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Impact of Sulphur and Nitrogen Application on Seed and Xanthotoxin Yield in *Ammi majus* L.

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ABSTRACT Field experiments were conducted to determine the physiological and biochemical basis of the interactive effect of sulphur (S) and nitrogen (N) application on seed and xanthotoxin yield of *Ammi majus* L. Six treatments were tested (T_1 = control-without manure and fertilizers, T_2 = manure @ 9 kg plot^{-1}-10 t ha^{-1}, $T_3 = S_0N_{50}K_{25}P_{25}$, $T_4 = S_{40}N_{50}K_{25}P_{25}$, $T_5 = S_{40}N_{100}K_{25}P_{25}$, $T_6 = S_{20+20}N_{50+50}K_{25}P_{25}$). Nitrate reductase (NR) activity and ATP-sulphurylase activity in the leaves were measured at various phenological stages, as the two enzymes catalyze rate-limiting steps of the assimilatory pathways of nitrate and sulphate, respectively. The activities of these two enzymes were strongly correlated with seed and xanthotoxin yield. The highest nitrate reductase activity, ATP-sulphurylase activity and xanthotoxin yield were achieved with the treatment T_4. Any variation from this treatment decreased the activity of these enzymes, resulting in a reduction of the seed and xanthotoxin yield in *Ammi majus* L. The higher seed and xanthotoxin yield achieved in *Ammi majus* L. at treatment T_4 could be due to optimization of leaf soluble protein and photosynthetic rate, as these parameters are influenced by S and N assimilation.

Keywords : *Ammi majus* L., sulphur, nitrogen, xanthotoxin, ATP-sulphurylase, nitrate reductase (NR)

***Ammi majus* L.** (Apiaceae) commonly known as atrilal in India, has been used in the treatment of skin diseases (skin depigmentation, vitiligo) for centuries worldwide (Parrish *et al.*, 1974). *Ammi majus* L. contains four main linear furocoumarins: xanthotoxin (8-MOP), bergapten (5- MOP), isopimpinelin and imperatorin. Xanthotoxin appears to be most promising molecule from the pharmacological point of view (Pande *et al.*, 2002; Ahmad *et al.*, 2004). *Ammi majus* and other photosensitizing plants are cultivated even today in some parts of the world as a source of these medicinally important compounds. Some furocoumarins are also synthesized for medicinal use in treating leucoderma and, more recently psoriasis. At present, xanthotoxin resources are limited. Chemical synthesis is still very expensive. Hence, the molecules are commercially extracted from the *Ammi majus*, but their production is insufficient to meet the increasing demand of these molecules by pharmaceutical industries. The information related with the cultivation and harvesting of *Ammi majus* plants for the optimal production of xanthotoxin is meager. Therefore, it is necessary to increase the yield of these metabolites in the plant by developing a better agrotechnology for the cultivation of this plant.

Sulphur (S) is increasingly being recognized as the fourth major plant nutrient after nitrogen, phosphorous and potassium. Sulphur uptake and assimilation in higher plants is one of the crucial factors determining crop yield, quality and even resistance to pests and environmental stresses.

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The interactive effect of sulphur and nitrogen application on the growth and yield of agricultural crops has already been reported (Fazli *et al.*, 2005a & b, Ahmad *et al.*, 1999a; Ahmad and Abdin, 2000; Ahmad *et al.*, 2001; Jamal *et al.*, 2005). To our knowledge, no study has been conducted so far to study the interactive effect of S and N on growth and secondary metabolite yield in medicinal plants particularly *Ammi majus* L. In this study, we have attempted to analyze the physiological and biochemical basis of the yield responses of *Ammi majus* L. to the combined application of S and N.

MATERIALS AND METHODS

Plant materials

The seeds of *Ammi majus* L. were collected from Herbal Garden of Hamdard University, New Delhi, India located at 28°38'N, 77°11'E and 228 m altitude. Sowing was done during 2004-2005 at the experimental field of the Hamdard University. The soil was a sandy loam with pH 7.3 and deficient in S (0.001%).

