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Synergistic Effect of Sulphur-Nitrogen-Interaction on Cowpea (*Vigna unguiculata* (L.) Walp) Yield and Seed Macronutrient Content

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Abstract: Pot experiments were performed in the greenhouse of the National Research Center to study the synergistic interaction between nitrogen fertilizer levels when combined with sulphur on cowpea (*Vigna unguiculata* (L.) Walp) yield and seed NPK contents. The plants were fertilized with N (N1, N2 and N3) at 0.7, 1.4 and 2.1 g pot^{-1} and combined with sulphur (S0, S1, S2 and S3) at 0, 5, 10 and 15 g pot^{-1}. The results showed that cowpea dry weight plant^{-1} positively responded to combined nitrogen at 2.1 g and sulphur at 15 g pot^{-1} (N3 × S3 treatment). Cowpea pod number and weight plant^{-1} as well as seed yield plant^{-1} showed significant positive responses to nitrogen and sulphur interaction. Meanwhile, the interaction between nitrogen and sulphur showed more beneficial effects on yield characters than each element applied alone. The data of N, P and K concentrations in cowpea seeds indicated that application of nitrogen levels alone did not induce increases in P or K concentrations. However, such tendency changed when sulphur was combined with nitrogen. The best response due the combined nitrogen and sulphur on NPK seed content occurred when sulphur was applied at 10 or 15 g (S3 or S4) in combination with nitrogen at 1.4 or 2.1 g pot^{-1} (N2 or N3). Application of nitrogen significantly increased protein percentage in cowpea seeds; also, the interaction between sulphur and nitrogen resulted in the greatest protein percentage in seeds. The combined effect of nitrogen and sulphur application showed that product of the individual responses was greater that each single product > 1(1.49 and 1.33) indicating that there were synergistic effect due to sulphur application under moderate (N2) or high (N3) of nitrogen application which resulted in 33-49% increase in cowpea yield. It can be concluded from this study that sulphur application to cowpea plants which receive nitrogen may improve yield and increase the efficiency of NPK uptake in cowpea seeds. Such improved responses to nitrogen permit a lower and perhaps more economic nitrogen fertilizer when growing cowpea in similar soils.

Key words: Cowpea · Yield · Seed element content · Nitrogen · Sulphur · Synergism · Antagonism

INTRODUCTION

Sulphur (S) is considered to be one of the most important nutritive elements for plant growth and productivity. Different crops especially legume or oil crops require a relatively large amount of mineral sulphur [1]. Sulphur deficiency can reduce yield and impacts on the quality of harvested products [2]. Sulphur Application could result in soil properties and may increase nutrient uptake [3, 4]. Many field crops especially pulses require high sulphur rates to improve yields like soybean and beans [5]. Some of the interaction between nitrogen and sulphur metabolism comes from O-acetylserine, the immediate precursor of cysteine that does not itself contain sulphur. Crops must contain adequate levels of this precursor for sulphate assimilation [6]. Choudhary [7] found that nitrogen and sulphur concentrations in cluster bean seeds and straw increased significantly due to S application at 40 kg ha^{-1} as well as nutrient uptake while Chandra *et al*. [8] found that seed yield and protein content increased with increasing levels of sulphur. Venkatesh *et al*. [9] reported that the application of 30 kg sulphur ha^{-1} proved superior to other levels in respect to protein content and sulphur uptake by groundnut.

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Nowadays in Egypt sulphur receives increased attention in Egyptian agriculture [10]. Sulphur is required for nodulation and protein synthesis. It is evident that there is a relationship between sulphur and yield or chemical constituents of different field crops especially legumes. Several investigators have proved the efficiency of including sulphur treatments for legumes which receive nitrogen and a strong relationship for the interaction of sulphur and nitrogen on seed yield was found in different crops [11-18].

Cowpea (*Vigna unguiculata* (L.) Walp) is an important pulse crop as it is considered as protein and other nutrients source. It is consumed as green pods and mature seeds by people all around the world, especially in the poor communities [19, 20]. Cowpea has the advantage like most pules in N fixing up to 200 kg N ha^{-1} [21-24] and result in positive soil N balance up to 92 kg ha^{-1} [25, 23]. According to FAO, cowpea was grown on an in Africa and the area of cultivated dry cowpea was estimated by 12.3 million ha in Africa in 2014 with the bulk of production occurring on 10.6 million ha [26]. It was found that cowpea seed yield and chemical composition greatly affected due to the applied sulphur level. Meanwhile, the major nutrient NPK concentration and total amount can be changed by sulphur application [27].

