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Impact of Legume Ameliorating with Sulphur and Phosphorus on seed Protein, S-Amino Acids and P Content

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Abstract

A pot trial was conducted in the greenhouse of the National Research Centre, Dokki, Cairo, Egypt to study the effect of two levels of phosphorus P1 (1.44) and P2 (2.16) g $P2O_5$ pot^{-1} and four Sulphur levels S0, S1, S2 and S3 at (0, 10, 15 and 20 g pot-1) on yield components of fababean, N and seed protein content as well as S-amino acid content (Methionine and Cysteine). The results showed that phosphorus and sulphur did not exhibit a significant effect on pod number while, seed number was decreased by P and S-treatments. Seed yield was increased at high P-level under S-levels, up to 15 g S pot^{-1}, thereafter it declined. The best effect on both pod and seed weight was obtained by 10 and 15 g S pot-1 under the higher P-level. Further increase of S-addition (20 g pot^{-1}) caused significant reductions in pod and seed yield as compare with those without S at higher P-level. The chemical composition of fababean revealed that N-concentrations generally increased by P and S additions, but crude protein content decreased with the highest application rate of S (20 g pot^{-1}). S effects were more pronounced at the high P level than at the lower one. The maximum increase in Cysteine and Methionine contents due to S addition were respectively 17.6 and 24.6% expressed as % of dry matter and 11.11 and 27.8% expressed as)g/16 g N). The results showed a slight increase in phosphorus concentration by increasing S levels up to 15 g pot-1 at the two P levels. The beneficial effect was evident from the interaction between S and P indicating synergism of both nutrients which is explained by the individual responses to each element and it was greater than the unity (1.17 and 1.66). Such relation between both elements was pronounced under moderate (P1) or high (P2) which reflected on seed yield and achieving 17-66% increase. The results of the study highlight the importance of the combined S and P in improving yield and chemical constituents of fababean.

Key words: Vicia faba, P, S, Protein, S-amino acids, Yield, Synergism

1. Introduction

Fababean (*Vicia faba* L.) is the main diet for breakfast in Egypt. The gap between production and consumption is increasing annually due to the reduction in the planted area and increasing population. Moreover, it is considered to be one of the world's most promising pulse crops. According to FAOSTAT [1], from 2009 to 2019, the area planted by fababean globally averaged 2.44 million hectares occupying the fourth place in the world in terms of acreage relative to other cultivated pulses. As a nutitional crop it is an excellent crop for protein and carbohydrate content as well as vitamins and and S-a amino acids, lysine and L -Dopa [2, 3] It has beneficial effects on soil quality and productivity and important nutritional crop for the Egyptians [4, 5] while [6] revealed that it is a renewable N input for crops and soil via biological ambient N_2 fixation. Sulfur (S) is considered one of the most important plant nutrients affecting growth and yield. Various

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crops, especially legumes or oilseeds, have relatively high demand for mineral sulfur [7]. Sulfur deficiency adversely affects crop yield and quality [8]. Sulfur fertilizers can lead to soil acidification and ultimately affect nutrient uptake [9-10]. Gikogno *et al.* [11] reported the possibility of improving the productivity of high sulphur crops demand by using proper sulfur fertilizers. Several interactions between sulfur and specific nutrients could affect metabolism of biochemical substances which considered a direct precursor of cysteine, which does not itself contain sulfur. Plants must contain sufficient amounts of this precursor to absorb sulfate [12]. Chowdhary [13] noted that the application of 40 kg sulfur per hectare significantly increased nitrogen and sulfur concentrations in seed and straw, as well as uptake in soybean seeds. Chandra *et al.* [14] conducted an experiment in summer and found that grain yield and protein content increased with increasing sulfur content. Venkatesh *et al.* [15] reported that the application of 30 kg sulfur per hectare was effective in terms of protein content and sulfur absorption in peanuts. Sulfur is essential for nodule formation and protein synthesis. It is evident that there is a relationship between sulfur and the yield or chemical composition of various crops, particularly legumes. Sweed and Awad [16] showed a growing interest in sulfur in Egyptian agriculture. Several researchers have demonstrated th e effectiveness of incorporating sulfur treatment into nitrogen-receptive legumes and found a strong relationship between sulfur and nitrogen interactions and seed yield. In other researches; Ahmad and Abdin [17], Fazli *et al.* [18-20], Verma and Swarnkar [21] and Jamal *et al.* [22-24] pointed out to this relationship. Phosphorus (P) is one of the important elements that greatly affect plant growth and metabolism for legumes [25]. Phosphorus also enhances plant growth, yield increased the photosynthetic efficiency and biological productivity due to its importance as an energy storage and transporter in metabolic processes [26-27]. The P requirement of agricultural crops is generally in the range 10-25% of the quantity of N removed by crop plants from soil [28](Cooke, 1982) whereas typically contain about half as much P as they do N. Consequently, more P is applied than can be utilized by growing crops such that P potentially accumulates in the cultivated layer of the soil following repeated applications of P. However, the long-term accumulation of P in P-treated soils has caused concern principally for two reasons. Firstly, it is suggested that P concentrations in drainage water may eventually be increased due to the possible saturation of the soil binding capacity for P causing leaching and impacts on water resources. However, data from long-term field trials with P demonstrate that this is unlikely in practice [29](Johnston 1981). Indeed, the mobility of P-borne P is significantly lower than that contained in the wastes of farm animals or supplied in inorganic fertilizers. The second potential problem concerning P accumulation in agricultural soils is run-off and surface water contamination. This is generally recognized as the principal environmental impact arising from the application of P fertilizers on agricultural soils. It is well established that P environmental concerns could be mitigated through its interaction with sulphur which minimize the fixation or accumulation it in alkaline soils.

