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EFFECT OF LITHOVIT ON GROWTH, SEED YIELD COMPONENTS AND ANATOMY OF STEM AND LEAF OF COWPEA PLANTS UNDER DIFFERENT MINERAL NITROGEN LEVELS

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ABSTRACT: This work was carried out during the two autumn seasons of 2015 and 2016 at Ghazala Experimental Farm, Fac. Agric., Zagazig University to study the effect of mineral nitrogen fertilizer levels (15, 25 and 35 kgN/fad.) and lithovit levels (0, 2, 4 and 6 g/l) as well as the interaction between them on growth, seed yield and anatomical traits of cowpea plants grown in clay soil. Fertilizing cowpea plants with mineral N at 35 kg/fad., increased plant height and number of both leaves and branches/plant, total dry weight/plant, as well as seed yield/fad., and N, P, K, total carbohydrates and total protein in seeds. Whereas N at 25 kg/fad., increased dry weight of leaves and branches/plant as well as N,P and K uptake in leaves and branches compated with low used level of 15 kg N/ faddan. Spraying cowpea plants with lithovit at 6g/l induced all previous characters as well as N, P and K uptake in leaves and branches. The interaction between N at 25 kg/fad., and lithovit at 6 g/l increased plant height, number of both leaves and branches/plant, total dry weight/plant, N,P and K uptake by leaves and branches, seed yield/fad., and N, P, K total protein in seeds. As for the effect of N levels and foliar application with lithovit on anatomical structure of vegetative organs of cowpea, it could be stated that 25 kg mineral nitrogen appeared a decrease in the main stem diameter, thickness of cortex, fiber, phloem, xylem and pith diameter, number of vascular bundle and diameter of vessel compared with high level of 35 kg N/fad., whereas the same level of N and lithovit at 6 g/l showed an increase in all previous characters except fiber thickness. Application of 25 kg mineral N/fad., caused in reduction in midvein, lamina of leaflet blades, palisade and spongy tissue thickness and dimensions of midvein bundle compared with high level of 35 kg N/fad., but the same level of N and lithovit reflected an increase in thickness of midvein region, width of midvein bundle, number of vessels/ midvein bundle and average diameter of vessel, but decreased the thickness of leaf lamina.

Key words: Cowpea, lithovit, mineral nitrogen, stem and leaf anatomy, growth and seed yield.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) considers one of the most important vegetable legumes due to its high protein content, heat tolerant, low fertilization requirements and can grow easily in the new reclaimed lands. The protein content in cowpea seeds is high and rich in amino acids. It is considered as one of the major summer vegetable crops grown in Egypt for local consumption and exportation.

The favorable effects of nitrogen on stimulating the meristematic activity for producing more tissues and organs, since nitrogen plays an important role in protein and nucleic acids synthesis, as well as protoplasm formation (**Marschner, 1995**). **Hussaini *et al.* (2004)** illustrated that small doses of applied N (from 30 to40 kg N/fad.) may be synergistic and stimulate nodulation and symbiotic fixation in cowpea and even improve seed yield. Moreover, **Geetha and Varughese (2001) and El-Waraky**

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and Kasem (2007) stated that cowpea plants fertilized with 30 kg N/fad., produced the greatest pod yield, also increasing nitrogen fertilizer level up to 40 kg N/fad., gradually increased cowpea plant growth, yield and its components. N Fertilization at 45or 60kg/fad., of cvs Cream-7 and Dokki-331, increased dry weight of leaves and total dry weight/ plant, and seed yield per plant and per fad., in both seasons **(Attia,2012).** Adding 40 kg N/fad., significantly increased plant height, number of leaves/plant, number of branches /plant and chlorophyll content in both seasons, while adding 30 kg N/fad., gave the highest mean values of seed yield/ plant, seed yield/fad., number of pods/ plant and 100-seed weight **(El- Atawy and Kasem, 2011 ; Moursi *et al.*, 2013).**

Lithovit is a natural calcium carbonate (nano-$CaCO_3$), foliar fertilizer supplemented with calcium micronutrient, which delivers fine particles (<10 μm) that can easily be adsorbed directly through the stomata of plant leaves. The micronutrients supplied with lithovit influence plant metabolism and cell wall formation (Ca), resulting in a product that has potential to increased and sustain improved plant metabolism. Inside the leaf intercellular spaces, lithovit particles break down and release gaseous CO_2, enhancing CO_2 concentration at the photo-synthetically active area within the plant leaves. The normal concentration of CO_2 in the atmosphere is approximately 0.04%, which means that the most cultivated plants failed to achieve the optimum level of photosynthetic rate, and is achieved at near to 0.1 vol. (%) CO_2. The lithovit particles that enter the intracellular "compartment" dock, with its negative surface of the cell membrane, where they produce a negative potential. Lithovit's mode of action is to increase CO_2 levels within the plant leaf structure and by implication enhance the photosynthetic efficiency. Lithovit consists of calcium carbonate 79.19%, gave a direct effects of increasing carbon dioxide (CO_2) on plant growth and development **(Abdelrehem, 2014).** The highest photosynthesis intensity was observed in variant treated with lithovit foliar fertilizer based on CO_2. Lithovit fertilizer particles, finally sprayed on the leaf surface are absorbed and transformed in CO_2 **(Carmen *et al.*, 2014).**

The aim of this study was to reduce mineral nitrogen in production of cowpea plants by using lithovit (nano-$CaCO_3$) as foliar fertilization.

MATERIALS AND METHODS

This work was carried out during the two summer seasons of 2015 and 2016 at Ghazala Experimental Farm, Fac. Agric., Zagazig University Egypt, to study the effect of mineral nitrogen fertilizer levels and foliar spray with lithovit (nano-$CaCO_3$) on growth, seed yield and anatomical traits of cowpea under flood irrigation system.

Physical and chemical properties of the experimental soil are presented in Table 1.

Table 1. Physical and chemical properties of the experimental soil

Physical properties											Soil texture
Clay (%)		Silt (%)		Fine sand (%)		Coarse sand (%)					Clay
43.39		9.26		13.62		33.73					
Chemical properties											
Year	pH	EC m.mohs/cm	Organic mater (%)	Soluble cations (meq./l)				Soluble anions (meq. /l)			Available (ppm)		
				Mg^{++}	Ca^{++}	K^{+}	Na^{+}	Cl^{-}	HCO_3^{-}	SO_4^{--}	N	P	K
2015	7.87	0.95	0.52	2.8	1.5	1.3	3.8	4.5	1.5	3.4	170	830	710
2016	7.81	0.93	0.54	2.4	1.7	1.4	3.6	4.3	1.6	3.2	178	940	739

This experiment included 12 treatments, which were the combinations between three levels of mineral nitrogen (15, 25 and 35 kg/fad.) and four lithovit concentrations (0, 2, 4 and 6 g/l). These treatments were arranged in a split plot design with three replicates, where nitrogen levels were allotted in the main plots, while the concentrations of lithovit were assigned in the sub plots. The experimental unit area was 7.2 m^2 (four ridges with 3 m length for each and 60 cm width). One ridge was used for data collection and the others three ridges were used for yield determination.