According to Wallace [28] the potential relationship between sulphur and nitrogen interaction could be classified as synergism or antagonism or Zero-interaction. If the yield due to the combined application of two nutrients is more than the yield expected from the individual applications nutrient interaction is synergistic and it will be antagonistic if the yield due to the combined application of two nutrients is less than the yield expected from the individual applications. The third relationship is zero-interaction where no yield advantage or disadvantage occurred. Therefore, it is important to evaluate the interaction relationship between sulphur and nitrogen levels on cowpea yield and nutrient content.

Thus, the aim of this work is to study the effect of different levels of nitrogen when combined with other levels of sulphur on yield and seed macronutrient content in cowpea plants. Another target of this work is to evaluate the potential relationship between sulphur and nitrogen interaction as synergism or antagonism or zero-interaction.

MATERIALS AND METHODS

Two pot experiments were performed in 2018 and 2019 summer seasons in the greenhouse of the National Research Centre to study the response of cowpea (*Vigna unguiculata* (L.) Walp) to different nitrogen fertilizer levels when combined with sulphur on yield and seed chemical constituents. Cowpea cv. Kraim-7 seeds (local variety) were sown in 25 cm earthenware pots on 9th and 2nd June in 2018 and 2019 seasons respectively. Each pot contained 10 kg of sandy clay loam soil. The mechanical and chemical analyses of the soil are presented in Table (1).

The experiment included 12 treatments which were the combinations of three levels of nitrogen (0.7, 1.4 and 2.1 g N pot^{-1}) combined with other four elemental sulphur levels (0, 5, 10 and 15 g pot^{-1}). These levels represent the rates of 25, 50 and 75 kg N fd^{-1} as well as 0, 0.5, 1.0 and l.5 tone sulphur fd^{-1}. Nitrogen levels were applied as ammonium nitrate (33.5% N) while sulphur levels were applied as elemental sulphur, both of nitrogen and sulphur were applied 10 days after planting. The pots were arranged in completely randomized design with four replicates. After complete germination, cowpea plants were thinned and two plants pot^{-1} left to grow. At harvest time, the plants were taken; pods were separated and the total dry weight of the determined (stems + leaves) were recorded. Pod number and weight were determined, then the pods were shelled and seed yield plant^{-1} was recorded. Cowpea seeds were ground and a sample of each treatment was subjected to the chemical analysis. Plant samples were grinded, wet digested after dried at 70°C tell constant weight for estimation of; N, P, K concentrations. Nitrogen was determined by micro-Kjeldahl according to the [29]. After wet digestion of the samples P was determined by spectrophotometry, K by flame [30]. Protein content (%): was calculated according [29] by multiplying N% × 6.25; Nitrogen phosphorous and potassium uptake were determined by multiplying concentration of each element in seed yield plant^{-1}.

Table l: The mechanical and chemical analysis of the experimental soil

Mechanical Analysis	
Texture	Sandy Clay Loam
Sand	57.2 %
Silt	10.5 %
Clay	32.3 %
Ec	129 Mm hos/cm
Chemical Analysis	
Organic Matter	1.89 %
Calcium Carbonate	2.88 %
pH	7.73 %
Total N	0.08 %
Available P	13.6 PPM
Available S	10 ppm
Available Fe	19.32 PPM
Available Zn	2.53 PPM
Available Mn	15.20 PPM
Available Cu	3.54 PPM

Synergistic Effects Determination: Synergistic or antagonistic effects determination was carried out by calculating the yield expected (y_{ab}) on the basis of the individual responses (y_a and y_b) for both S and N according to Wallace [28] by using relative yields.

$$(y_{ab} / y_0 = y_a / y_0 \times y_b / y_0) \quad (1)$$

where y_0 is the yield in the reference or control treatment and (y_a and y_b) refers to both sulphur and nitrogen treatment yields.

