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The Role of Sulphur Application and Dicyandiamide Comparing with Mineral Fertilization Npk on Chemical Constituent of Lettuce Plant (Lactuca Sativa L.)

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ABSTRACT

A field experiment was conducted in a clayey soil at the experimental farm of the Faculty of Agric. El-Mansoura Univ.to study the effect of sulpher (S) application and Dicyandiamide, (DCD) comparing with mineral fertilization of NPK rates on chemical composition of lettuce plant (*Lactuca sativa* L.) in factorial design. Experiment included 9 treatments which were 3 rates of NPK fertilization (50, 75 and 100% from recommended dose) and 3 treatments of (DCD, S and DCD + S) with 3 replicates. Thus, the total number of the experimental plots was 27 plots.Results indicated that chlorophyll content, N, P, K and S concentrations, NO_3-N and NO_2-N content mg.l^{-1} as well as available N, P and K mg.l^{-1} in leaves were significantly increased due to increasing NPK fertilization rates, and the highest mean values were recorded with adding 100% NPK from recommended dose, while nitrate reducates activity was decreased. The highest average values of mentioned parameters indicated that using mix of (S & DCD) comparing with other treatments except NO_3-N, and NO_2-N mg.l^{-1} which recorded with adding sulphur. In the same time the effect of interaction among treatments on the traits illustrated that the highest values of all parameters in lettuce were connected with 100% NPK + S + DCD. While, nitrate reducates activity of lettuce recorded the highest mean values with S+DCD+ 50% NPK over the control

INTRODUCTION

Lettuce (*Lactuca sativa* L.) belongs to the botanical family Asteraceae and is an option in the olericulture for the cultivation both in protected environment and at the field. Commonly, the produced leaves are commercialized and freshly consumed in the form of salad, which is an alternative of healthy food in the Brazilian menu (Midan and Sorial, 2011). Lettuce leaves are a rich source of antioxidants, vitamins A and C, and phytochemicals which are anti-carcinogenic (Masarirambi *et al*., 2012). The cultivated area of lettuce in Egypt is about 3110 hectares, which produced about 68644 tons (MALR, 2012).

Mineral fertilizers were used to provide soil nutrients in order to maintain optimum soil fertility conditions and healthy growth of plants and quality yield. NPK fertilizers are required greatly by crops for healthy development and crop quality. Furthermore, N is an important factor for higher yield and average head weight of lettuce (Hosseny and Ahmed, 2009). However, the increase in the nitrogen fertilization rate enables obtaining a higher yield but at the same time conveys the risk of deteriorating yield quality resulting from an excessive nitrate accumulation. It particularly refers to leaf vegetables. Phosphorus (P) is the second major nutrient for plant growth as it is an integral part of different biochemical like nucleic acids, nucleotides, phospholipids and phosphoproteins. Inadequate P supply will result in a decreased synthesis of RNA, the protein maker, leading to decreased growth. Grain yield is often severally reduced with P deficiency (Jones *et al*., 2003). Potassium is required in least amount but in soil it is required in large amount by many crops and it is important for maintaining the osmotic potential and rigidity of plant cells; hence it plays a vital role in water relations in the plant (Okoli and Nweke, 2015).

Dicyandiamide (DCD containing 66.7% N) is the most widely used nitrification inhibitor because it is cheaper, nontoxic, less volatile, relatively water soluble, and relatively benign to non-target microbial communities (O'Callaghan *et al*., 2010 and Di *et al*., 2011). Many studies have shown that DCD can significantly decrease NO_3 leaching and NO_2 emissions from cropping systems or grazed pasture systems (Di *et al*., 2007 and Cui *et al*., 2011). The nitrification inhibitor DCD decreases NO_3^- leaching by inhibiting the growth and activity of the ammonia-oxidizing bacteria in the soil, thus slowing down the rate of nitrification and keeping the N in the NH_4^+ form which is adsorbed onto the soil exchange surfaces and is available for plant uptake (Asing *et al*., 2008, Di *et al*., 2010).