Cowpea seeds were sown on 2nd and 4th July in 2015 and 2016 seasons, respectively. Cowpea seeds were sown in hills (2-3 seeds/hill) at 20 cm apart and then thinned after completely emergency to leave one plant/hill. The used cultivar Cream -7 was produced by Hort. Res. Inst., Agric. Res. Center, Egypt.

Plants of cowpea were sprayed and fertilized with both of lithovit and different levels of mineral nitrogen, respectively three times at 25,35and 45 days after seed sowing .The lithovit was obtained from Agrolink Company as a powder, and the source of mineral nitrogen fertilizer was ammonium sulphate (20.6% N).

All experimental units received equal amounts of calcium superphosphate (15.5% P_2O_5) at 150 kg/fad., and potassium sulphate (48%K_2O) at 50 kg/faddan. All amounts of phosphorus fertilizer and one third of potassium sulphate were added during soil preparation and before seed sowing, while the other two third of potassium sulphate was added at 30 and 60 days after sowing. The other cultural practices such as weed, pest and disease control on all plots were carried out according to the recommendations of Ministry of Agriculture, Egypt.

Data Recorded

Plant growth characters

Three plants from each experimental unit were randomly taken at 55 days after sowing and plant height (cm), number of leaves/plant and number of branches/ plant were recorded.

Dry weight (g)

The different plant organs; *i.e.,* leaves, branches and pods were oven dried at 70° C till constant weight and the following data were recorded: dry weight of leaves/ plant, dry weight of branches/plant, dry weight of pods/plant, and total dry weight/whole plant (leaves+ branches + pods).

Determination of macro-nutrients

Nitrogen, phosphorus and potassium contents were determined on the basis of dry matter in branches and leaves at 55 days after sowing in the 2nd season only according to the methods described by **Bremner and Mulvaney (1982**), **Olesen and Sommers (1982)** and **Jackson (1970**), respectively. Also N, P and K uptake were calculated.

Anatomical studies

A comparative micro-scopical examination was performed on plant material for treatment which showed a remarkable response. In addition to the control, tested materials included the main stem at its median portion and terminal leaflet from the compound leaf developed on the median portion of the main stem. Specimens were taken throughout the second growing season at the age of 10 weeks. Specimens from the control and chosen treatment, including stems and leaves, were killed and fixed for at least 48 hr. in F.A.A. (10 ml formalin, 5 ml glacial acetic acid and 85 ml ethylalcohol 70%). The selected materials were washed in 50% ethylalcohol, dehydrated in a normal butylalcohol series, embedded in paraffin wax of melting point 56°C, sectioned to a thickness of 20 micrometers (µm), double stained with safranin and fast green, cleared in xylene and mounted in Canada balsam **(Nassar and El- Sahhar, 1998).**

Yield and its components

At maturity stage, a random sample of three plants were taken to determine the number of pods/ plant and after that, 15 dry pods from each plot were randomely taken to determine pod weight, diameter, length, number of seeds/pod, 100 seed weight and average seed weight of plot were recorded, as well as total yield/fad., and the relative increases in total yield/fad., over control were calculated.

Seed quality

In the second season only, samples of dry seeds were fine ground then N, P and K contents were determined in dry seeds, as previously explained in the plant chemical composition. Total carbohydrates (%) were determined according to the method described by **Dubois *et al.* (1956)** and total protein (%) was calculated by multiplying total nitrogen in seeds x 6.25.

Statistical Analysis

The data in this experiment were subjected to proper statistical analysis of variance according to **Snedecor and Cochran (1980)** and means separation were done according to LSD at 5% level.

RESULTS AND DISCUSSION

Vegetative Growth

Plant height and number of both leaves and branches/plant were significantly increased with increasing of N levels up to 35 kg/fad., (Table 2). Spraying cowpea plants with lithovit at 6g/l increased plant height, number of leaves /plant and number of branches/plant. The interaction between N at 25 kg/fad., and spraying with lithovit at 6 g/l increased plant height, while the interaction between N at 35 kg/fad., and spraying with lithovit at 6g/l increased No. of both leaves and branches /plant in both growing seasons.

Dry weight

Fertilizing cowpea plants with N at 25 kg/ fad., increased dry weight of leaves and branches/plant, whereas N at 35 kg/fad., increased dry weight of pods and total dry weight/plant with no significant differences with 25 kg N/fad., with respect to total dry weight/ plant in the 2nd season (Table 3). Dry weight of leaves, branches and pods/plant as well as total dry weight/plant, significantly increased with increasing lithovit at 6 g/l. The interaction between fertilizing plants with N at 25 kg/fad., and spraying with lithovit at 6g/l increased dry weight of leaves , branches and total dry weight/ plant, whereas the interaction between N at 35 kg/fad., and lithovit at 6g/l increased dry weight of pods/plant, in both seasons.

The increase in dry weight might be attributed to the favorable effects of nitrogen on stimulating the meristematic activity for producing more tissues and organs, since nitrogen plays an important role in protein and nucleic acids synthesis as well as protoplasm formation (**Marschner, 1995**).

Adding 40 kg N/fad., significantly increased plant height, number of leaves/ plant, number of branches/plant and chlorophyll content in both seasons **(El-Atawy and Kasem, 2011; Moursi *et al.*, 2013)**. Fertilization of cvs Cream-7 and Dokki-331 with rhizobium at 200 g/fad., and mineral at 45 or 60 kg N/fad., increased dry weight of leaves and total dry weight/plant **(Attia, 2012)**. Plant height, number of both leaves and branches/plant and dry matter production/plant of cowpea were the highest with the highest level of nitrogen at 30 kg N/ha than 10and 20 kg N /ha or control treatment (**Upadhyay and Singh, 2016**).

The increase in plant growth as response to lithovit may be due to, its role as a long term reservoir supplying plants with CO_2; thus, it can enhance the plant growth and productivity, where elevated CO_2 concentrations generally increased carbon assimilation, biomass and leaf area of plants (**Bilal, 2010**). Spraying plants with lithovit gave the best results for increasing plant growth than unsprayed plants with lithovit **(Byan, 2014 ; Abo-Sedera *et al*., 2016** on snap bean).