Treatments

Six treatments, T_1 = control-without manure and fertilizers, T_2 = manure @ 9 kg plot^{-1}-10 t ha^{-1}, $T_3 = S_0N_{50}K_{25}P_{25}$, $T_4 = S_{40}N_{50}K_{25}P_{25}$, $T_5 = S_{40}N_{100}K_{25}P_{25}$, $T_6 = S_{20+20}N_{50+50}K_{25}P_{25}$ were used. The fertilizer treatments included two levels of nitrogen and two levels of sulphur viz., 50 and 100 kg ha^{-1} and 0 and 40 kg ha^{-1} in the following combinations. In the treatments T_4 and T_5, nitrogen and sulphur were applied as single basal dose, while in the treatment T_6 these were applied in two equal splits. All plots received 25 kg ha^{-1} of phosphorus and potassium at the time of sowing. The experiments were conducted using a randomized block design with three replicates of each treatment. The plot size was 9 m^2 (3 m × 3 m) with 9 rows and row-to-row distance of 45 cm and one uniform irrigation was done before sowing and subsequently, whenever needed. Weeding was carried out frequently to keep the plant free from weeds.

Sampling

Samples were collected at an interval of 45 days after sowing till harvest. The seeds were removed from the plant, packed in polythene bags and brought to the laboratory.

Estimation of dry weight of plants

The plant samples (leaves, stem, root and seeds) were collected at different phenological stages. Three plants were taken from each plot randomly. The plants were cut at the root-shoot junction and dried in oven at 65℃±2 until constant weight was reached.

Estimation of soluble protein

Soluble protein content in the developing seeds was estimated by the method of Bradford (1976) after precipitation with trichloroacetic acid; using bovine serum albumin as standard.

Measurement of photosynthetic rate

The photosynthetic rate of intact leaves in the field was measured at different phenological stages of *Ammi majus* using a portable photosynthesis system (Model Li 6200, Li-COR Inc., USA).

Assay of nitrate reductase activity

In vivo nitrate reductase activity in leaves was measured at different phenological stages according to the procedure of Hageman and Hucklesby (1971) with slight modification (Ahmad *et al.*, 1998). Fresh leaf tissue of *Ammi majus* was cut into 2 mm slices and placed in ice cold incubation medium containing 3.0 ml of potassium phosphate buffer (0.05 M, pH 7.8 and 0.15 M, pH 7.2 and 3.0 ml of 0.4 M KNO_3 solution). The tubes were evacuated with a vacuum pump and then incubated in a water bath at 35℃ for 75 min under dark conditions. At the end of incubation period, tubes were kept in boiling water bath for 5 min to stop the enzyme activity and complete leaching of the nitrite in the medium. Nitrite was estimated by the method of Evans and Nason (1953). 0.2 ml of the aliquot from reaction mixture was taken and 1.0 ml each of 1.0 per cent sulphanilamide in 1N-HCl and 0.02 per cent N-(1-Napthyl)-ethylene diammonium dichloride (NEDD) in distilled water were added. The pink colour due to diazotisation was allowed to develop for 30 mins after which the volume

was made upto 6.0 ml with distilled water. The absorbance was read at 540 nm, using UV-Vis spectrophotometer (Model DU 640B, Beckman, USA). The calibration curve was prepared using sodium nitrite solution. The enzyme activity was expressed as μ mole NO_2 g^{-1} fw hr^{-1}.

Assay of ATP-sulphurylase activity

In vitro ATP-Sulphurylase activity in fresh leaves was determined by the method of Wilson and Bandurski (1958). 0.5 g of fresh tissue was homogenized in 5 ml of extraction buffer with the help of mortar and pestle placed in ice. The homogenate was centrifuged at 5000 rpm for 15 mins at 4℃. 0.1 ml aliquot was taken in the test tube, to which 0.4 ml of reaction mixture was added. It was then incubated in water bath at 33℃ for 30 mins. The reaction was terminated in hot water. 1 ml of ammonium molybdate solution and 0.1 ml of reducing agent were added. Volume was made to 10 ml with DDW. After 20 mins, absorbance was read at 660 nm (Model DU 640, Beckman, USA). Calibration curve was prepared by different concentrations of KH_2PO_4 solution. The enzyme activity was expressed as μ mol Pi mg^{-1} Protein min^{-1}.

Estimation of xanthotoxin by high pressure liquid chromatography (HPLC)

Standard curve of xanthotoxin: A stock solution (1 mg ml^{-1}) of the authentic sample (xanthotoxin) was prepared in methanol. From the stock solution, various dilutions were made so as to have xanthotoxin concentration in the range of 1-5 μg. These were analyzed independently by HPLC and standard curves were plotted between concentrations and peak areas. The injected quantities and peak area showed good linearity.