Sulfur (S) is one of nature's super nutrients and one of the oldest elements known. It is the thirteenth most abundant element in the earth's crust. S is essential for the production of three amino acids found in both plants and animals. These amino acids are the building blocks in the synthesis of proteins. Without proteins, plants and animals simply could not exist. Mehana and Farag (2000) and El Sayed *et al*., (2015) mentioned that adding elemental sulphur had an important role in soil pH reduction, that increased availability of some nutrient elements such as P, Fe, Zn, Mn and Cu which were reflected on plant uptake and plant growth.

The main objective of the present investigation was to study the effect of sulphur (S) application and Dicyandiamide, (DCD) comparing with mineral fertilization NPK rates on chemical constituent of lettuce plant (*Lactuca sativa* L.)

Keywords: sulphur, Dicyandiamide, NPK fertilization and lettuce plant

MATERIALS AND METHODS

A field experiment was conducted in a clayey soil at the experimental farm of the Faculty of Agric. El-Mansoura Univ.to investigate the application effect of sulpher(S) and Dicyandiamide, (DCD) comparing with mineral fertilization with NPK on chemical composition of lettuce plant (Lactuca sativa L.).

The soil of the experimental field was clayey with pH 7.9, EC: 3.87 dSm^{-1}, $CaCO_3$: 36.8 g kg^{-1}, OM: 17.6 g kg^{-1} .Available N, P and K contents were 43.4,

5.21 and 190 mg kg^{-1} during the season of the experiment, respectively which determined according to Sahlemedhin and Taye (2000). Experiment included 9 treatments which were 3 rates of NPK fertilization (50, 75 and 100%) and 3 treatments of (DCD, S and DCD + S) with 3 replicates. Thus, the total number of the experimental plot was 27 plot comparing with 100% NPK from recommended doses as control.

Seeds of lettuce cv. dark green, were sown on January 5rd, at 30 cm apart between each seedling and the other on both sids of ridgs (3m long and 70 cm wide). Each plot consisted of 3 ridgs making an area of about 6.3 m^2.

Mineral fertilizers used in this investigation were urea (U) (46.5% N), calcium super phosphate (7 % P) and potassium sulphate (40 % K). N fertilizer was added in 2 equal doses directly before the first and second irrigation, while the P fertilizer was added during soil preparation for sowing and K fertilizer was given before the first irrigation. Dicyandiamide 4% N was added as a nitrification inhibitor by mixing it with nitrogen fertilization (U). Sulphur rate was added at the rate of 200 kg fed^{-1} sulphur twice after 20 days from sowing and a week after.

At marketing stage; 90 days after sowing (May 3rd) during the season of lettuce seedling, five plants were randomly taken from each plot. The plants were weighed and then determined nutrient, where N, P, K, S% (Mertens, 2005a & b), NO_3-N, NO_2-N $mg.l^{-1}$ and nitrate reducates activity (*S*ingh, 1988 and Hageman and Reed, 1980) as well as chlorophyll a and b (Gavrilenko and Zigalova (2003), then calculate the total chlorophyll. Soil available forms of N, P and K $mg.l^{-1}$ were measured in the soil samples after harvesting as determined by Haluschak, 2006 and Reeuwijk, (2002).

Data reported for chemical content of lettuce plant and soil availability were assessed by analyses of variance (ANOVA), Duncan's and the least significant difference (L.S.D) method was used for any significant differences at the $P< 0.05$ levels between the means of treatment values to the methods described by Gomez and Gomez, (1984). All the analyses were conducted using software computer CoSTATE.

RESULTS AND DISCCUSION

- **Chlorophyll contents:**

Results presented in Table 1 declare the effect of various concentrations of NPK fertilization, sulphur and DCD and their interactions comparing with treatment of 100% NPK from recommended dose on photosynthetic pigments during 2016.