This work highlights the different responses of fababean to sulphur interactions with P levels and their effects on yield, protein, p content and bioassay of S-amino acids in fababean seeds.

2. MATERIALS AND METHODSUSE

2.1. Experimental

A pot experiment was carried out in 2022 winter season in the greenhouse of the National Research Centre to study the effect of phosphorus and sulphur interaction on protein, S-amino acids, and p content of fababean seeds as well as yield and yield components. Fababean (Giza-716) was sown in 25 cm earthenware pots on 9th November. Each pot contained 10 kg of sandy clay loam soil. The mechanical analysis of the experimental soil was Sandy Clay Loam (Sand 57.2 %, Silt 10.5%, and Clay 32.3%). The chemical analysis was : EC 0.24dsm^{-1}, OM 0.73 %, Ca CO3 2.88 %, pH 7.73, N 1400 ppm; P 132 pm; K 826 ppm; Fe 3694 ppm; Mn 56.8 ppm; Zn 17.8 ppm; Cu 3.78 ppm. Eight treatments were tested in the experiment which was the combinations of two levels of phosphorus (1.44 (P1) and 2.16 (P2) g P_2O_5 pot^{-1}) combined with other four elemental sulphur levels (S0, S1, S2 and S3: 0, 10, 15 and 20 g pot-1, respectively). The chosen levels were equal to 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}. P was applied as calcium super phosphate 15.5 P_2O_5 while sulphur levels were applied as elemental sulphur, both of phosphorus and sulphur were applied 10 days after planting. The pots were arranged in completely randomized design with four replicates. After complete germination, fababean plants were thinned and two plants pot^{-1} left to grow. At harvest time, the plants were taken; pods were separated and pod number and weight were determined, then the pods were shelled and seed yield plant-1 was recorded. Fababean seeds were grounded and a sample of each treatment was subjected to the chemical analysis. Seed samples were wet digested after drying at 70 ºC tell constant weight for estimation of; N and P concentrations. Nitrogen was determined by micro-Kjeldahl according to A.O.A.C. [30]. After wet digestion of the samples P was determined by spectrophotometry, K by flame according to Jackson [31]. Protein content (%): was calculated by multiplying N% × 6.25; Methionine and Cysteine in seeds without coat were estimated microbiologically as described by Balasubramanian and Ramachandran [32].

2.2. Synergistic effects determination:

Synergistic or antagonistic effects determination was carried out by calculating the yield expected (yab) on the basis of the individual responses (ya and yb) for both S and P according to Wallace [33] by using relative yields.

$$(yab / y0 = ya / y0 \times yb / y0) \quad (1)$$

where y0 is the yield in the reference or control treatment and (ya and yb) refer to both sulphur and phosphorus treatment yields.

2.3. Statistical analysis:

Analysis of variance of the complete randomized block design was carried out using MSTAT-C [34] Computer Software. Means of the different treatments were compared using the least significant difference (LSD) test at $P < 0.05$.

3. RESULTS AND DISCUSSION

Results given in Table (1) and Fig. (1) clearly show that pH values of the experimental soil were slightly reduced by S application. The reduction in pH was gradual and reached < 7 under P2 level. Such effect was expected to be temporal due to soil buffering but it is reported that it causes beneficial effects for crop plants. Sulphur application can be considered an effective way to lower high soil pH values and thus improve micronutrient availability and uptake by plants. It has been reported that the application of sulphur-containing fertilizers can lead to soil acidification and ultimately affect nutrient uptake [9-10].