Statistical Analysis: The analysis of variance of complete randomized design was carried out using MSTAT-C Computer Software [31], after testing the homogeneity of the error according to Bartlett's test, combined analysis for both seasons were done. Means of the different treatments were compared using the least significant difference (LSD) test at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of Different Nitrogen and Sulphur Levels on Cowpea Yield Characters: Significant effects on cowpea dry weight (stems + leaves), number and weight of pods and seed yield plant^{-1} were reported due to nitrogen and sulphur application as well as their interaction. The total dry weight of cowpea (stems + leaves) at harvest significantly increased as nitrogen level increased. Meanwhile, increasing sulphur level from 0 (S0) to 15 g (S3) pot^{-1} resulted in successive increases in cowpea dry weight plant^{-1}. Table (2) and Fig. (1) shows the positive effect on cowpea dry weight plant^{-1} when nitrogen level (N3) was combined with sulphur at 15 g (S3) pot^{-1}.

From Table (2), it can be noticed that increasing nitrogen level from (N1) to (N3) pot^{-1} resulted in significant pod number increase plant^{-1}. The highest number of pods of cowpea plants were recorded when sulphur was applied at (S2) level. The data also shows that the positive response resulted from the interaction between nitrogen and sulphur on pod numbers plant^{-1} especially when nitrogen at (N1) was combined with sulphur at (S2) level. Similar trends were observed on cowpea pod weight plant^{-1} due to nitrogen and sulphur application (Table 2). The plants which received (N3) produced the heaviest pod weight plant^{-1} compared with those which received the other levels. At the same time, sulphur application at (S2) pot^{-1} increased pod weight plant^{-1}. Concerning the interaction between nitrogen and sulphur, it is clear that application of the low level of nitrogen (N1) in combination with (S2) pot^{-1} could produce similar pod weight plant^{-1} to the plants which received only (N3) pot^{-1}.

Seed yield plant^{-1} was significantly affected by nitrogen and sulphur application as well as the interaction between them. Cowpea plants which received nitrogen at 0.7 or 1.4 (N1or N2)g pot^{-1} surpassed those which received (N3) level. Also, sulphur application at (S2) level out yielded the greatest seed yield plant^{-1} compared to the other sulphur levels. The combined nitrogen and sulphur application reveals positive effects on cowpea seed yield plant^{-1} where the greatest yield was obtained when (S2) to (S3) sulphur was combined with (N1) and (N3) nitrogen (Fig. 2).

These results emphasize the beneficial effect of the combined nitrogen and sulphur application on yield characters of cowpea. Such positive responses may be due to the lowering of pH value which increase soil acidity, which in turn allow better circumstances for cowpea growth and yield. Several investigators came to similar conclusions on the positive response of crops to the combined nitrogen and sulphur. In this respect, Choudhary [7], Chandra *et al.* [8] and Venkatesh *et al*. [9] pointed out that S application have positive effects on seed yield or protein percentage and nutrient uptake Similar results were reported by [32-34].

Effect of Nitrogen, Sulphur and Their Interaction on Chemical Constituents of Cowpea Seeds: Data presented in Table (3) show significant increase in cowpea nitrogen concentration (mg g^{-1} seed) due to application of nitrogen levels. However, application of nitrogen levels to cowpea plants did not induce such effect on either phosphorus or potassium concentrations in the seeds. The data also shows that sulphur application at (S2) and (S3) levels gave the highest concentrations of nitrogen, phosphorus and potassium of seeds. The interaction between sulphur and nitrogen induced significant increases in either nitrogen or potassium concentrations in cowpea seeds especially when (N3) nitrogen was combined with (S2) or (S3) levels of sulphur. Choudhary [7] observed positive effects of S on cluster bean seed and stover yields and significantly increased nitrogen and sulphur concentration as well as their uptake in seeds.

In contrast of nitrogen, phosphorus and potassium concentrations in cowpea seeds were influenced by different applications of nitrogen, this may be due to the dilution of these elements with regard the increase in cowpea seed yield. The best response due to the combined effect of nitrogen and sulphur applications

Table 2: Effect of different nitrogen and sulphur levels on cowpea yield characteristics