N, P and K contents

Mineral nitrogen at 35 kg/fad., gave the highest values of N, P and K contents in leaves and K in branches ,otherwise,without significant differences with N at 25 kg/fad., of N and P in branches (Table 4). Spraying lithovit at 6g/l increased N, P and K contents in vegetative parts of cowpea plants. The interaction between N at 25 or 35 kg /fad., and spraying with lithovit at 6g/l recorded maximum values of of N, P and K contents in leaves and branches . **Attia (2012)** reported that the contents of N,P and K in branches and leaves, as well as its uptake of cowpea were significantly increased with increasing N up to 60 kg N/fad. Moreover spraying snap bean with Lithovit at 3 g/l recorded the highest values of N, P and K contents in the plant foliage than unsprayed plants **(Abo-Sedera *et al*., 2016)**.

Table 2. Effect of mineral nitrogen fertilizer levels, lithovit as foliar spray and their interactions on vegetative growth of cowpea plants at 55 days after sowing during 2015 and 2016 seasons

Treatment		Plant height (cm)		Number of leaves/plant		Number of branches/plant	
		First season	Second season	First season	Second season	First season	Second season
N levels (kg/fad.)		**Effect of nitrogen levels (kg/fad.)**					
15		34.75	38.50	67.00	69.88	11.00	14.75
25		40.25	47.33	75.75	79.38	12.00	14.50
35		42.25	51.50	79.38	83.38	13.75	15.75
LSD (0.05)		1.21	1.44	1.72	2.10	0.47	0.71
Lithovit (g/l)		**Effect of lithovit (g /l)**					
Control (Tap water)		30.8	33.8	54.6	60.9	9.3	12.3
2		34.8	41.8	69.9	74.4	11.5	14.8
4		36.8	41.8	71.1	77.0	12.5	14.8
6		44.0	50.3	85.1	85.0	14.3	16.0
LSD (0.05)		1.12	1.37	1.77	1.92	0.45	0.63
N levels (kg/fad.)	**Lithovi (g/l)**	**Effect of interaction**					
15	**Control**	26.0	35.0	50.0	57.5	8.0	11.0
	2	37.0	35.0	66.5	70.0	10.0	16.0
	4	32.0	34.0	70.5	71.0	13.0	15.0
	6	44.0	50.0	81.0	81.0	13.0	17.0
25	**Control**	33.0	36.3	57.5	66.5	9.0	12.0
	2	37.0	48.0	75.0	80.0	13.0	16.0
	4	42.0	49.0	77.5	77.0	13.0	15.0
	6	49.0	56.0	93.0	94.0	13.0	15.0
35	**Control**	39.0	43.0	61.5	71.0	12.0	15.0
	2	41.0	53.0	81.5	83.5	13.0	15.0
	4	45.0	54.0	76.5	86.0	12.0	15.0
	6	44.0	56.0	98.0	93.0	18.0	18.0
LSD (0.05)		1.98	2.41	3.11	3.38	0.79	1.12

Table 3. Effect of mineral nitrogen fertilizer levels, lithovit as foliar spray and their interactions on dry weight of different parts of cowpea plants at 55 days after sowing during 2015 and 2016 seasons

Treatment		Dry weight of leaves/plant (g)		Dry weight of branches/plant (g)		dry weight of pods/plant (g)		Total dry weight/plant (g)	
		First season	Second season	First season	Second season	First season	Second season	First season	Second season
N levels (kg/fad.)		**Effect of nitrogen levels (kg/fad.)**							
15		13.95	15.45	14.51	16.80	2.25	2.40	30.71	34.65
25		16.25	18.18	18.36	21.32	2.38	2.71	36.98	42.20
35		17.13	17.50	18.93	20.08	2.80	3.10	38.85	40.68
LSD (0.05)		0.74	1.44	0.93	0.86	0.12	0.22	0.90	1.44
Lithovit (g/l)		**Effect of lithovit (g /l)**							
Control (Tap water)		10.03	11.93	13.70	13.97	2.07	2.17	25.80	28.07
2		17.07	12.61	15.10	16.97	2.37	2.78	34.53	32.35
4		16.07	20.13	15.68	21.13	2.30	2.63	34.04	43.90
6		19.93	23.49	24.58	25.52	3.17	3.37	47.68	52.38
LSD (0.05)		0.68	1.49	2.00	0.82	0.13	0.21	2.18	1.84
N levels (kg/fad.)	**Lithovit (g/l)**	**Effect of interaction**							
15	**Control**	8.40	11.30	11.10	11.40	2.00	1.70	21.50	24.40
	2	15.20	12.33	13.90	16.90	2.20	2.50	31.30	31.73
	4	15.40	19.00	12.43	17.70	2.10	2.40	29.93	39.10
	6	16.80	19.17	20.60	21.20	2.70	3.00	40.10	43.37
25	**Control**	10.60	12.10	14.30	15.20	2.00	2.30	26.90	29.60
	2	16.60	12.40	14.90	17.60	2.30	2.93	33.80	32.93
	4	15.50	20.30	15.60	24.20	2.20	2.50	33.30	47.00
	6	22.30	27.90	28.63	28.27	3.00	3.10	53.93	59.27
35	**Control**	11.10	12.40	15.70	15.30	2.20	2.50	29.00	30.20
	2	19.40	13.10	16.50	16.40	2.60	2.90	38.50	32.40
	4	17.30	21.10	19.00	21.50	2.60	3.00	38.90	45.60
	6	20.70	23.40	24.50	27.10	3.80	4.00	49.00	54.50
LSD (0.05)		1.20	2.63	3.52	1.44	0.23	0.38	3.84	3.29

Table 4. Effect of mineral nitrogen fertilizer levels, lithovit as foliar spray and their interactions on N, P and K (%) in leaves and branches of cowpea plants at 55 days after sowing during 2016 season

Treatment		Leaves			Branches		
		N	P	K	N	P	K
N levels (kg/fad.)		**Effect of nitrogen levels (kg/fad.)**					
15		2.21	0.360	1.60	2.08	0.30	1.19
25		2.39	0.370	1.67	2.42	0.35	1.26
35		2.44	0.410	1.75	2.40	0.36	1.30
LSD (0.05)		0.04	0.008	0.07	0.04	0.005	0.03
Lithovit (g/l)		**Effect of lithovit (g/l)**					
Control (Tap water)		2.17	0.340	1.41	1.98	0.31	1.16
2		2.14	0.350	1.52	2.21	0.32	1.11
4		2.34	0.370	1.84	2.34	0.34	1.32
6		2.74	0.450	1.92	2.67	0.38	1.40
LSD (0.05)		0.09	0.010	0.06	0.06	NS	0.03
N levels (kg/fad.)	**Lithovit (g/l)**	**Effect of interaction**					
15	**Control**	1.99	0.338	1.32	1.96	0.295	1.15
	2	2.20	0.358	1.41	1.83	0.293	1.06
	4	2.15	0.331	1.79	2.01	0.299	1.25
	6	2.49	0.396	1.87	2.53	0.312	1.29
25	**Control**	2.10	0.346	1.45	1.87	0.298	1.07
	2	2.16	0.325	1.49	2.43	0.327	1.16
	4	2.43	0.362	1.80	2.55	0.352	1.36
	6	2.87	0.455	1.95	2.81	0.420	1.46
35	**Control**	2.41	0.349	1.47	2.10	0.340	1.25
	2	2.06	0.360	1.65	2.38	0.344	1.12
	4	2.43	0.430	1.92	2.46	0.355	1.36
	6	2.87	0.491	1.94	2.67	0.401	1.46
LSD (0.05)		0.17	0.019	0.11	0.12	0.017	0.05