Table 1

Effect of P level and Sulphur application on pH value after 60 days from sowing

S-level	S0	S1	S2	S3
P1	7.65	7.40	7.2	7.0
P2	7.70	7.15	6.9	6.7

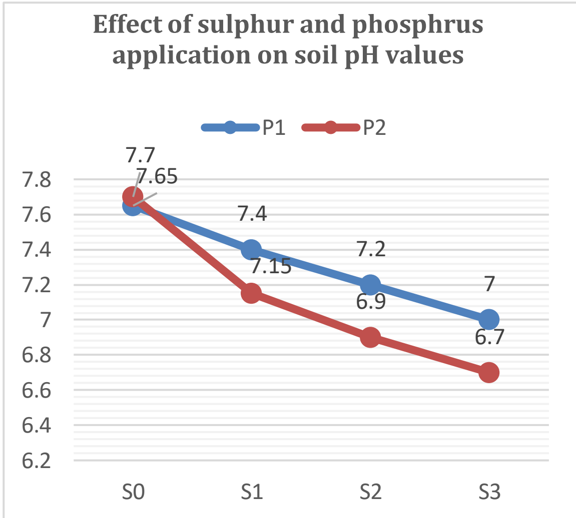
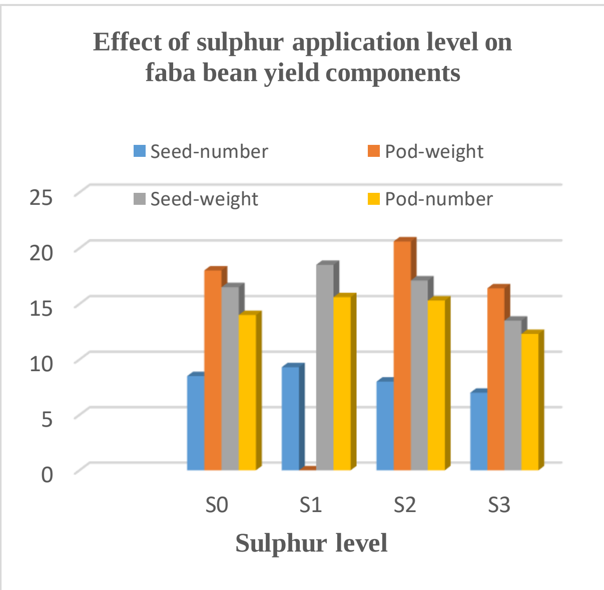


Fig. 1: Effect of P level and Sulphur application on pH value after 60 days from sowing

Significant effects due to P and S application on seed number plant^{-1} as well as pod and seed weight plant^{-1} was reported. It is clear that the fababean seed number exhibits a significant reduction by P and S treatments. However, P alone or S alone did not reveal a significant effect on pod number. Meanwhile, the combined effect (P × S) was significant on pod number. Such effect might be attributed to the increase in size of both pods and seeds which are sink that need more assimilates as a pulse crop during the pod filling stage. On the other hand, a significant decrease in seed number plant^{-1} at the higher level of P and S was recorded. Fertilizing fababean with the levels of S1 and S2 significantly increased pod and seed weights under the higher P level (Table 2 and Figs. 2 and 3). Regarding the P × S interaction, positive responses of fababean yield due to S application was more pronounced when the P level was at higher than when it was at the lower level (Fig. 3). Further increase of S addition at S3 caused a significant reduction in pod and seeds weight of fababean compared with the treatment without S under high P level. The beneficial effect of the combined P-S application may be attributed to the acidic effect of S which raises the soil pH temporarily after its addition and hence neutralizes or lowers partly the soil pH (Helal and Al-Badrawy, 1980 and 1985)[35,36]. Regarding the P × S interaction, positive responses of fababean yield due to S application was more pronounced when the P level was at higher than when it was at the lower level (Fig. 3). Further increase of S addition at S3 caused a significant reduction in pod and seeds weight of fababean compared with the treatment without S under high P level. The beneficial effect of the combined P-S application may be attributed to the acidic effect of S which raises the soil pH temporarily after its addition and hence neutralizes or lowers partly the soil pH [35, 36]. In general, such results indicate the enhancing role of sulphur in increasing nutrient uptake by fababean plants when it was combined with phosphorus hence such effect was greater than the single application of each nutrient. The beneficial effect of the combined phosphorus - sulphur application may be attributed to the acidic effect of urea which rises the soil pH temporirely after its addition and hence neutralises even through partly the acidic effect of sulphur in the soil [35] obtained results are in harmony with those obtained by Eppendorfer [37] .