(B) Nitrogen (g pot^{-1})	(A) Sulphur (g pot^{-1}) 0 (S0)	5 (S1)	10 (S2)	15 (S3)	Mean
		Dry weight (stems + leaves) g plant^{-1}			
0.7 (N1)	26.4	25.0	27.7	24.1	25.8
1.4 (N2)	20.0	27.0	29.0	35.2	27.8
2.1 (N3)	27.2	30.0	31.9	38.2	31.8
Mean	24.5	27.3	29.5	32.5	
LSD at 0.05		(A) = 1.8	(B) = 2.3	(AB) = 4.6	
		Number of pods plant^{-1}			
0.7 (N1)	33.0	37.0	50.9	21.0	35.5
1.4 (N2)	38.0	37.0	43.0	40.0	39.5
2.1 (N3)	50.0	36.0	48.0	30.0	41.0
Mean	40.3	36.7	47.3	30.3	
LSD at 0.05		(A) = 4.4	(B) = 1.1	(AB) = 6.2	
		Pod weight (g plant^{-1})			
0.7 (N1)	25.3	21.6	32.3	21.2	25.1
1.4 (N2)	26.2	23.3	29.7	23.2	25.6
2.1 (N3)	33.8	21.6	27.8	27.3	27.6
Mean	28.4	22.2	29.9	23.9	
LSD at 0.05		(A) = 1.7	(B) = NS	(AB) = 4.2	
		Seed yield (g plant^{-1})			
0.7 (N1)	19.3	14.2	23.4	17.2	18.5
1.4 (N2)	16.7	15.0	22.5	20.0	18.6
2.1 (N3)	12.9	14.2	18.5	23.4	17.3
Mean	16.3	14.5	21.5	20.2	
LSD at 0.05		(A) = 2.0	(B) = 0.9	(AB) = 2.9	

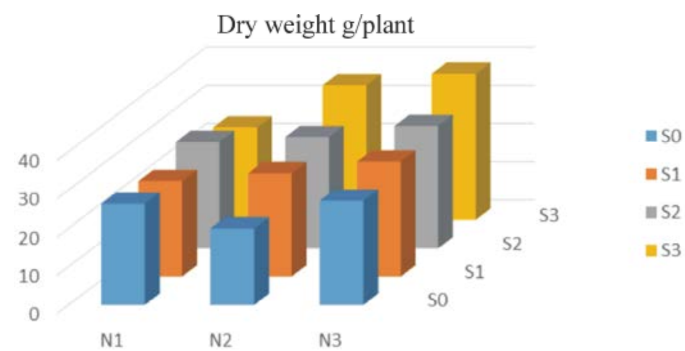

Fig. 1: Effect of sulphur and nitrogen interaction on cowpea dry weight (g plant^{-1})

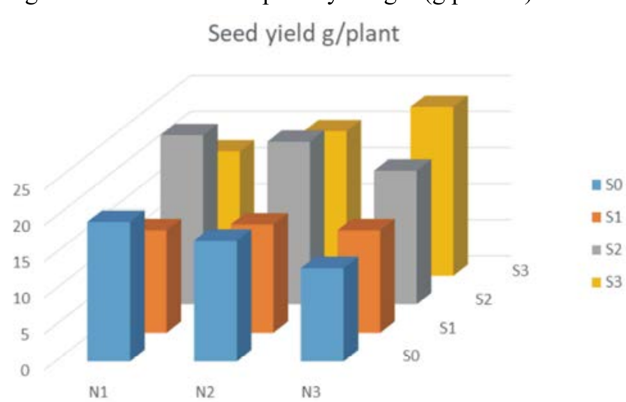

Fig. 2: Effect of sulphur and nitrogen interaction on cowpea seed yield (g plant^{-1})

Table 3: Effect of different nitrogen and sulphur levels on N, P and K concentrations in cowpea seeds

	(A) Sulphur (g pot^{-1})				
(B) Nitrogen (g pot^{-1})	0 (S0)	5 (S1)	10 (S2)	15 (S3)	Mean
		Nitrogen (mg g^{-1})			
0.7 (N1)	39.6	46.4	36.8	50.4	43.3
1.4 (N2)	42.0	36.8	44.0	40.0	40.7
2.1 (N3)	46.4	35.2	54.4	50.0	46.5
Mean	42.7	39.5	45.1	46.8	
LSD at 0.05		(A) = 1.2	(B) = 2.2	(AB) = 8.4	
		Phosphorus (mg g^{-1})			
0.7 (N1)	10.2	9.2	10.2	11.2	10.2
1.4 (N2)	9.4	10.1	10.2	11.7	10.4
2.1 (N3)	9.9	9.2	11.1	10.5	10.2
Mean	9.8	9.5	10.5	11.1	
LSD at 0.05		(A) = 1.10	(B) = NS	(AB) = NS	
		Potassium (mg g^{-1})			
0.7 (N1)	20.8	22.8	21.6	24.8	22.5
1.4 (N2)	22.4	21.2	22.4	24.0	22.5
2.1 (N3)	24.0	22.4	24.0	23.2	23.4
Mean	22.4	22.1	22.7	24.0	
LSD at 0.05		(A) = 1.1	(B) = NS	(AB) = NS	

