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Potential use of Mascagnite [$(NH_4)_2.SO_4$]: A way to ameliorate crop yield and nitrogen fixation capacity of *Lens culinaris* L.

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Abstract
A field experiment was carried out to evaluate the effect of different levels of mascagnite solution on some bio-chemical parameters such as: proline, Leghaemoglobin, chlorophyll, nitrogen, phosphorous, protein, and nitrate and nitrite reductase enzymes activity in lentil crop. The experiments were conducted in the research farm of the Botany Department of C.C.S. University, Meerut in Rabi season (2017-18). Mascagnite, a source of nitrogen and sulfur nutrients has the potential agronomic benefits in increasing crop production over other commonly used N and S fertilizers. 1.5% of ammonium sulphate was found beneficial for the lentil crop productivity as compared to others remaining 0%, 0.5%, 1.0% treatments. 1.5% of mascagnite solution had the better potential to reduce the stress conditions and enhanced the biochemical pathways to provide essential elements such as nitrogen or sulfur.

Keywords: *Lens culinaris.* L., ammonium sulphate, fertilizer, chlorophyll, leghaemoglobin, nitrate reductase enzyme

Introduction

World population is predicted to be doubled by 2050 (http://www.fao.org), imposing an increasing demand for food that comes together with an increasing concern on environment and food security. Legumes are important food and feed crops as the main component of nutritious diet [1]. It has an ever increasing role as forage producing crop for high-quality meat and milk production [2]. Legumes are also gaining importance for their health benefits [3]. They also have the unique ability to form a symbiotic relationship with soil bacteria collectively called "Rhizobia" [4].

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) which is a diploid (2*n* = 14 chromosomes) as an autogamous annual legume with a haploid genome size of an estimated 4063 Mbp [5] is the second most important Rabi legume crop after chickpea and is cultivated on 1.47 million hectares area with an annual production of 1.04 million tones with a productivity around 705 kg/ha during 2014 (Agricultural Statistics Division, 2014-2015). It is variously grown as rain fed crop in area of various states of India [6].

Plant nutrient management is critical to the sustainability of agricultural production systems. Nitrogen, phosphorus, potassium and sulfur are the four macronutrients required for crop growth in order to maintain agricultural sustainability (nutrients that are removed from the soil by crops) and the soil fertility. Balanced fertilizer sources (macro - and micro - nutrients) are incorporated into soil regularly [7].

Leguminous plants are able to biologically fix atmospheric nitrogen so that there is no or very little need of external nitrogen input [8] but plants are not able to fix sulfur as they fix nitrogen by the process of nitrogen fixation. However, sulfur is also very essential for synthesis of amino acids like cystine and methionine. These amino acids are component of vitamin A and activates certain enzyme systems in leguminous plants [9]. Obviously it is required for proper growth and yield of plants in sufficient amount. Continuous removal of sulphur from soil by plant led to widespread deficiency which affected soil sulphur budget. Sulphur deficiency leads accumulation of amino acids, which is assumed to down to regulate nitrogen uptake and assimilation.

Mascagnite fertilizer (Ammonium sulfate) is produced from the reaction of ammonia with sulfuric acid. It contains 21% nitrogen and 24% sulphur. It is known to be as one of the best fertilizers for providing essential elements like nitrogen and sulphur for alkaline and calcareous soils [10].

Since little work has been done as a nitrogen and sulphur fertilizer simultaneously in the relation of lentil therefore, Keeping in view the present study was focused on the following objectives: To evaluate the effect of Mascagnite on some bio-chemical parameters and the efficiency of nitrogen fixing capacity of *Lens culinaris* L.

Method and material

a) Geographical situation

The Choudhary Charan Singh University campus, Meerut (U.P. India) is situated between 29^0 01N latitude and 77^0 45E longitudes at an altitude of 237 meters above sea level. The total geographical area of Meerut district is 2564 km^2. The district falls under western plain zone of Uttar Pradesh, sub-region of Upper Gangetic plain.

b) Material

- Certified seeds of *Lens culinaris.* L. (Pusa vaibhav) was procured from Indian Agriculture Research Institute, New

Delhi.