Fig. 2: Effect of Sulphur levels and on fababean yield components



These results are in general in accordance with those reported on the beneficial effect of the combined phosphorus -sulphur application by Elsagan *et al.* [5] who reported that sulphur treatments generally showed improvement of values in all growth and yield parameters of fababean when compared with control treatment. Also this interaction affords a nutrient source if applied in quantities aligned to the local P level recommendations [38-41] came to similar conclusions.

The results of the interaction between S × P were found to be significant, indicate a synergistic relationship. These results are confirmed by Aulakh *et al.* [42]; Nagar *et al.*[40]. Moreover, it seems that the effect of each nutrient is not independent but integrated which lead to better plant growth and development. Similar results were reported by Kaiser *et al.* [38]; Mendel and Bittner [39]; Majumdar *et al.* [41]; Tomar *et al.* [43]; Scherer [44]; Khan and Mazid [45].

Chemical constituents

Total N concentration:

The effects of both P and S treatments on total nitrogen concentration are presented in Table (3). It is evident that P fertilizer had an enhancing effect on total N concentration. It can also be seen that total N concentration generally tended to be increased by S addition particularly at S lower level. The highest values were obtained from S2 level under both P levels and 20 g S pot^{-1} while the highest values for S2 level was recorded at P1 level. In other words, it can be said that S effects were more pronounced at the high P level than at the low one. These results were in agreement with that obtained by Eppendorfer [37].

These results are in general in accordance with those reported on the beneficial effect of the combined phosphorus -sulphur application by Elsagan *et al.* [5] who reported that sulphur treatments generally showed improvement of values in all growth and yield parameters of fababean when compared with control treatment. Also this interaction affords a nutrient source if applied in quantities aligned to the local P level recommendations [38 - 41] came to similar conclusions. The results of the interaction between S × P were found to be significant, indicate a synergistic relationship. These results are confirmed by Aulakh *et al.* [42] and Nagar *et al.* [40]. Moreover, it seems that the effect of each nutrient is not independent but integrated which lead to better plant growth and development. Similar results were reported by Majumdar *et al.* [41]; Tomar *et al.* [43]; Khan and Mazid [45]; Kaiser *et al.* [38]; Mendel and Bittner [39]; Scherer [44].

Crude protein yield (g plant^{-1} seeds):

Results in Table (3) indicate that increasing P level from 1.44 to 2.16 g pot^{-1} led to an increase in the crude protein yield of fababean. This might indicate that P encouraged N assimilation in fababean seeds, these results were similar to those reported by Yasmin *et al*. [46] who found that the crop fertilized with 40 kg P ha^{-1} produced the highest seed protein content (38.17%). Also, the addition of S induced an increase in crude protein content, except at the higher S level (20 g pot^{-1}) which exhibits a depression than the control. Crude protein content increased by increasing S level up to 15 g S pot^{-1} at the higher P level. Meanwhile, further addition of S (20 g pot^{-1}) at the higher P level showed a depression than that without S treatment. This occurred through an unbalance between nutrients that may have caused the above-mentioned decrease in crude protein yield. The increase in crude protein by using S application is in agreement with those reported by Yasmin *et al.* [46] who found that the crop fertilized with 40 kg P ha^{-1} produced the highest seed protein content (38.17%) and Eppendorfer [37] who reported similar findings.

S-amino acids content:

The present results in Table (3) show that increasing S application raised Methionine concentration from 0.134 to 0.167 and Cysteine from 0.26 to 0.30 (% DM), meanwhile these criteria have not been affected by P application. Concentrations expressed as (g Methionine/16 g N) as means of two P-levels, were increased by 13.9, 8.8 and 27.8 % by application of 10, 15 and 20 g S pot^{-1}, respectively., also, concentrations of Cysteine expressed as (g/16 9 N) were 10, 15 and 20 g S pot^{-1}, respectively (Fig. 5). From the same table, the results showed a slight increase in phosphorus concentration by increasing S levels up to 15 g pot^{-1} at the two P levels. In this connection, Rakha and El-Said [47] found that P concentration, tended to be stable in broad bean seeds. It is well known that its function may be limiting factor for the utilization of other nutrients, such as nitrogen and potassium. In this regard, the early work of Eppendorfer [37] emphasized that the N concentration generally increases with the addition of S, but decreases with many applications of N and P. Significant interactions were found for S × P. He added that the maximum increases in Cysteine and Methionine contents from S-application were respectively 39.1 and 13.7% expressed as % of dry matter and 37.9 and 12.3% expressed as (g/16 g N). Similar results were obtained by Glowacka *et al.* [48], they reported an increase of Cysteine and Methionine in common beans in southeast Poland. On contrast, Barlóg *et al.* [49] showed no effect of S on Cysteine and Methionine in fababean. Deficiency of the S-containing amino acids cysteine and methionine can limit the nutritional value of foods and feeds [50].