Sample preparation for xanthotoxin estimation: Plant sample (seed) from *Ammi majus* were collected from the experimental field of Hamdard University at different developmental stages. Extraction and isolation of xanthotoxin from the seed was performed by the method as reported earlier (Ahmad *et al.*, 2004). 1 g of each dry sample (oven dried) was extracted using a soxlet apparatus with methanol (20 ml). The presence of xanthotoxin in the methanolic extract was confirmed by co-chromatography of seed extract and authentic xanthotoxin by TLC (Thin Layer Chromatography). Methanolic extract of seed sample along with authentic sample was resolved on aluminum sheets coated with Silica gel 60 F254 (20×20 cm, Merk, Germany). The plates were developed in an airtight chromatographic chamber saturated with 200 ml of di-butyl ether and chloroform solvent system. The plates were activated at 50℃ in an oven. The developed chromatogram was visualized under UV light in UV cabinet (Matrex, New Delhi, India). The yellow fluorescence observed, confirmed the presence of xanthotoxin in *Ammi majus* seed extract. The R_f value was 0.39 for authentic xanthotoxin and in compounds detected in seed extract, respectively.

Xanthotoxin estimation was done by the method of Kavli and Volden (1984). Each methanolic sample (seed extract) was filtered with Millipore filter paper and the 20 μl of this filtrate was injected in HPLC. Reverse phase HPLC (Waters, USA) was performed with methanol: water (1:1, HPLC grade) as mobile phase at a flow rate of 1 ml min^{-1} maintained by Waters 600 controller pump. Separation was done in RP-18 (5 μm) columns. The xanthotoxin was resolved on the column and monitored at 251 nm using Waters Photodiode Array Detector (water delta 996). The area of the relevant peak of each sample was interpolated with the standard curve to determine the quantity of xanthotoxin (in mg g^{-1} dry wt. of plant sample). At least three samples from each treatment were analyzed.

Seed yield and xanthotoxin yield

The total seed produce from one meter square area was cleaned and weighed to compute the seed yield in g m^2 and q ha^{-1} (quintal/hectare, 100 kg = 1 q). The percent xanthotoxin content when multiplied with seed yield gave the xanthotoxin yield. The xanthotoxin yield was also expressed in q ha^{-1}.

Statistical analysis

The statistical analysis was performed following the method of Nageswar (1983).

RESULTS AND DISCUSSION

The present study was undertaken to develop an integrated nutrient management technology with the use of different combinations of sulphur and nitrogen applied at various phenological stages for optimizing biomass accumulation, seed and xanthotoxin yield in *Ammi majus* L.

Dry matter, soluble protein and photosynthetic rate

The result (Table 1) revealed that the treatment T_4 showed best results and the percent increment in biomass accumulation was 28.05 at vegetative stage and 32.76 at harvest followed by treatment T_6 (24.50% at vegetative stage and 27.64% at harvest), when compared with control (T_1). Application of S and N in various combinations significantly enhanced leaf soluble protein over control (T_1) (Table 2). Among the various treatments, T_4 showed best result at different growth stages. The percent increment in leaf soluble protein due to the treatment T_4 at vegetative and flowering stage was 29.39 and 30.97, respectively followed by T_6

Table 1. Effect of sulphur (S) and nitrogen (N) on biomass accumulation (g plant^{-1}) in *Ammi majus* L. at different phenological stages.

TREATMENT (T)	PHENOLOGICAL STAGES (S)				
Ammi majus L.	Vegetative stage	Pre-flowering stage	Flowering stage	Post-flowering stage	At harvest
T_1	1.64	18.97	39.42	36.48	32.78
T_2	1.76	19.92	41.65	37.94	33.83
T_3	1.93	21.45	45.86	41.28	37.90
T_4	2.10	24.31	51.54	47.92	43.51
T_5	2.02	22.40	48.34	44.40	40.30
T_6	2.05	22.91	50.45	46.64	41.84
L.S.D. (0.05)					
S	1.0033				
T	1.0991				
S x T	2.4576				

T_1 = control-without manure and fertilizers, T_2 = manure @ 9 kg plot^{-1}-10 t ha^{-1}, $T_3 = S_0N_{50}K_{25}P_{25}$, $T_4 = S_{40}N_{50}K_{25}P_{25}$, $T_5 = S_{40}N_{100}K_{25}P_{25}$, $T_6 = S_{20+20}N_{50+50}$
T : treatment, S : phenological stages
LSD : Least significant differences

Table 2. Effect of sulphur (S) and nitrogen (N) on soluble protein content (mg g^{-1} fw) in leaves of *Ammi majus* L. at different phenological stages.