N, P and K uptake

Fertilizing cowpea plants at 25 kg N/fad., in most cases or spraying lithovit at 6g/l or the interaction between them recoded maximum values of N,P and K uptake in leaves and branches compared with low used level of 15 kg N/ fad., (Table 5).

Anatomical studies

Anatomy of the main stem

Results in Table 6 and microphotographs shown in Fig. 1 note that cowpea plants obtained from treatment of the nitrogen mineral fertilization at 25 kg/fad., appeared a decrease in the main stem diameter by 5.6% less than N at 35 kg/faddan. The decrease in stem diameter, could be imputed to the prominent deficiency includes all tissues, except the hollow pith diameter, which is only 2.9% higher than N at 35 kg/fad. The decrements less than the N at 35 kg/fad., were 5.3, 45.5, 10, 2.5 and 16.3% for thickness of cortex, fiber, phloem, xylem and pith diameter; respectively. Moreover, a decrement of 27.8 and 13.5% in number of vascular bundle and diameter of vessel less than N at 35 kg/faddan. On the other hand, vascular cylinder thickness and number of vessel/ vascular bundle were not affected.

Also, results in Table 6 and microphotographs shown in Fig. 1 also indicate that cowpea plants sprayed with lithovit and mineral nitrogen fertilizer at 25 Kg/fad., showed an increase in diameter of the main stem by 7.5% more than stem diameter of N at 35 kg/faddan. The increase in stem diameter could be imputed to the increases in thickness of cortex, vascular cylinder, phloem, xylem and pith diameter by 52.6, 11.3, 20.1, 5 and 15.2% over the N at 35 kg/fad., respectively, but thickness of fiber decreased by 36.5%. However, a decrement of 1.3% below N at 35 kg/fad., was observed for diameter of hollow pith. Moreover, an increment of 7.8 and 26% in number of vascular bundle and diameter of vessel more than N at 35 kg/ faddan.

Anatomy of the leaf

Results in Table 7 and microphotographs shown in Fig. 2 note that cowpea plants that received nitrogen mineral fertilization at 25kg N/fad., exhibited reduction in midvein and lamina of leaflet blades of cowpea plants by 35 and 51.6% less than the N at 35 kg/fad., respectively. The decrease in lamina thickness due to adding N at 25 kg/fad., could be attributed to the decrease in palisade and spongy tissue thickness. The decrements below the N at 35 kg/fad., were 53.6 and 63.6% for thickness of palisade tissue and thickness of spongy tissue, respectively. However, reduction observed in dimensions of midvein bundle valued 35.4 and 45.2% in both length and width of midvein bundle less than N at 35 kg/fad., respectively. Also, the previous treatment resulted in a decrease in the number of vessels/midvein bundle and vessels diameter by 33.3 and 29.8%, respectively. The thickness of upper and lower epidermis were not affected.

Moreover, results revealed that cowpea plants sprayed with lithovit and fertilized with 25 kg/fad., mineral nitrogen resulted in an increase in thickness of midvein region by 16.6% more than leaves of N at 35 kg/faddan. At the same time, the thickness of leaf lamina showed a decrease of 26.7% below N at 35 kg/ fad., due to the decrease of 21.5% in thickness of palisade tissue and 41% in the thickness of spongy tissue below N at 35 kg/ faddan. The increase in midvein thickness could be attributed to the increases in dimensions and components of midvein bundle. The increments over the N at 35 kg/fad., were 43.1, 1.1 and 21.9% for width of midvein bundle, number of vessels/ midvein bundle and average diameter of vessel, respectively. On the other hand, length of midvein bundle decrease by 19.65%. The thickness of upper and lower epidermis were not affected. Worthy to note that all measurments induced from treatment of 25kg N/fad.+lithovit were decidedly higher than those recorded by treatment of 25 kg N/fad., alone.

Seed yield and its components

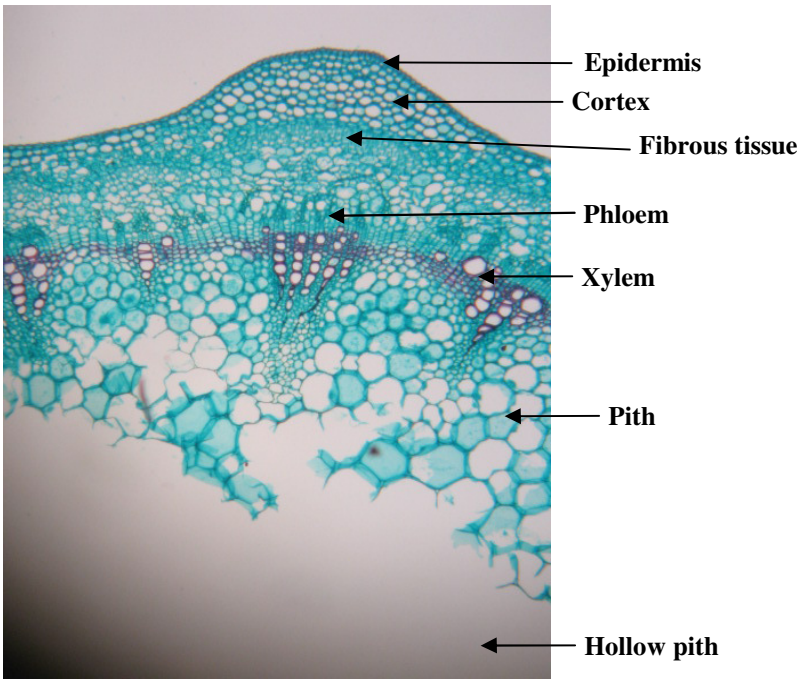
Nitrogen at 25 and 35 kg/fad., increased seed yield in the 1st and 2nd seasons, respectively, whereas N at different rates did not effect on seeds number/pod and 100 seed weight in both seasons (Table 8). Seed number/pod and seed yield/ fad., significantly increased with increasing the concentration of lithovit up to 6 g/l in both seasons. In general, the interaction between N at

