction increases. Salinity which is a result of osmotic pressure leads to reduction in water absorption so, cell division and differentiation reduce and reduction of plumule and radicle length will be explainable.

3- *Seed vigor:*

The highest effect on seed vigor index caused by different salt concentrations (400 and 500 mM) which recorded (zero value) followed by 300 mM (0.39), 200 Mm (0.86) and 100 Mm (0.8). Generally, race and percentage of germination and seed vigor index is related to a special impact of ions and reduction of environmental water potential in the presence of salinity. Results showed that when salinity increases (reduction of environmental osmotic potential), seed characteristics decrease. These results are in accordance with the finding of Kader and Jutzi,(2004).

4- *Wet and dry weight:*

Impact of salinity stress concentrations on wet and dry weights of the seedlings of *Cuscuta trifolii* Bab was shown in Table (2) and Figure (4). Salinity stress on wet and dry weight had been decreased with the increase of salinity. By enhancing salinity levels, seedlings wet weight amounts decrease extremely with 300 mM (0.039). The highest wet weight occurred by 100mMin mean (0.043) but, the highest dry weight occurred by 100mM (0.003). The lowest dry wet weight occurred by 400 and 500mM (a value of zero).

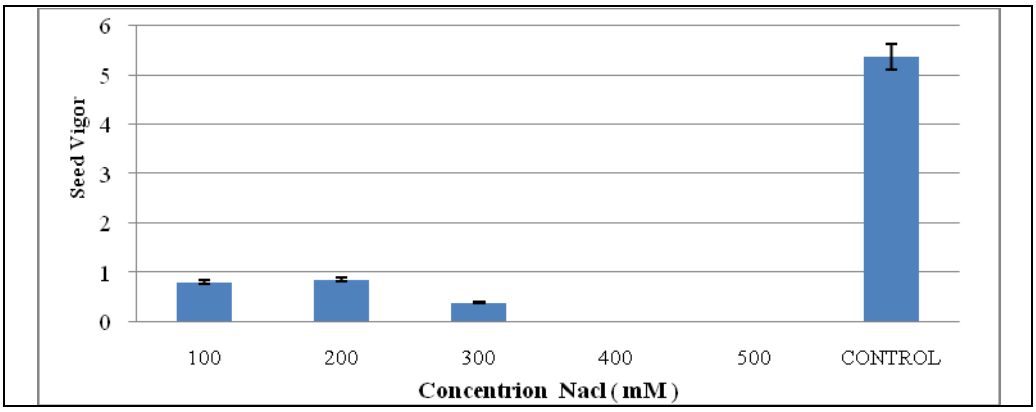

Fig. 4: Effect of different concentrations of NaCl on seed vigor of *Cuscuta trifolii* Bab

These results agree with many authors such as Al-Jibury and Clor (1986) who demonstrated similar results with bitter lentil (*Securigera securidaca*). They found that for seedling growth, osmotic pressure had a greater effect on seed germination. Also, our results are in line with Egan *et al.* (1997) who tested the effects of sodium chloride, potassium chloride, sodium sulfate, and potassium sulfate on *Atriplex prostrate* seed germination and early growth. they found similar results to those of the present study. We found that germination of *A. acanthocarpa* was inhibited by an osmotic effect as opposed to specific ion toxicity. Etesami and Galeshi (2008) reported that salinity is the cause of reduction in germination percentage, race and homogeneity of germination and dry weight of barley (*Hordeum vulgare*) seedling. whoever, Massai *et al*. (2004) mentioned that salinity is delaying plant growth under reduction of photosynthesis effects, it causes closing stomata and reduction of water entrance into the plant and so that cause duplicate reduction in plant weight. Also, Redman *et al*. (1994) showed that this reduction in dry weight of plumule and radicle as results of enhancing the salinity concentration is a normal phenomenon and probably it is the result of low water absorption by germinating seeds.

Protein Content and Activity

SDS-PAGE analysis showed considerable variations in protein profiles in both *Trifolium alexandrinum* and *Cuscuta trifolii* Bab (Table 3 and Fig.5). One specific band was unique for each species, the first band (45 KDs) detected for *Trifolium alexandrinum*, while with *Cuscuta trifolii* Bab different band appeared (35 KDs). That indicates difference in gene expression and total protein content in both species responsible to different salt concentrations. For the first one *Trifolium alexandrinum*, it was noted that the intensity of band expression (116 KDa and 45KDa) appeared with different concentration of salinity (100, 200, 300, 400, 500mM) of NaCl.