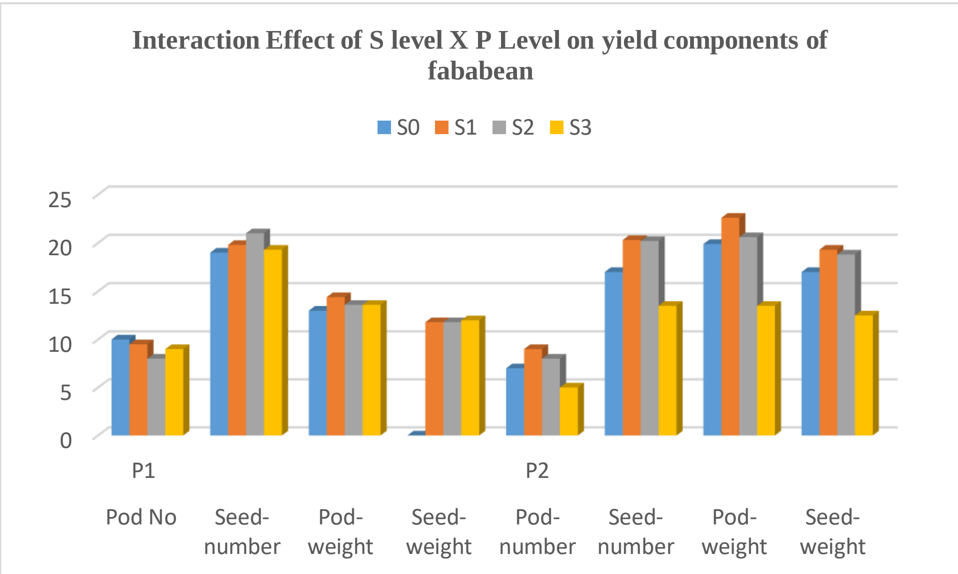


Fig. 3: Effect of sulphur and phosphorus levels and their interaction on fababean yield components

Table 2
Effect of sulphur and phosphorus levels and their interaction on fababean yield components

Yield characteristics	**S-level / P level**	**S0**	**S1**	**S2**	**S3**	**Mean**
Pod number plant^{-1}	**P1**	**10.0**	9.5	8.0	9.0	9.13
	P2	**7.0**	9.0	8.0	5.0	7.3
	Mean	**8.5**	9.3	8.0	7.0	
Seed number plant^{-1}	**P1**	**19.0**	19.8	21.0	19.3	19.8
	P2	**17.0**	20.3	20.2	13.5	17.9
	Mean	**18.0**	20.1	20.6	16.4	
Pod weight plant^{-1} (g)	**P1**	**13.0**	14.4	13.6	13.6	13.6
	P2	**19.9**	22.6	20.6	13.5	19.2
	Mean	**16.5**	18.5	17.1	13.5	
Seed yield plant^{-1} (g)	**P1**	**11.1**	11.8	11.8	12.0	11.7
	P2	**17.0**	19.3	18.8	12.5	16.9
	Mean	**14.0**	15.6	15.3	12.3	
Pod number plant^{-1}		**8.5**	9.3	8.0	7.0	0.27
Seed number plant^{-1}		**18.0**	20.1	20.6	16.4	0.28
Pod weight plant^{-1} (g)		**16.5**	18.5	17.1	13.5	
Seed yield plant^{-1} (g)		**14.0**	15.6	15.3	12.3	
LSD at 0.05			p	S	P × S	
	Pod number plant^{-1}		N.S	N.S	1.5	
	Seed number plant^{-1}		1.3	1.8	2.2	
	Pod weight plant^{-1} (g)		2.5	1.6	1.5	
	Seed yield plant^{-1} (g)		2.1	1.1	1.4	

Table 3
Effect of Sulphur and Phosphorus levels and their interaction on fababean seed chemical constituents