Table 5. Effect of mineral nitrogen fertilizer levels, lithovit as foliar spray and their interactions on N, P and K uptake (mg) in leaves and branches of cowpea at 55 days after sowing during 2016 season

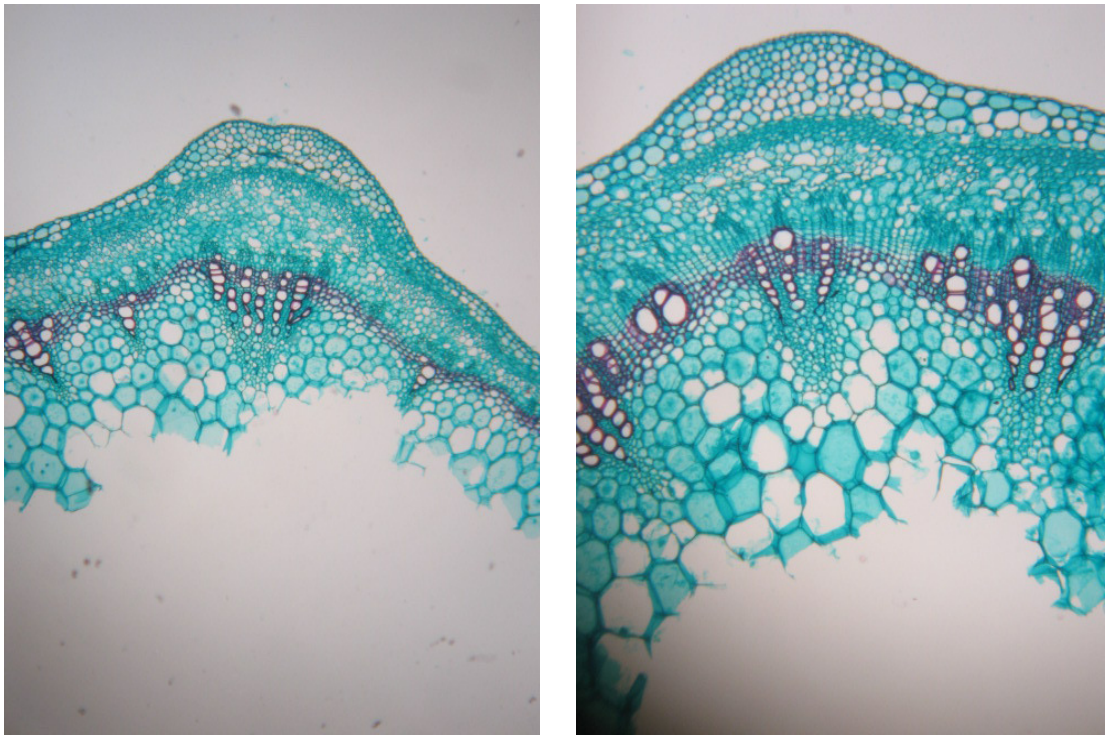
Treatment		Leaves			Branches		
		N	P	K	N	P	K
N levels (kg/fad.)		**Effect of nitrogen levels (kg/fad.)**					
15		345.93	55.26	255.42	355.77	50.53	201.70
25		453.90	70.63	317.59	531.16	76.70	277.40
35		438.25	74.05	314.36	491.41	73.33	265.88
LSD (0.05)		35.60	4.70	31.52	22.76	3.01	11.22
Lithovit (g/l)		**Effect of lithovit (g /l)**					
Control (Tap water)		259.26	41.13	168.96	276.33	43.65	161.96
2		269.76	43.91	191.49	375.90	54.50	189.18
4		471.49	75.66	370.42	500.75	71.49	280.98
6		650.26	105.88	452.30	684.80	97.76	361.18
LSD (0.05)		41.09	8.10	35.47	24.17	4.11	17.77
N levels (kg/fad.)	**Lithovit (g/l)**	**Effect of interaction**					
15	**Control**	225.25	38.23	149.16	223.44	33.63	131.48
	2	271.18	44.20	173.59	309.27	49.52	179.70
	4	409.13	62.83	340.73	354.71	52.89	221.43
	6	478.17	75.76	358.20	535.65	66.07	274.19
25	**Control**	253.70	41.87	175.85	284.75	45.35	162.64
	2	268.25	40.33	185.17	427.68	57.55	204.16
	4	492.61	73.42	365.40	617.91	85.26	329.12
	6	801.03	126.91	543.95	794.29	118.62	413.68
35	**Control**	298.84	43.28	181.87	320.79	51.97	191.76
	2	269.86	47.20	215.71	390.76	56.42	183.68
	4	512.73	90.73	405.12	529.62	76.33	292.40
	6	671.58	114.97	454.74	724.47	108.58	395.66
LSD (0.05)		72.10	14.22	62.24	42.41	7.22	31.18

Table 6. Influence of mineral nitrogen fertilizer levels and lithovit on the anatomical structure of median portion of the main stem of cowpea plant at 70 days after sowing during 2016 season

Character (µm)	Treatment				
	35 kg N/fad.	25 kg N/fad.	±% to 35 kg N/fad.	25 kg N/fad. + lithovit	±% to 35 kg N/fad.
Stem diameter	4807	4538.8	- 5.6	5170	+ 7.5
Epidermis thickness	18.8	18.8	-	18.8	-
Cortex thickness	119	112.8	- 5.3	181.7	+ 52.6
Fiber strands thickness	68.9	37.6	- 45.5	43.8	- 36.5
Vascular cylinder thickness	332.1	332.1	-	369.7	+ 11.3
Phloem tissue thickness	62.6	56.4	- 10	75.2	+ 20.1
Xylem tissue thickness	250.6	244.4	- 2.5	263.2	+ 5
Number of vascular bundle	21.6	15.66	- 27.8	23.3	+ 7.8
Number of vessel / vascular bundle	5	5	-	5	-
Vessel diameter	43.8	37.9	- 13.5	55.2	+ 26
pith diameter	788	660.2	- 16.3	907.8	+ 15.2
Hollow pith diameter	2153.3	2215.8	+ 2.9	2125.5	- 1.3



(A)



(B) **(C)**

Fig. 1. Transverse sections through median portion of the main stem of cowpea plant under the effect of mineral nitrogen fertilizer levels and lithovit at 6g per liter, 70 days after sowing (X40)

A- From plant treated with 35 kg/fad., mineral nitrogen fertilizer.
B- From plant treated with 25 kg/fad., mineral nitrogen fertilizer.
C- From plant treated with 25 kg/fad., mineral nitrogen fertilizer + lithovit.