TREATMENT (T)	PHENOLOGICAL STAGES (S)			
Ammi majus L.	Vegetative stage	Pre-flowering stage	Flowering stage	Post-flowering stage
T_1	6.15	8.41	7.05	ND
T_2	6.46	8.84	7.42	ND
T_3	7.14	9.80	8.21	ND
T_4	7.95	10.67	9.23	ND
T_5	7.23	10.21	8.59	ND
T_6	7.42	10.22	8.65	ND
L.S.D. (0.05)				
S	0.2241			
T	0.3169			
S x T	NS (Non-significant)			

T_1 = control-without manure and fertilizers, T_2 = manure @ 9 kg plot^{-1}-10 t ha^{-1}, $T_3 = S_0N_{50}K_{25}P_{25}$, $T_4 = S_{40}N_{50}K_{25}P_{25}$, $T_5 = S_{40}N_{100}K_{25}P_{25}$, $T_6 = S_{20+20}N_{50+50}$
T : treatment, S : phenological stages, ND : not determined
LSD : Least significant differences

(20.66% at vegetative and 22.70% at flowering stage), when compared with control (T_1). The maximum photosynthetic rate (Table 3) was recorded at pre-flowering stage (22.85 μ mole CO_2 m^{-2} sec^{-1}), when compared with vegetative (20.06 μ mole CO_2 m^{-2} sec^{-1}) and flowering stages (18.98 μ mole CO_2 m^{-2} sec^{-1}), respectively. Among the various treatments, T_4 showed best result. The percent increment in photosynthetic rate due to the treatment T_4 at vegetative and flowering stage was 20.20 and 22.70, respectively followed by T_6 (17.10% at vegetative and 20.16% at flowering stage), when compared with control (T_1).

The *Ammi majus* plant accumulated more biomass when both S and N were applied as single basal dose (T_4), when compared with control (T_1) at all the phenological stages. Many workers have reported that the application of these macronutrients caused significant increase in growth and yield in *Ammi majus* (Randhawa *et al.*, 1985; Gill and Samra, 1986; Lakshmipathaiah *et al.*, 1999). An increase in plant height, number of branches and leaf area per plant in Isabgol due to the balanced application of the macronutrients viz. N, P and K has already been reported (Ramesh *et al.*, 1989). Data on leaf soluble protein content and photosynthetic rate revealed that the application of S along with N (T_4) resulted in significant enhancement in both the soluble protein content and the photosynthetic rate over the application of control (T_1). The low soluble protein content observed in leaves of *Ammi majus* L. with other combinations of S and N (T_2) could be due to the imbalanced supply of these nutrients to the plants. When plants are grown with insufficient S, non-protein N accumulates in the vegetative tissue at the expense of protein N and growth is retarded (Eppendorfer, 1971). The increase in non-protein N in S-deficient plants is characterized by an accumulation of amides, usually asparagines (Stewart and porter, 1969). The decline in soluble protein due to either low S supply or an imbalanced supply of nitrogen and sulphur is mainly a consequence of the linkage of N and S metabolism at the level of protein synthesis. Since RUBISCO (the enzyme responsible for carbon fixation) constitutes 50-70% of total soluble protein content, a change in soluble protein content affects the rate of photosynthesis. Maintenance of photosynthesis by leaves throughout the growth period especially at the seed filling stage is a major requirement for the production of adequate carbohydrate to give large seeds and high yield (People *et al.*, 1980). Hence, the high seed and xanthotoxin yield obtained in our study with the treatment T_4 could be due to the production of an adequate amount of carbohydrate during seed growth of *Ammi majus* L., as both soluble protein and rate of photosynthesis were optimum in this treatment.

Table 3. Effect of sulphur (S) and nitrogen (N) on rate of Photosynthetic rate (μ mole CO_2 m^{-2} sec^{-1}) in leaves of *Ammi majus* L. at different phenological stages.