Table 3: Effect of different salt concentration in total seed protein as analyzed on SDS-PAGE. Lanes control, 1,2,3,4 and 5represented proteins extracted from control and NaCl treated seed after 24 h.from application and lane M represents a molecular weight marker

		***Cuscutatrifolii* Bab**						***Trifoliumalexandrinum***					
	KDa	**Co.**	1	2	3	4	5	**Co.**	1	2	3	4	5
1	116	**1**	1	1	1	1	1	**1**	1	1	1	1	1
2	66.2	**1**	1	1	1	1	1	**0**	0	0	0	0	0
3	45	**0**	0	0	0	0	0	**0**	1	1	1	1	1
4	35	**1**	1	1	1	1	0	**0**	0	0	0	0	0
5	25	**0**	0	0	0	0	0	**0**	0	0	0	0	0
6	18.4	**0**	0	0	0	0	0	**0**	0	0	0	0	0
7	14.4	**0**	1	1	1	1	1	**1**	1	0	0	0	0

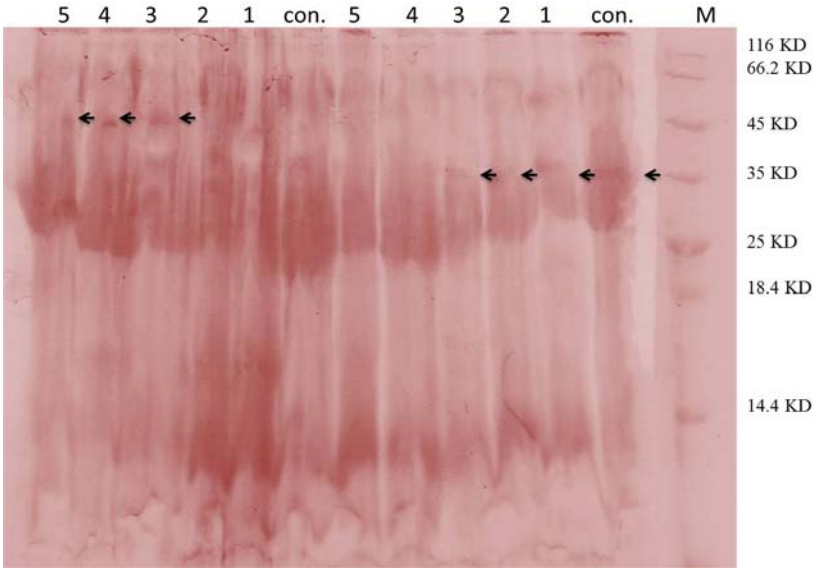

Fig. 5: Effect of different concentration of NaCl on polypeptide patterns of total seed protein as analyzed on SDS-PAGE. Lanes control, 1,2,3,4 and 5represented proteins extracted from control and NaCl treated seed after 24 h. from application and lane M represents a molecular weight marker.

Whereas, all concentrations of NaCl effect on the band expression. While, after using the concentration 100 mM of NaCl the band expression 14.4 KDa appeared. After using the same concentration of NaCl against the seeds of *Cuscuta trifolii* Bab SDS-PAGE analysis showed considerable variations in protein profiles. It was noted that the intensity of band expression (116, 66.2 and 14.4 KDa) were appeared with different concentrations of salinity (100, 200, 300, 400, 500mM) of NaCl. Whereas, all concentrations of NaCl affect the band expression except for the band expression 35 KDa which appeared with all level of salinity except for the concentration 500mM of NaCl. Proteins generally deposit in plants grown under stress condition and they may supply a storage form of nitrogen which plays a vital role in osmotic adjustment, which is utilized after stress (Singh *et al*., 1978).Our results are in line with Agastian *et al.* (2000) who founded hat soluble protein increases at low salt concentration and decreases at elevated levels in mulberry. Similarly, Bozarth *et al.* (1987) noted the deposition of membrane protein in the cell wall portion of the soybean stem when seedlings were treated with abiotic stress.

The effects of different concentrations of NaCL on seed germination and protein of *Cuscuta trifolii* Bab and *Trifolium alexandrinum* cleared that the latter more is more tolerant to salinity than *Cuscuta trifolii*

Bab. So, we can use these different concentrates of NaCl as pre emergency herbicides to control seeds of *Cuscuta trifolii* Bab aiming to reduce the effect of the harmful herbicides on the environment and the same time. On the other hand, it will be more safe considering the animal feed.

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