Table 7. Influence of mineral nitrogen fertilizer levels and lithovit on the anatomical structure of the terminal leaflet from the compound leaf developed on the median portion of the main stem of cowpea plant, 70 days after sowing during 2016 season

Character (µm)	Treatment				
	35 kg N/fad.	25 kg N/fad.	±% to 35 kg N/fad.	25 kg N /fad.+lithovit	±% to 35 kg N/fad.
Thickness of midvein	1128	733.2	- 35	1316	+ 16.6
Thickness of lamina	351	170	- 51.6	257	- 26.7
Thickness of palisade tissue	175.4	81.4	- 53.6	137.8	- 21.5
Thickness of spongy tissue	137.8	50.1	- 63.6	81.4	- 41
Dimensions of the main vascular bundle of midvein:					
Length	194.2	125.3	35.4-	156.6	- 19.6
Width	319.6	175.4	45.2-	457.4	+ 43.1
Number of vessels/midvein bundle	9	6	- 33.3	9.1	1.1+
Vessels diameter	28.2	19.8	- 29.8	34.4	+ 21.9
Thickness of upper epidermis	18.4	18.4	-	18.4	-
Thickness of lower epidermis	18.4	18.4	-	18.4	-

Table 8. Effect of mineral nitrogen fertilizer levels, lithovit as foliar spray and their interactions on seed yield and its components of cowpea plants during 2015 and 2016 seasons

Treatment		Seeds number/pod		100 seed weight (g)		Seed yield/fad. (kg)		Relative increases in seed yield (%)	
		First season	Second season	First season	Second season	First season	Second season	First season	Second season
N levels (kg/fad.)		Effect of nitrogen levels (kg/fad.)							
15		10.18	10.75	13.35	12.68	628.34	657.52	100.00	100.00
25		10.80	10.52	13.43	13.20	740.63	709.58	117.87	107.92
35		11.27	10.51	12.95	13.05	673.29	731.30	107.15	111.22
LSD (0.05)		NS	NS	NS	NS	17.52	21.8	--	--
Lithovit (g/l)		Effect of lithovit (g/l)							
Control (Tap water)		9.10	8.67	12.33	12.23	509.75	526.04	100.00	100.00
2		10.78	11.01	13.13	12.13	705.43	739.37	138.39	140.55
4		11.39	10.68	13.57	13.33	743.75	748.71	145.90	142.33
6		11.74	12.01	13.93	14.20	764.08	783.72	149.89	148.98
LSD (0.05)		0.58	0.49	NS	NS	18.31	31.48	--	--
N levels (kg/fad.)	Lithovit (g/l)	Effect of interaction							
15	Control	9.21	8.00	12.10	11.70	440.05	441.00	100.00	100.00
	2	10.31	12.00	14.00	11.00	629.15	725.01	142.97	164.40
	4	11.11	11.00	13.80	13.40	702.07	705.02	159.54	159.87
	6	10.10	12.00	13.50	14.60	742.08	759.04	168.64	172.12
25	Control	9.01	9.01	13.30	13.20	624.19	529.03	141.85	119.96
	2	10.02	10.02	12.10	11.60	767.14	748.07	174.33	169.63
	4	11.05	11.03	14.10	14.20	782.11	762.09	177.73	172.81
	6	13.11	12.00	14.20	13.80	789.07	799.11	179.31	181.20
35	Control	9.07	9.00	11.60	11.80	465.01	608.10	105.67	137.89
	2	12.00	11.00	13.30	13.80	720.00	745.04	163.62	168.94
	4	12.01	10.01	12.80	12.40	747.07	779.03	169.77	176.65
	6	12.01	12.03	14.10	14.20	761.09	793.01	172.96	179.82
LSD (0.05)		1.02	0.87	NS	NS	32.14	55.24	--	--

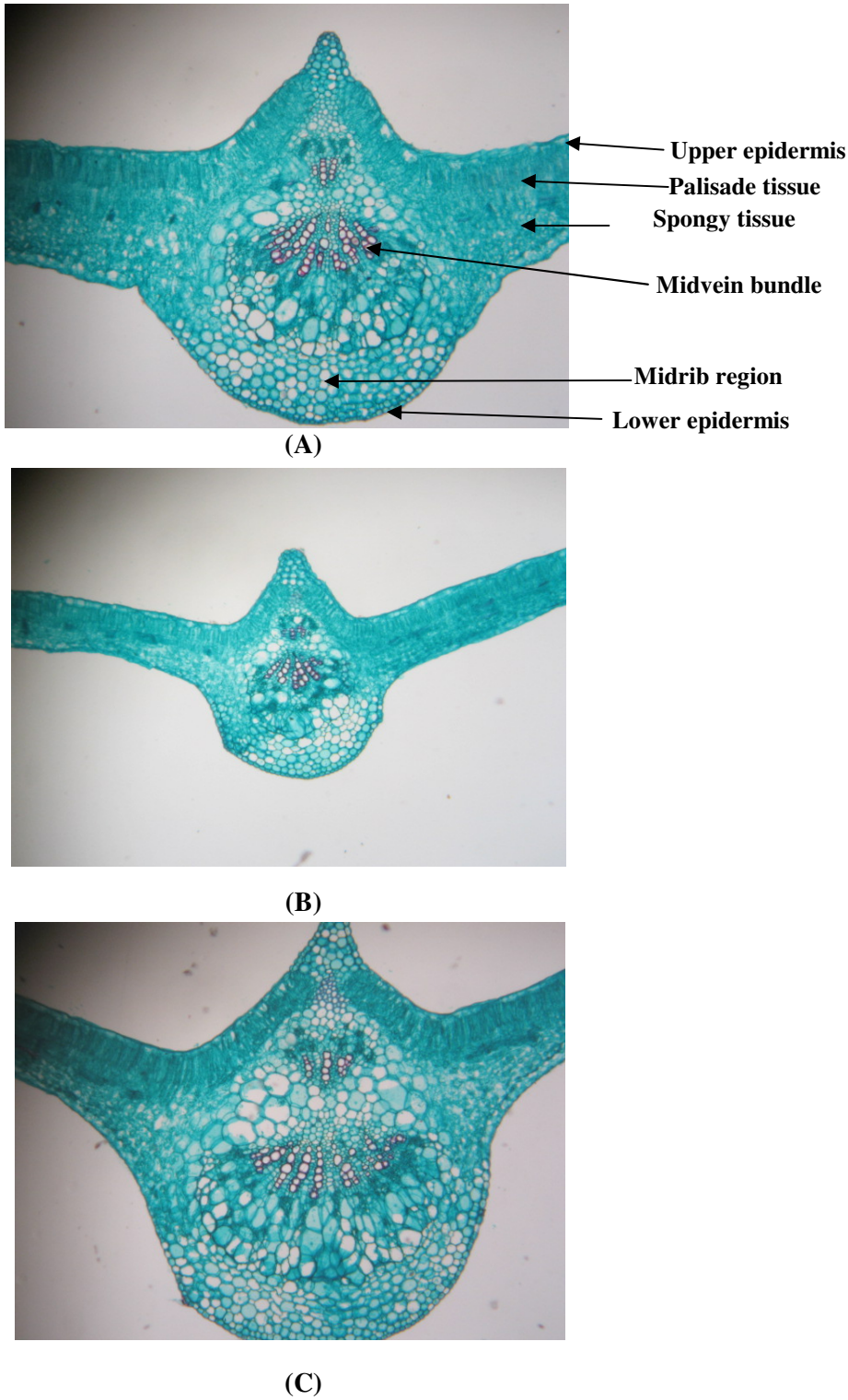


Fig. 2. Transverse sections in terminal leaflet from the compound leaf developed on the median portion of the main stem of cowpea plant under the effect of mineral nitrogen fertilizer level and lithovit at 6g per liter, 70 days after sowing (X 40)