- Mascagnite [$(NH4)_2.SO_4$] was received from the Fisher scientific company.

c) Experimental site

The field experiment was conducted during the Rabi season in the month of November to January during 2017-2018 to evaluate the "Potential use of mascagnite [$(NH_4)_2.SO_4$]: a way to ameliorate crop yield and nitrogen fixation capacity of lens culinaris L."

d) Preparation of stock solution

Three samples of different concentrations of ammonium sulphate solution were prepared. For this 5, 10 and 15 gm of ammonium sulphate were dissolved in each of 1 litre of de-ionized water.

1. 05 gm + 1000 ml de-ionized water (0.5%)
2. 10 gm + 1000 ml de-ionized water (1.0%)
3. 15 gm + 1000 ml de-ionized water (1.5%)

e) Experimental Details

Four levels of ammonium sulphate as 0, 5, 10, and 15 gm/1000 ml distilled water in soil were used in this experiment, resulting in a total of 4 treatments (control, 0.5%, 1.0 %, 1.5% $(NH_4)_2.SO_4$). Four small land plots, each of 1X1 meter^2 were sprayed with each of four solution of treatments i.e. control, 0.5%, 1.0 %, 1.5% $(NH_4)_2.SO_4$.

f) Recorded Data

Seed Viability Test The seeds were checked for their viability by using the test made by Lamarca (2014) [11].

Proline Content: The proline content was estimated by the protocol given by Bates *et al*. (1973) [12].

Leghaemoglobin Content: Leghaemoglobin content was estimated by the protocol given by Bergersen method (1980) [13].

Chlorophyll content: Chlorophyll content was estimated by using Arnon's (1949) Method [13].

Estimation of nitrogen content: Total nitrogen content was estimated by the method adopted by Snell and Snell (1967) [14].

Estimation of Protein Content: The protein content was estimated by Bradford method (1976) [15].

Determination of Nitrate Reductase: Total nitrate reductase was estimated by Hageman method (1980) [16].

Determination of Nitrite Reductase: Total nitrite reductase was estimated by Guerrero method (1982) [17].

Determination of phosphorous: Total phosphorous was estimated by Olsen method (1954) [18].

Result & Discussion

Protein content

With the increase in use of concentration of mascagnite solution, increase in the protein content was observed in the seeds (Table 1). The high protein content was observed in 1.5% mascagnite treated plots. It may be due to the stimulatory effects of mascagnite on proteolyses enzymes and enhancement of biosynthesis of amino acids and proteins [20]. Mascagnite contains 24% sulfur and 21% nitrogen and both of these elements play crucial role in protein synthesis. When sulfur is limiting, protein synthesis is inhibited, resulting in lower yields. Many globulins that increase in response to sulfur deficiency (vicilins and convicilins) contain few S–containing amino acids (approximately 0.6% of their sequence), whereas the globulins whose levels decreased contained a higher proportion of S–containing amino acids (approximately 1.5%) [21]. Second element of mascagnite nitrogen increases the ammonia assimilation in roots which enhances amino acids concentration in roots which ultimately responsible to increases the protein contents of the seeds [20]. Under optimum growth conditions, plants accumulate nitrogen and sulfur which are proportional to that of incorporated into protein. Present results are conformity with Scherer (2006) [22].

Proline Content

Proline as a stress amino acid indicator accumulates under stress conditions and helps induce tolerance for stress in plants. Increase in proline level may help maintain osmotic potential of cytoplasm of cells which is important for survival of plants under stress [23]. In the present investigation, maximum proline accumulation was observed in 1.0% mascagnite treated plots (0.106) while minimum proline accumulation in 0.5% mascagnite treated plots (0.092) (Table 1). However, 1.5% mascagnite treated plots show moderate level (0.100) of proline content. It may be due to 1.5% amount of mascagnite reducing the stress condition (to provide better internal environment for metabolic activity of the plants). A similar kind of findings has also been reported by Saha *et al*. 2006 [24].