Table 4: Effect of different nitrogen and sulphur levels on N, P, K uptake and protein % in cowpea seeds

	(A) Sulphur (g pot^{-1})				
(B) Nitrogen (g pot^{-1})	0 (S0)	5 (S1)	10 (S2)	15 (S3)	Mean
		Nitrogen (mg plant^{-1})			
0.7 (N1)	764.3	658.9	860.1	866.9	787.6
1.4 (N2)	701.4	552.0	990.0	800.0	760.9
2.1 (N3)	590.6	499.8	1006.4	1170.0	816.7
Mean	685.4	570.2	952.2	945.6	
LSD at 0.05		(A) = 289	(B) = NS	(AB) = 380	
		Phosphorus (mg plant^{-1})			
0.7 (N1)	197.2	130.2	237.7	192.8	189.5
1.4 (N2)	156.8	152.8	229.9	234.4	193.5
2.1 (N3)	128.2	130.6	206.3	146.6	152.9
Mean	160.7	137.9	224.6	191.3	
LSD at 0.05		(A) = 88	(B) = NS	(AB) = NS	
		Potassium (mg plant^{-1})			
0.7 (N1)	401.4	323.8	505.4	426.6	414.3
1.4 (N2)	374.1	348.0	504.0	480.0	426.5
2.1 (N3)	309.6	318.1	444.0	542.9	403.7
Mean	361.7	330.0	484.5	483.2	
LSD at 0.05		(A) = 104	(B) = NS	(AB) = NS	
		Protein % in seeds			
0.7 (N1)	24.7	28.9	22.9	31.4	27.0
1.4 (N2)	26.2	22.9	27.4	27.9	26.1
2.1 (N3)	28.9	21.9	33.9	31.2	29.0
Mean	26.6	24.6	28.1	30.2	
LSD at 0.05		(A) = 0.9	(B) = 1.4	(AB) = 4.7	

occurred when nitrogen at (N2) and (N3) pot^{-1} and sulphur at (S2) and (S3) pot^{-1} were added, respectively. Pareek [32] reported that the content and uptake of N improved significantly both in seed and stover with increasing levels of sulphur (S) up to 30 kg ha^{-1}. Interactions between nutrients occur when the supply of one nutrient affects the uptake, distribution, or function of another nutrient. Interactions can be assessed by examining the relationship between nutrient supply and nutrient concentrations in plants [35].

Table 5: The yield expected due to the interaction (y_{ab}) on the basis of the individual responses (y_a and y_b)

	5 (S1)	10 (S2)	15 (S3)	y_a/y_0	y_b/y_0	$y_{ab}*/y_0$
0.7 (N1)	0.74	1.21	0.89	0.88	1.13	0.99
1.4 (N2)	0.77	1.17	1.04	1.31	1.14	1.49
2.1 (N3)	0.74	0.96	1.21	1.25	1.06	1.33

*$y_{ab}/y_0 = y_a/y_0 \times y_b/y_0$

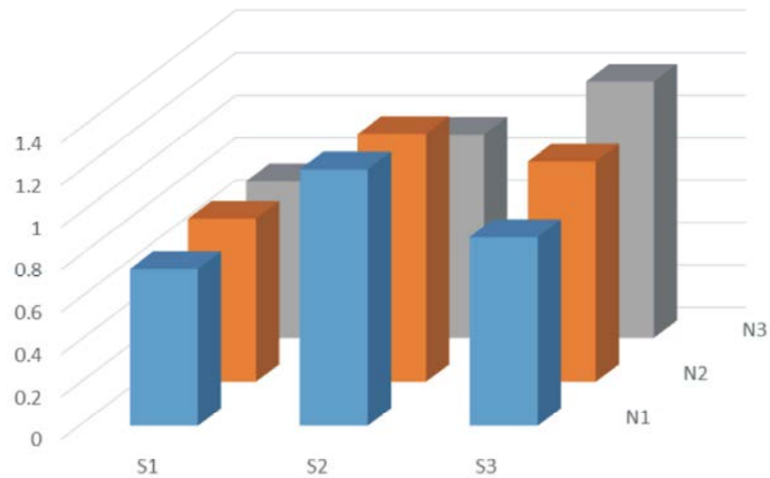

Fig. 3: Expected yield (y_{ab}) as a product of the individual nitrogen and sulphur responses