Data showed generally, that there was an increasing in chlorophyll a, b, and a+b with increasing NPK fertilization. The increasing of change in pigments was significantly increased by increasing NPK levels. The significant increases were found in plants grown under high NPK levels i.e. 0.668, 0.481 and 1.149 mg g^{-1} FW of Chl a, Chl b and total, respectively over rate of 100% NPK from recommended dose. The satisfactory effects of mineral application on the content of chlorophyll may be attributed to its prominent role in the chlorophyll pigment synthesis or chlorophyll molecule in the plant tissues. These findings are corresponding to those reported by Saif-El-Deen (2000); Nabih (2002) and Abd-Elfattah, (2012).

In the same table using individual rates of DCD, S and S + DCD significantly affected chlorophyll a, b, and a+b, the highest average values were indicated with using mix of S + DCD comparing with other treatments. These results may be due to the beneficial effect of the applied-S as a one of many elements required for plant growth, its important in the formation of protein and chlorophyll (Morris *et al.,* 1984). The obtained results are in harmony with those reported by Cuppett *et al.,* (1999), Al-Redhaiman *et al.,* (2003); Wright *et al.,* (2010) and Sanchez and Silvertooth (2016).

It is obvious from the same data in Table 1 that all characteristics are affected by interaction. In general, plants received sulphur at 200 kg fed^{-1} with DCD gave the highest values of chlorophyll content investigated during the season under all treatments of NPK fertilization comparing with 100% NPK from recommended dose (control). The highest values of traits were recorded with 100% NPK+ S + DCD.

Table1. Effect of sulpher application, Dicyandiamide (DCD) and NPK mineral fertilization rates on chlorophyll content.

Char. Treat.		Chlorophyll a mg g^{-1} FW	Chlorophyll b mg g^{-1} FW	Total a+b chlorophyll mg g^{-1} FW
NPK fertilization				
50% RD		0.638c	0.450c	1.088c
75% RD		0.659b	0.470b	1.129b
100% RD		0.668a	0.481a	1.149a
LSD at 5%		0.002	0.006	0.006
S, DCD and S+DCD fertilization				
DCD		0.650b	0.461b	1.111b
S		0.629c	0.442c	1.071c
DCD +S		0.686a	0.497a	1.183a
LSD at 5%		0.005	0.005	0.007
Interaction				
100% RD		0.650f	0.460f	1.110f
50% RD	DCD	0.623i	0.434h	1.057i
	S	0.615j	0.427i	1.041j
	DCD +S	0.678c	0.488c	1.166c
75% RD	DCD	0.659e	0.469e	1.128e
	S	0.631h	0.444g	1.075h
	DCD +S	0.687b	0.496b	1.183b
100% RD	DCD	0.668d	0.481d	1.149d
	S	0.642g	0.454f	1.096g
	DCD +S	0.694a	0.507a	1.202a
LSD at 5%		0.007	0.007	0.010

RD (recommended dose), DCD (Dicyandiamide), S (sulphure), FW (Fresh weight)

- **N, P, K and S concentration:**

Data in Table 2 show that N, P, K and S concentrations in leaves were significantly increased due to supplying the plants with NPK fertilization at different rates. The highest mean values were recorded with adding 100% NPK from recommended dose. As

for the increasing N, P and K concentration with increasing rates of NPK fertilization, it may be owed to the availability of N, P and K elements for plant and improving root growth, hence increasing the absorbing area of root. These results agree with findings of Gairola *et al*., (2009); Mirdad, (2009); Kawthar *et al*., (2014); Singh *et al*., (2014); Hossain *et al*., (2014) and Nemadozi, (2015) who showed that N, P, K concentrations were increased with increasing rates of NPK fertilization and chlorophyll content.