A- From plant treated with 35 kg/fad., mineral nitrogen fertilizer.
B- From plant treated with 25 kg/fad., mineral nitrogen fertilizer.
C- From plant treated with 25 kg/fad., mineral nitrogen fertilizer + lithovit.

25 kg/fad., and lithovit at 6 g/l gave the highest values of seed number/pod and seed yield/fad., with no significant differences with the interaction between the same N level with other lithovit concentrations and with the interaction between N at 35 kg/fad., and lithovit at 6 g/l. Nitrogen at different levels, lithovit at different concentrations and their interactions had no significant effects on 100 seed weight in both seasons.

The stimulative effect of N at 25 kg/fad., and lithovit at 6 g/l on total dry weight N, P and K uptake by leaves and branches and seed yield/fad., may be due to N level at 25 kg/fad., with lithovit at 6 g/l increased main stem diameter, thickness of cortex, phloem, xylem and pith diameter, number of vascular bundle and diameter of vessel and also increased, midvein, and dimensions of midvein bundle at leaves compared at N at 35 kg/fad., (Tables 6 and 7 and Figs. 1 and 2).

Lithovit's mode of action is to increase CO_2 levels within the plant leaf structure and by implication enhance the photosynthetic efficiency. Lithovit consists of calcium carbonate 79.19% gave a direct effect of increases CO_2 on plant growth and development (**Abdelrehem, 2014**).

Regarding the effect of N fertilization and lithovit on yield of cowpea, **Attia (2012)** reported that fertilization of cowpea plants with 45 or 60 kg N/fad., gave the highest values of number and weight of pods/plant, number of seeds/ plant and yield of seeds/plant and per fad., compared to 30 kg N/faddan. Adding 40 kg N/fad., gave the highest mean values of seed yield/ plant, seed yield/fad., number of pods/ plant and 100-seed weight **(El-Atawy and Kasem, 2011; Moursi *et al.*, 2013)**. In addition, **Upadhyay and Singh (2016**) demonstrated that the maximum cowpea yield of seeds were recorded with the highest level of nitrogen at 30 kg N/ha, than 10, 20 kg N/ha or control treatment.

Lithovit a natural CO_2 foliar fertilizer is a new nanotechnological fine powdered created by tribodynamic activation and micronization. Lithovit particles, sprayed finely onto the leaf surface, are taken up directly through the stomata and converted into carbon dioxide. In this way, lithovit can considerably increase the photosynthesis rate, since the essential factor limiting photosynthesis, leading to yield increases (**Farouk, 2015**). Obtained results coincided with those reported by **Byan (2014)** and **Abo-Sedera *et al.* (2016)** who found that spraying snap bean plants with lithovit increased green pod characters than unsprayed plants.

Dry pod characters

Nitrogen at 25 or 35 kg/fad., increased pod number/plant in both seasons and at 35 kg/fad., increased pod length in the 1st season (Table 9).

Foliar spray with lithovit at 6 g/l gave the highest values of pod number/plant and pod length in both seasons. The interactions between N at 25 kg/fad., and lithovit at 6 g/l and N at 35 and lithovit at 4 or 6 g/l, increased pod number/ plant, whereas the interactions between N at 15, 25 and 35 kg/fad., and lithovit at 6 g/l increased pod length. Nitrogen at different levels, lithovit at different concentrations and their interactions had no significant effects on both dry weight and diameter of pod.

Respecting the effect of nitrogen fertilizer, the longest pod of cowpea was recorded with the highest level of nitrogen than other levels (**Upadhyay and Singh, 2016**)**.** Moreover, the highest number of pods/plant of cowpea were obtained with 45 kg N/ha, while the lowest value was obtained with unfertilized plants (**Jacob, 2016**). In addition, spraying snap bean with lithovit at 3 g /l gave the longest pods than unsprayed plants **(Abo-Sedera *et al.*, 2016)**

Seed quality

Fertilizing cowpea plants with N at 35 kg/fad., spraying with lithovit at 6 g/l or the interaction between N at 25 or 35 kg/fad., and lithovit at 6 g/l increased N, P, K, total protein and total carbohydrates in seeds (Table 10). The nutritive value of (N, P, K, TSS, protein and carbohydrate content) were gradually and significantly increased by increasing the level of nitrogen application **(Mahmoud *et al.*, 2010)**. Spraying snap bean with Lithovit at 3 g/l gave the maximum values of total carbohydrates and total protein in dry pods at harvesting time (**Abo-Sedera *et al.*, 2016**).

Table 9. Effect of mineral nitrogen fertilizer levels, lithovit as foliar spray and their interactions on cowpea dry pod characteristics during 2015 and 2016 seasons