Leghaemoglobin Content

The single most abundant protein that the plant host makes in the nodule is leghaemoglobin. In present investigation, maximum leghaemoglobin accumulation was found in 1.5% mascagnite treated plots (0.0152), while minimum amount of leghaemoglobin content was found in 0.0% mascagnite (0.0111) treated plots (Table 2). With the increasing concentrations of mascagnite, leghaemoglobin amount increases proportionally in the plant nodules due to the availability of the energy supply to the nodules which enhance the nitrogenase enzyme activity [25]. Nitrogen fixation is reduced as a consequence of low nitrogenase enzyme activity in plants subjected to nutrient stresses [26]. It may also be because, sulphur as a central component of this Fe–S cluster enzyme [27]. Sulfur deficiency ultimately affects the production of leghaemoglobin content in leguminous plants. Similar findings also have been reported by Saha *et al*. (2016) [24].

Chlorophyll Content

The effects of different treatment of mascagnite solutions on photosynthetic pigments are presented in Table 2. The highest amount of chlorophyll was found in 1.5 mascagnite treated plots.The results of this experiment reveal that applications of mascagnite at the higher amount had a significant positive effects on activities of catalase, ascorbate peroxidase, guaiacol peroxidise enzymes, and chlorophyll synthesis. The decrease in the chlorophyll and Rubisco contents observed with sulfur deficiency has also been reported in rapeseed, spinach, and wheat. Similarly, application of sulphur significantly increased the chlorophyll content of the leaves. Sulphur deficiency affects the photosynthetic apparatus severely and the chlorophyll content gets reduced by 49% because of a general reduction of PS I and PS II [28]. Such kind of observations has been reported earlier by Bashir *et al*. (2015) [29].

Phosphorous Content

Treatment of 1.5% mascagnite resulted in the highest phosphorous amount; however minimum amount of phosphorous was found in 0.5% mascagnite treated plots (Table 3). The results of this experiment revealed that application of mascagnite at the higher doses has a significant positive effect on phosphorous amount in plants. It may be due to at sulfur concentration increases the uptake of the phosphorous by plants also increases. It indicates a strong synergetic relationship between phosphorous and sulfur in plants. The results are in agreement with the finding of Kannan *et al*. (2017) [30].

Nitrogen Content

Nitrogen concentration of the leaves of lentils is influenced by the application of mascagnite clearly shown in the (Table 3). Maximum nitrogen content (0.545) was obtained with the application of 1.5% mascagnite treated plots as compared to 0.0% (0.398), 1.0% (0.499), 0.5% (0.412) treatment respectively. These results show positive impact of mascagnite on nitrogen content of leguminous crops. However, sulfur deficiency resulted in lower nitrogen concentrations of leaves. The nitrogen concentrations decreases under sulfur deficiency imply a parallel decrement in N_2 fixation. It may be an indication of a sulfur limitation in plants. Sulfur deficiency leads to the accumulation of amino acids, which is assumed to down regulate nitrogen uptake and assimilation. Lower nitrogen concentrations as well as a reduced N_2 fixation under suboptimum sulfur conditions were also established in other legumes [31], which may be partly due to limited synthesis of the enzymatic machinery for reducing inorganic nitrogen [32]. Sulfur assimilation is inter- connected with carbon and nitrogen metabolism [33]. In particular, there are interactions between the amino acid pathways, balancing the flux of sulfur, carbon and nitrogen, as are essential for the aspartate pathways leading to synthesis of methionine and two other essential amino acids (threonine and lysine) [34]. In legumes, previous studies showe that sulfur deficiency decreases nitrogen assimilation and nitrogen fixation [35].