Data in Table 2 show that the sulphur application + DCD inhibitor had a significant effect on N, P, K and S concentrations in leaves of lettuce. All elements concentrations in lettuce leaves were significantly increased with adding sulphur+DCD during the season. These results may be related to the positive effect of sulphur on the availability of some nutrients, which was reflected on plant uptake and plant growth (Mehana and Farag, 2000). An additional advantage of Urea mix with DCD is improving the uptake of macro and micronutrients. When plant roots take up ammonium ions protons (H^+) are excreted for charge equilibration in the roots. Thus, the pH of the rhizosphere is decreased, resulting in greater mobility and uptake of some nutrients by the plant. These results are in accordance with the finding of Choudhury and Kennedy (2005), Shaviv (2005), Amberger (2008) and Trenkel (2010).

Table2. Effect of sulpher application, Dicyandiamide (DCD) and NPK mineral fertilization rates on N, P, K and S% in lettuce leaves.

Char. Treat.		N%	P%	K%	S%
NPK fertilization					
50% RD		1.97b	0.197c	2.20c	0.251c
75% RD		2.11a	0.217b	2.41b	0.293b
100% RD		2.20a	0.227a	2.51a	0.333a
LSD $_{at\ 5\%}$		0.14	0.05	0.04	0.005
S, DCD and S+DCD fertilization					
DCD		2.02b	0.210b	2.33b	0.224c
S		1.80c	0.187c	2.11c	0.315b
DCD +S		2.44a	0.244a	2.68a	0.338a
LSD $_{at\ 5\%}$		0.13	0.003	0.05	0.005
Interaction					
100% RD		2.01cde	0.208f	2.32e	0.209i
50% RD	DCD	1.77fg	0.183i	2.05h	0.192j
	S	1.66g	0.173j	1.97i	0.270f
	DCD +S	2.47a	0.234c	2.59c	0.292e
75% RD	DCD	2.11cd	0.219e	2.41d	0.230h
	S	1.82efg	0.189h	2.13g	0.312d
	DCD +S	2.39ab	0.243b	2.69b	0.338c
100% RD	DCD	2.20bc	0.227d	2.53c	0.252g
	S	1.93def	0.200g	2.22f	0.364b
	DCD +S	2.47a	0.254a	2.77a	0.384a
LSD $_{at\ 5\%}$		0.20	0.006	0.07	0.008

It is obvious from data in Table 2 that the interaction between S and DCD had significant effects on all chemical constituents in lettuce under all treatments of NPK rats. On the other hand, the highest values of elements concentration in lettuce are connected with 100% NPK + S + DCD which were 2.47, 0.254, 2.77 and 0.384 for N, P, K and S%, respectively.

- **NO_3-N, NO_2-N and nitrate reducates activity:**

Obtained results in Table 3 illustrated the effect of different rates of NPK fertilization, sulphur, DCD and their interactions NO_3-N, NO_2-N and nitrate reducates activity during 2016.

Table3. Effect of sulpher application, Dicyandiamide (DCD) and NPK mineral fertilization rates on NO_3-N, NO_2-N and nitrate reducates activity in lettuce leaves.

Char. Treat.		NO_3-N mg.l^{-1}	NO_2-N mg.l^{-1}	Nitrate reducates activity mg.min .g^{-1} FW
NPK fertilization				
50% RD		120.3c	1.04c	2.09a
75% RD		130.6b	1.16b	1.93b
100% RD		138.5a	1.28a	1.75c
LSD $_{at\ 5\%}$		3.34	0.04	0.02
S, DCD and S+DCD fertilization				
DCD		127.9c	1.13b	1.89b
S		160.4b	1.51a	1.39c
DCD +S		101.2a	0.83c	2.49a
LSD $_{at\ 5\%}$		2.66	0.05	0.04
Interaction				
100% RD		181.7a	1.78a	1.09j
	DCD	116.4g	0.99g	2.09d
50% RD	S	150.5d	1.37d	1.54g
	DCD +S	93.9j	0.75	2.63a
	DCD	128.1f	1.12f	1.89e
75% RD	S	161.8c	1.52c	1.37h
	DCD +S	102.0i	0.82h	2.52b
	DCD	139.2e	1.27e	1.68f
100% RD	S	168.7b	1.64b	1.24i
	DCD +S	107.6h	0.92g	2.31c
LSD $_{at\ 5\%}$		4.21	0.08	0.06