Treatment		Pod number/ plant		Dry pod weight (g)		Pod diameter (cm)		Pod length (cm)	
		First Season	Second season	First Season	Second season	First Season	Second season	First Season	Second season
N levels (kg/fad.)		**Effect of nitrogen levels (kg/fad)**							
15		28.00	29.50	1.79	1.81	0.65	0.58	10.90	12.20
25		30.36	31.36	1.89	1.93	0.58	0.58	12.23	12.38
35		30.59	31.53	1.94	1.93	0.58	0.60	13.46	12.78
LSD (0.05)		1.08	0.87	NS	NS	NS	NS	0.65	NS
Lithovit (g/l)		**Effect of lithovit (g /l)**							
Control (Tap water)		23.33	25.33	1.70	1.68	0.57	0.60	11.81	11.27
2		30.15	31.15	1.88	1.93	0.57	0.53	11.48	12.43
4		31.92	32.92	1.88	1.90	0.60	0.53	11.17	12.00
6		33.20	33.78	2.02	2.03	0.67	0.67	14.33	14.10
LSD (0.05)		1.09	0.84	NS	NS	NS	NS	0.64	0.56
N levels (kg/fad.)	**Lithovit (g/l)**	**Effect of interaction**							
15	**Control**	21.40	23.40	1.60	1.53	0.60	0.60	10.20	10.30
	2	27.60	29.60	1.80	1.90	0.70	0.60	8.80	11.90
	4	31.35	32.35	1.80	1.80	0.60	0.50	10.20	11.80
	6	31.65	32.65	1.95	2.00	0.70	0.60	14.40	14.80
25	**Control**	23.15	25.15	1.60	1.70	0.50	0.60	11.80	11.00
	2	32.80	32.80	1.95	2.00	0.50	0.50	11.93	12.50
	4	31.80	32.80	1.90	1.90	0.70	0.50	11.00	12.00
	6	33.70	34.70	2.10	2.10	0.60	0.70	14.20	14.00
35	**Control**	25.45	27.45	1.90	1.80	0.60	0.60	13.42	12.50
	2	30.05	31.05	1.90	1.90	0.50	0.50	13.70	12.90
	4	32.60	33.60	1.95	2.00	0.50	0.60	12.30	12.20
	6	34.25	34.00	2.00	2.00	0.70	0.70	14.40	13.50
LSD (0.05)		1.92	1.48	NS	NS	NS	NS	1.13	0.99

Table 10. Effect of mineral nitrogen fertilizer levels, lithovit as foliar spray and their interactions on seed quality of cowpea during 2016 season

Treatment		N (%)	P (%)	K (%)	Total protein (%)	Total carbohydrates (%)
N levels (kg/fad.)				**Effect of nitrogen levels (kg/fad.)**		
15		3.34	0.48	2.69	20.83	57.80
25		3.60	0.52	2.87	22.45	59.17
35		3.69	0.50	3.02	23.08	61.16
LSD (0.05)		**0.04**	**0.03**	**0.05**	**0.22**	**1.36**
Lithovit (g/l)				**Effect of lithovit (g/l)**		
Control (Tap water)		3.39	0.46	2.44	21.19	56.50
2		3.51	0.49	2.67	21.94	58.68
4		3.52	0.48	3.03	21.98	60.97
6		3.74	0.56	3.29	23.37	61.37
LSD (0.05)		0.06	0.03	0.06	0.22	1.66
N levels (kg/fad.)	**Lithovit (g/l)**			**Effect of interaction**		
15	**Control**	3.06	0.45	2.31	19.10	55.69
	2	3.32	0.46	2.45	20.73	57.80
	4	3.31	0.48	2.86	20.67	58.90
	6	3.65	0.51	3.15	22.83	58.82
25	**Control**	3.50	0.47	2.55	21.85	55.99
	2	3.57	0.48	2.53	22.31	56.70
	4	3.45	0.51	3.10	21.54	62.70
	6	3.86	0.61	3.29	24.10	61.30
35	**Control**	3.62	0.45	2.46	22.63	57.82
	2	3.64	0.53	3.04	22.77	61.53
	4	3.80	0.46	3.14	23.73	61.30
	6	3.71	0.57	3.43	23.17	63.99
LSD (0.05)		0.12	0.05	0.12	0.39	2.92

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تأثير الليثوفيت على النمو، مكونات محصول البذرة وتشريح سيقان وأوراق نباتات اللوبيا تحت مستويات مختلفة من النيتروجين المعدني

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أجري هذا العمل خلال خريف موسمي ٢٠١٥و٢٠١٦ بمزرعة التجارب بغزالة التابعة لكلية الزراعة- جامعة الزقازيق، لدراسة تأثير معدلات التسميد المعدني النيتروجيني (١٥ – ٢٥ – ٣٥ كيلوجرام للفدان) ومستويات الليثوفيت (صفر، ٢، ٤، ٦ جرام /لتر) والتفاعل فيما بينهم على النمو ومحصول البذور والصفات التشريحية لسيقان وأوراق نباتات اللوبيا المزروعة في أرض طينية، أدى تسميد نباتات اللوبيا بمعدل ٣٥ كيلوجرام نيتروجين للفدان إلى زيادة ارتفاع النبات، وعدد كل من الأوراق والأفرع على النبات، والوزن الجاف الكلي للنبات، و محصول البذور للفدان بجانب محتوى البذور من النيتروجين والفوسفور والبوتاسيوم والكربوهيدرات الكلية والبروتين، كذلك أدى رش النباتات بالليثوفت بتركيز ٦جرام/ لتر إلى زيادة في جميع الصفات السابقة، بالإضافة لزيادة في الكميات الممتصة من النيتروجين والفوسفور والبوتاسيوم بالأفرع والأوراق، أدى التفاعل بين تسميد اللوبيا بمعدل ٢٥ كيلو جرام نيتروجين للفدان والرش بالليثوفيت بتركيز ٦جرام/ لتر إلى زيادة إرتفاع النبات، وعدد كل من الأوراق والأفرع على النبات، والوزن الجاف الكلي للنبات، والكميات الممتصة من النيتروجين والفوسفور والبوتاسيوم بالأفرع والأوراق ومحصول البذور للفدان ومحتوى البذور من النيتروجين والفوسفور والبوتاسيوم والكربوهيدرات الكلية والبروتين، بينما أدى تسميد اللوبيا بمعدل ٢٥ كيلو جرام نيتروجين/فدان إلى زيادة الوزن الجاف للأوراق والأفرع والكميات الممتصة من النيتروجين والفوسفور والبوتاسيوم بواسطة الأفرع والأوراق، أما بالنسبة لتأثير معدلات النيتروجين المعدني والرش بالليثوفيت على التركيب التشريحي للمجموع الخضري لنبات اللوبيا، أظهرت المعاملة ب٢٥ كيلوجرام من التسميد النيتروجيني انخفاض في قطر الساق الرئيسي، سمك القشرة، الألياف، اللحاء، قطر الخشب وقطر النخاع، عدد الحزم الوعائية وقطر الوعاء مقارنة باستخدام ٣٥ كيلو جرام نيتروجين/ فدان، بينما نفس المستوى من النيتروجين مع الرش بالليثوفيت قد أعطى زيادة في جميع الصفات السابقة، إضافة ٢٥ كيلوجرام من السماد النيتروجيني المعدني أظهر نقص في منطقة العرق الوسطى، سمك القطاع، وسمك النسيج العمادى والاسفنجى مقارنة باستخدام ٣٥ كيلو جرام نيتروجين/فدان، ولكن نفس المستوى من السماد النيتروجيني مع الليثوفيت عكس زيادة في سمك منطقة العرق الوسطى، عرض حزمة العرق الوسطى، وعدد الأوعية ومتوسط قطر الوعاء ولكن هذه المعاملة أدت إلى نقص في سمك القطاع.

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