TREATMENT (T)	PHENOLOGICAL STAGES (S)			
Ammi majus L.	Vegetative stage	Pre-flowering stage	Flowering stage	Post-flowering stage
T_1	20.06	22.85	18.98	ND
T_2	20.24	22.64	19.07	ND
T_3	22.32	24.39	21.27	ND
T_4	24.12	26.26	23.29	ND
T_5	23.43	25.81	22.39	ND
T_6	23.49	25.85	22.81	ND
L.S.D. (0.05)				
S	0.4288			
T	0.6063			
S x T	NS (Non-significant)			

T_1 = control-without manure and fertilizers, T_2 = manure @ 9 kg $plot^{-1}$-10 t ha^{-1}, $T_3 = S_0N_{50}K_{25}P_{25}$, $T_4 = S_{40}N_{50}K_{25}P_{25}$, $T_5 = S_{40}N_{100}K_{25}P_{25}$, $T_6 = S_{20+20}N_{50+50}$

T : treatment, S : phenological stages, ND : not determined

LSD : Least significant differences

In vivo nitrate reductase and *in vitro* ATP-sulphurylase activity

The NR activity (Table 4) in the leaves was increased continuously till pre-flowering stage and was recorded maximum at this stage (4.59 μ mole NO_2 g^{-1} fw h^{-1}) when compared with vegetative (4.07 μ mole NO_2 g^{-1} fw h^{-1}) and flowering stages (3.82 μ mole NO_2 g^{-1} fw h^{-1}), respectively. Among the various treatments, T_4 showed best results at different growth stages. The percent increment in NR activity due to the treatment, T_4 at vegetative and flowering stage was 49.34 and 50.52, respectively followed by T_6 (47.62% at vegetative and 45.99% at flowering stage), when compared with control (T_1). The *in vitro* ATP-sulphurylase activity in the leaves (Table 5) varied from 13.96 μ mole Pi mg^{-1} protein min^{-1} at vegetative stage to 13.46 μ mole Pi mg^{-1} protein min^{-1} at flowering stage. The maximum ATP-sulphurylase activity was observed at pre-flowering stage (15.58 μ mole Pi mg^{-1} protein min^{-1}). Application of S and N in various combinations significantly enhanced ATP-sulphurylase activity over control (T_1). Among

Table 4. Effect of sulphur (S) and nitrogen (N) on NR Activity (μ mole NO_2 g^{-1} fw h^{-1}) in leaves of *Ammi majus* L. at different phenological stages.

TREATMENT (T)	PHENOLOGICAL STAGES (S)			
Ammi majus L.	Vegetative stage	Pre-floweringstage	Flowering stage	Post-flowering stage
T_1	4.07	4.59	3.82	ND
T_2	4.41	4.87	3.93	ND
T_3	5.23	5.77	4.89	ND
T_4	6.07	6.34	5.75	ND
T_5	5.93	6.17	5.45	ND
T_6	6.00	6.21	5.58	ND
L.S.D. (0.05)				
S	0.1399			
T	0.1979			
S x T	NS (Non-significant)			

T_1 = control-without manure and fertilizers, T_2 = manure @ 9 kg $plot^{-1}$-10 t ha^{-1}, $T_3 = S_0N_{50}K_{25}P_{25}$, $T_4 = S_{40}N_{50}K_{25}P_{25}$, $T_5 = S_{40}N_{100}K_{25}P_{25}$, $T_6 = S_{20+20}N_{50+50}$
T : treatment, S : phenological stages, ND : not determined
LSD : Least significant differences

Table 5. Effect of sulphur (S) and nitrogen (N) on *in vitro* ATP sulphurylase activity (μ mole Pi mg^{-1}protein min^{-1}) in leaves of *Ammi majus* L. at different phenological stages.

TREATMENT (T)	PHENOLOGICAL STAGES (S)			
Ammi majus L.	Vegetative stage	Pre-flowering stage	Flowering stage	Post-flowering stage
T_1	11.03	12.80	10.25	ND
T_2	11.82	13.15	11.24	ND
T_3	13.93	15.65	13.59	ND
T_4	16.02	17.73	15.66	ND
T_5	15.21	16.98	14.84	ND
T_6	15.72	17.18	15.20	ND
L.S.D. (0.05)				
S	0.2547			
T	0.3602			
S x T	NS (Non-significant)			

T_1 = control-without manure and fertilizers, T_2 = manure @ 9 kg $plot^{-1}$-10 t ha^{-1}, $T_3 = S_0N_{50}K_{25}P_{25}$, $T_4 = S_{40}N_{50}K_{25}P_{25}$, $T_5 = S_{40}N_{100}K_{25}P_{25}$, $T_6 = S_{20+20}N_{50+50}$
T : treatment, S : phenological stages, ND : not determined
LSD : Least significant differences

the various treatments, T_4 showed best result at different phenological stages. The percent increment in ATP-sulphurylase activity due to the treatment, T_4 at vegetative and flowering stage was 45.27 and 52.80, respectively followed by T_6 (42.49% at vegetative and 48.31% at flowering stage), when compared with control (T_1).