It is evident from these results that treating lettuce plants with NPK fertilization significantly increased NO_3-N and NO_2-N in lettuce leaves and decreased the nitrate reducates activity, Generally, the most favorable treatment for enhancing NO_3-N and NO_2-N mg.l^{-1} ,in leaves were the treatment of 100% NPK from RD, while nitrate reducates activity recorded the highest values with 50% from RD. This trend was true during season of the experimentation. Abd-Elfattah, (2012) illustrated that the highest nitrate content due to chemical fertilization maybe attributed to that mineral fertilizer salts are soluble and nitrogen is immediately available for plant uptake soon after fertilizer application.

It is clear from data in Table 3 that the positive effects were happened in NO_3-N, NO_2-N mg.l^{-1} , and nitrate reducates activity of lettuce with using of DCD,

sulphur, S + DCD. The highest values of NO_3-N, and NO_2-N mg.l^{-1} were recorded with adding sulphur alone comparing with other treatments while nitrate reducates activity of lettuce was connected with DCD+S. These results could be attributed to the sulfur's role in the activation of the enzyme nitrate reducates, which is necessary for the conversion of nitrate to amino acids in plants. Low nitrate reducates activity depresses soluble protein levels, while raises nitrate concentrations in plant tissue. The obtained results are in harmony with those reported by Cuppett *et al.,* (1999), Al-Redhaiman *et al.,* (2003) Wright *et al.,* (2010) and Sanchez and Silvertooth (2016). Because accumulation of nitrate in plants depended on its concentration in the soil solution; an addition of nitrification inhibitors to ammonical N-fertilizers will decrease the concentration of nitrate in the soil and consequently its contents in the plant. Similar results were reported by Pasad *et al.,* (2001b), Amberger (2006), Shaviv (2005) and Grant (2005).

Data in Table 3 reveal that NO_3-N, NO_2-N mg.l^{-1} and nitrate reducates activity of lettuce was significantly affected during 2016 with the interaction among the treatments comparing with the control (100% NPK). The lowest NO_3-N, NO_2-N mg.l^{-1} were obtained by applied S+DCD+ 50% RD from NPK comparing with the control (100% NPK) which recorded the highest mean values. While, nitrate reducates activity of lettuce recorded the highest mean values with S+DCD+ 50% over the control which was 2.63 mg.l^{-1}.

- **Available N, P and K mg.l^{-1}:**

Data in Table 4 indicate that available N, P and K mg.l^{-1} in the soil after harvesting significantly affected with application of NPK fertilization rates during the season of the experiment. Moreover, application of 100% NPK from recommended dose was more effective than the other treatments to increase the average values. The obtained results are in accordance with Titilola (2006); Ayeni and Adetunji (2010) and Onwudiwe *et al*., (2014).

Data illustrated in Table 4 show the effect of DCD, sulphur, S + DCD on availability of N, P and K mg.l^{-1} of lettuce. Such data indicate that the application of sulphur + DCD increased availability of N, P and K mg.l^{-1}. The positive effect of this sulphur level may be due to lowering soil pH value, improving soil structure, soil chemical properties and increased the availability of certain plant nutrients such as N, P and K also, several micronutrients i.e. Fe, Zn and Mn. The obtained results are in accordance with those obtained by Al-Redhaiman *et al.,* (2003); Wright *et al.,* (2010) and Sanchez and Silvertooth (2016). Adding of DCD mix with N-fertilization in form of urea improved N, P and K availability because of Protons (H^+) are excreted for charge equilibration in the roots. Thus, the pH of the rhizosphere is decreased, resulting in greater mobility and elements become available for plant. These results are in accordance with the finding of Amberger (2008) and Trenkel (2010).