Nitrate Reductase

Nitrate reductase is an enzyme that reduces nitrate (NO^{3-}) to nitrite (NO^{2-}) and plays a critical role in the production of proteins in most of the crop plants. In present investigation maximum nitrate reductase activity (0.326) was observed in 1.5% mascagnite treated plots while minimum nitrate reductase activity (0.256) was found in 0.5% mascagnite treated plots in comparison to control (0.0% mascagnite) (Table 4). Nitrate reductase enzyme activity increases when relatively high levels of mascagnite are used. $(NH_4)_2$. SO_4 enhances the nitrate reductase enzyme activity at higher concentration but nitrate reductase enzymes activity reduces in the lower concentrations of mascagnite. Similar kind of results was also observed earlier by Filner (1966) [36].

Nitrite Reductase

Nitrite reductase is an enzyme that reduces nitrite (NO_2^-) to ammonia (NH_4^+) and plays a critical role in the production of proteins in the most of the crop plants. In present investigation maximum nitrite reductase activity (0.569) was observed in 1.5% mascagnite treated plots while minimum nitrite reductase activity (0.549) was found in 0.5% mascagnite treated plots (Table 4). Nitrite reductase enzyme activity increases when relatively high amount of mascagnite is used. Availability of nitrite (which is product of nitrate enzyme product) enhances the nitrite reductase activity in the present tested plants.

Tables

Table 1: Effect of mascagnite solution (0.0%, 0.5%, 1.0% and 1.5%) on protein and proline content of *Lens culinaris.*

Parameters	**Treatments sample**			
	Mascagnite (0.0%)	**Mascagnite (0.5%)**	**Mascagnite (1.0%)**	**Mascagnite (1.5%)**
Protein content (mg/gm fresh wt)	0.349	0.408	0.516	0.717
Proline content (mg/gm fresh wt)	0.097	0.092	0.106	0.1

Table 2: Effect of mascagnite solution (0.0%, 0.5%, 1.0% and 1.5%) leghaemoglobin and chlorophyll content of *Lens culinaris.*

parameters	**Treatments sample**			
	Mascagnite (0.0%)	**Mascagnite (0.5%)**	**Mascagnite (1.0%)**	**Mascagnite (1.5%)**
Leghaemoglobin mM(g.f.M.)-1	0.0111	0.0129	0.0143	0.0152
Chlorophyll a (mg/g fresh wt)	1.97	2.16	1.56	1.89
Chlorophyll b (mg/g fresh wt)	0.62	0.57	0.46	0.57

Wt = weight

Table 3: Effect of Mascagnite solution (0.0%, 0.5%, 1.0% and 1.5%) on phosphorous and nitrogen of *Lens culinaris.*

parameters	**Treatments sample**			
	Mascagnite (0.0%)	**Mascagnite (0.5%)**	**Mascagnite (1.0%)**	**Mascagnite (1.5%)**
Phosphorous content (mg/gm fresh wt)	0.367	0.454	0.479	0.554

Nitrogen content (mg/gm dry wt)	0.398	0.412	0.499	0.545

Table 4: Effect of mascagnite solution (0.0%, 0.5%, 1.0% and 1.5%) on protein, pro line, Leghaemog lob in, chlorophyll, phosphorous, nitrogen content and nitrate and nitrite reductase enzymes activity of *Lens culinaris.*

parameters	Treatments sample			
	Mascagnite (0.0%)	**Mascagnite (0.5%)**	**Mascagnite (1.0%)**	**Mascagnite (1.5%)**
Nitrate reductase activity (µM/min/mg protein)	0.277	0.256	0.269	0.326
Nitrite reductase activity (µM/min/mg protein)	0.547	0.549	0.561	0.569

Conclusion

It can be concluded from the present study that Mascagnite has the positive impact on some biochemical parameters (protein, chlorophyll, Leghaemoglobin content, and other phosphorous and nitrogen content, nitrate and nitrite reductase enzymes activity). The present study revealed that that the 1.5% mascagnite solution application gives better results in comparison to the other treatments. In present time we need more food production to fulfill the requirement of a huge population of India or word as well as we has responsibilities to save the environment from the harmful effects of chemical fertilizers which we used drastically to increase the crop yield. So we need a proper management or concentration of chemical fertilizers which can improve the plant yield as well as should be eco-friendly. Essentially true this concentration (1.5%) of ammonium sulphate is useful for the legume crop.

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