In view of the key role played by the enzyme nitrate reductase in nitrate assimilation and of ATP-sulphurylase in sulphate assimilation, it is to be expected that the activities of these enzymes are related to seed and xanthotoxin yield. Application of S along with N ($T_4 > T_6 > T_5$) enhanced the activities of the two enzymes over the control (T_1). The highest NR and ATP-sulphurylase activities were observed during the various phenological stages of the crop with the treatment consisting of 40 KgS ha^{-1} applied along with 50 Kg N ha^{-1} applied as a single basal dose (T_4), when compared with the control (T_1). Increased NR and ATP-sulphurylase activities with this treatment (T_4) were an indicator of increased metabolic activity related to N and S assimilation as a result of the balanced supply of nitrogen and sulphur to the crop. These results are in consistent with those reported by other workers in different plants (Smith, 1975; Reuveny *et al.*, 1980; Clarkson *et al.*, 1989; Ahmad *et al.*, 1999b; Fazli *et al.*, 2005a; Ahmad *et al.*, 2006). Barney and Bush (1985) working on tobacco reported that +N-S treated plants had very low NR activity because of the lack of S. When plants were transferred from +N-S to -N+S, NR activity remained very low because of the lack of N. Similarly, -N+S treated plants had very low ATP-sulphurylase activity perhaps because the limited N supply in plant prevented SO_4^{2-} translocation from roots to shoots. Increased nitrate reductase activity with S fertilization was reported in tobacco (Pal *et al.*, 1976). The synthesis of cysteine as a result of the incorporation of a sulphide moiety into O-acetyl serine appears to be the meeting point between N and S assimilation.

Seed yield and xanthotoxin yield

All the combinations of S and N (T_4, T_5 and T_6) (Table 6) enhanced the seed yield and xanthotoxin yield significantly, when compared with control (T_1). Among the treatments, T_4 resulted in optimum seed and xanthotoxin yield (7.8 q ha^{-1} and 0.13 q ha^{-1}) which was followed by treatment T_6 (7.4 q ha^{-1} and 0.12 q ha^{-1}). The percent enhancement in seed and xanthotoxin yield with treatment T_4 was 70.6 and 93.71 when compared with control (T_1), respectively.

Our observation on increasing nitrate reductase activity, ATP-sulphurylase activity and yield of *Ammi majus* L. in response to treatment T_4 suggested that a strong correlation exists between yield and activities of these enzymes at various phonological stages. Similar findings were also observed in other crops, for example maize (Deckard *et al.*, 1973; Balasubramanian *et al.*, 1977) wheat (Croy and

Table 6. Effect of sulphur (S) and nitrogen (N) on seed and xanthotoxin yield (q ha^{-1}) of *Ammi majus* L.

TREATMENT (T)	ECONOMIC YIELD		HARVEST INDEX	
Ammi majus L.	Xanthotoxin Concentration (%)	Xanthotoxin yield (q ha^{-1})	Seed yield (q ha^{-1})	(%)
T_1	1.51	0.069	4.587	7.77
T_2	1.58	0.081	5.111	8.19
T_3	1.65	0.107	6.329	9.55
T_4	1.72	0.134	7.822	9.98
T_5	1.70	0.121	7.139	9.85
T_6	1.71	0.128	7.472	9.93
L.S.D. (0.05)				
T (Treatment)	0.044	0.007	0.437	0.859

T_1 = control-without manure and fertilizers, T_2 = manure @ 9 kg $plot^{-1}$-10 t ha^{-1}, $T_3 = S_0N_{50}K_{25}P_{25}$, $T_4 = S_{40}N_{50}K_{25}P_{25}$, $T_5 = S_{40}N_{100}K_{25}P_{25}$, $T_6 = S_{20+20}N_{50+50}$

T : treatment, (q ha^{-1}) = quintal / hectare (100 kg = 1 quintal)

LSD : Least significant differences

Hageman, 1970, Abrol *et al.*, 1976) and rapeseed-mustard (Abdin *et al.*, 2003; Ahmad *et al.*, 1999).

From these observations, it can be concluded that optimization of both NR and ATP-sulphurylase activities with the application of balanced doses of sulphur and nitrogen fertilizers may lead to higher seed and xanthotoxin yield in *Ammi majus* L.

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