Protein percentage in cowpea seeds was significantly affected by nitrogen and sulphur levels as well as their interaction (Table 4). The highest levels of nitrogen (N3) 2.1 g pot^{-1} and sulphur (S3) 15 g pot^{-1} when applied to cowpea plants resulted in the highest protein percentages in the seeds. In this respect, Pareek [32] reported that protein content in seed increased significantly with increasing levels of sulphur and molybdenum up to 30 kg and 1.0 kg ha^{-1}, respectively. Dhankar *et al.* [36] reported that when sulphur was applied to several legumes such as guar, mungbean and cowpea N, P and K concentrations and total amount improved and reached a maximum with 30-60 ppm and then decreased slightly with 90 ppm sulphur.

Application of nitrogen (N) and sulphur (S) resulted in increased uptake of these nutrients by plant, which may be due to their increased availability in soil. The increase in nutrient concentration might be attributed due to increase in supply of sulphur to plant, which activate greater absorption of N, P, S and Fe from soil. Another role of the sulphur applied is increasing growth and nutrient concentration in plant thus, higher nutrient uptake under the influence of sulphur application occurred [37]. Moreover, sulphur is known to interact with almost all essential macronutrients, secondary nutrients and micronutrients [38]. Application of sulphur can influence nutrient uptake through the pH lowering for some nutrients [39]. Without adequate sulphur, the quality or protein content; due to the inefficient use of applied nitrogen [40]. In wheat, it was found that continued use of nitrogen fertilizer without supplemental sulphur on low sulphur soils will reduce flour quality [41, 42].

Synergistic Effects of Nitrogen and Sulphur Interaction: Synergism nutrient interaction is synergistic when

$(y_{ab}/y_0 > y_a/y_0 \times y_b/y_0)$
while
Antagonism nutrient interaction is antagonistic when $(y_{ab}/y_0 < y_a/y_0 \times y_b/y_0)$ and

Zero-interaction when $(y_{ab}/y_0 = y_a/y_0 \times y_b/y_0)$

where y_0 is the yield in the reference or control treatment the yield expected (y_{ab}) on the basis of the individual responses (y_a and y_b) as a product of the individual responses of sulphur and N according to Eq. (1).

Data presented in Table (5) show that the interaction between (S1) and any level was antagonistic or did not reach the level of synergism. Also, application of moderate or higher levels of both sulphur and nitrogen (S2 × N1 or N2) and (S2 × N2 or N3). The same attitude was reported when (S2) was combined with (N3) level or when (S3) level was interacted with (N1) level indicating that there were antagonistic effect. Regarding the combined effect of nitrogen and sulphur application the results showed that product of the individual responses was greater that each single product >1 (1.49 and 1.33) indicating that there were synergistic effect due to sulphur application under moderate (N2) or high (N3) of nitrogen application to cowpea. In other words, synergism occurred through the interaction between sulphur and nitrogen at moderate or high levels of nitrogen supply by 33-49% increase.

That means that nitrogen fertilization to cowpea needs basically sulphur application at moderate or higher rate to achieve synergism on yield. The obtained results confirm those obtained by René *et al*. [43] who indicated that Interaction among plant nutrients can yield antagonistic or synergistic outcomes that influence nutrient use efficiency. Also, Robson and Pitman [35] explained that the interactions between nutrients occur when the supply of one nutrient affects the uptake, distribution, or function of another nutrient. Depending on the nutrient supply, the interaction can modify plant growth and yield [44]. Interactions occur when the supply of one nutrient affects the absorption and utilization of another nutrient. Also, Hesse *et al*. [6] mentioned that some of the interaction between nitrogen and sulphur metabolism comes from O-acetylserine, the immediate precursor of cysteine that does not itself contain sulphur. For assimilation of sulphate to occur, plants must contain adequate levels of this precursor and as an amino acid its concentration is dependent on nitrogen nutrition. Similar conclusions were reported by [45-47].

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