It is clear from data in Table 4 that there were significant interactions among treatments under study on availability of N, P and K mg.l^{-1} in the soil after harvesting during season of 2016. In general, plants fertilized by mix of S+DCD produced the highest values of traits under all rates of NPK fertilization comparing with the control (100% NPK). In the same time the highest values of available N, P and K realized with addition of 100% NPK+ S+ DCD over the control which recorded values near to that using DCD + 75% NPK.

Table4. Effect of sulpher application, Dicyandiamide (DCD) and NPK mineral fertilization rates on N, P and K mg.l^{-1} in the soil after harvesting.

Char. / Treat.		N mg.l^{-1}	P mg.l^{-1}	K mg.l^{-1}
NPK fertilization				
50% RD		61.22c	7.60b	253.4b
75% RD		69.09b	8.99c	276.7c
100% RD		72.91a	9.61a	286.8a
LSD $_{at\ 5\%}$		1.39	0.16	2.19
S, DCD and S+DCD fertilization				
DCD		65.90b	8.34c	266.7c
S		58.67c	6.93b	243.6b
DCD +S		79.66a	10.94a	306.7a
LSD $_{at\ 5\%}$		0.90	0.08	3.38
Interaction				
100% RD		66.47f	8.33f	267.3f
	DCD	55.30i	6.57i	236.2i
50% RD	S	54.90j	5.94j	228.9j
	DCD +S	76.47c	10.28c	295.2c
	DCD	69.70e	8.96e	277.4e
75% RD	S	58.50h	7.09h	245.4h
	DCD +S	79.07b	10.93b	307.4b
	DCD	72.70d	9.49d	286.5d
100% RD	S	62.60g	7.74g	256.4g
	DCD +S	83.43a	11.59a	317.4a
LSD $_{at\ 5\%}$		1.49	0.25	3.82

CONCLUSION

From the results of this study, it could be concluded that, application of S + DCD mixed with nitrogen fertilization is the recommended treatment for good chemical composition of lettuce. Based on the obtained results, combined application of nitrogen, phosphorous and potassium is the most suitable and recommendable dose for a good chemical composition of lettuce. So adding S + DCD+100% NPK from recommended dose was the best treatment for a good lettuce.

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دور اضافه الكبريت والمثبط مقارنه بالتسميد المعدنى على التركيب الكيماوى لنبات الخس

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اجريت تجربه حقلية لدراسه تاثير اضافه الكبريت مع المثبط مقارنه باضافه التسميد المعدنى على التركيب الكيميائى لنبات الخس خلال تجربه عامليه تشتمل على 9 معاملات وهى عباره عن 3 مستويات من التسميد المعدنى (50، 75 ، 100% من الموصي به) و 3 معاملات من (المثبط، كبريت، مثبط + كبريت) خلال 3 مكررات وبالتالى يصبح المجموع الكلى 27 وحده تجريبيه مقارنه بمعامله كنترول (100% موصي به من التسميد المعدنى فقط).اوضحت النتائج ان محتوى الكلوروفيل ، النسبه المئويه للنيتروجين، الفوسفور ، البوتاسيوم ، الكبريت فى الخس و النترات والنيتريت بالاضافه الى صلاحيه النيتروجين ، الفوسفور والبوتاسيوم فى التربه بعد الزراعه تاثرت معنويا باضافه مستويات التسميد المعدنى وسجلت اعلى القيم عند استخدام 100% من الموصى به بينما سجلت اعلى القيم نشاط انزيم النيتريت انخفضت مع استخدام التسميد المعدنى . و كانت اعلى القيم السابقه عند استخدام خليط من المثبط مع الكبريت مقارنه بباقى المعاملات ماعدا النترات والنيتريت سجلت اعلى القيم عند استخدام الكبريت فقط. فى نفس الاتجاه الاضافه المشتركه بين المعاملات تحت الدراسه على نفس الصفات اوضحت ان اعلى القيم سجلت عند استخدام خليط الكبريت و المثبط مع 100% تسميد معدنى من الموصى به بينما نشاط انزيم النيتريت سجلت عند استخدام نفس الخليط عند 50% من التسميد المعدنى مقارنه بمعامله الكنترول.