Seed chemical constituent	S-level / P-level	S0	S1	S2	S3	Mean
N%	**P1**	3.73	3.52	4.42	3.24	3.70
	P2	3.80	3.73	4.50	4.20	4.02
	Mean	3.75	3.63	4.45	3.72	
Relative content%		(100)	(96.8)	(118.7)	(100)	
Crude Protein (g plant^{-1} seeds)	**P1**	2.60	2.60	3.30	2.43	2.72
	P2	4.00	4.44	5.24	3.35	4.23
	Mean	3.28	3.52	4.25	2.84	
Relative content%		(100)	(107.3)	(129.6)	(86.6)	
g Methionine (g/100 g DM)	**P1**	0.130	0.142	0.174	0.17	0.154
	P2	0.137	0.151	0.160	0.16	0.152
	Mean	0.134	0.147	0.167	0.165	
Relative content%		(100)	(109.7)	(124.6)	(123.1)	
g Methionine/ 16 g N	**P1**	0.558	0.645	0.631	0.840	0.669
	P2	0.577	0.648	0.604	0.611	0.610
	Mean	0.568	0.647	0.618	o .726	
Relative content%		(100)	(113.9)	(108.8)	(127.8)	
g Cysteine (g/100 g DM)	**P1**	0.25	0.26	0.33	0.25	0.27
	P2	0.26	0.27	0.27	0.30	0.28
	Mean	0.26	0.265	0.30	0.275	
Relative content%		(100)	(103.9)	(117.6)	(107.8)	
g Cysteine /16gN	**P1**	1.07	1.18	1.20	1.23	1.17
	P2	1.09	1.16	1.20	1.14	1.16
	Mean	1.08	1.17	1.20	1.19	
Relative content%		(100)	(108.3)	(111.1)	(110.19	
P (g P/100 g DM	**P1**	0.12	0.13	0.19	0.07	0.13
	P2	0.18	0.18	0.20	0.07	0.16
	Mean	0.15	0.16	0.195	0.07	
Relative content%		(100)	(106.7)	(130)	(47)	

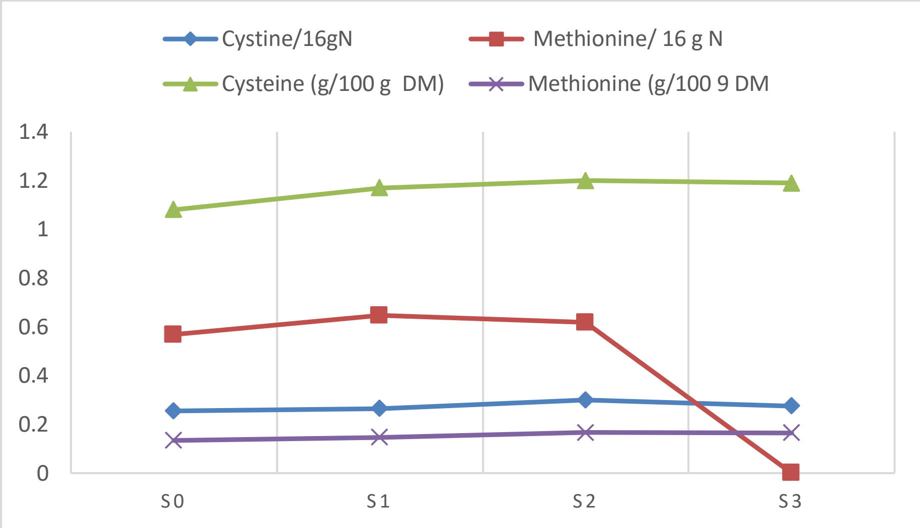


Fig. 5: Effect of sulphur level on Methionine and Cysteine concentrations in fababean seeds

Synergistic effects of phosphorus and sulphur interaction:

In general, the nutrient interaction is considered to be synergistic when yield responses follows the equation: (yab /y0 > ya /y0 × yb /y0) and the product of the yields derived from each nutrient applied was greater than unity (yab /y0 > 1), which means an interaction advantage while if the product derived from each nutrient applied was less than the unity it is considered antagonism and if the product equal the unity, there was not advantage of such interaction. The obtained results of yield responses due to the interaction between S and P indicate the effect of their combined effect on fababean yield (Table 4). The data showed that the interaction between S3 and P was not synergistic. However, the obtained results reveal synergistic relationships between the moderate or higher levels of both sulphur and P (S1 × P1 or P2) and (S2 × P1 or P2). A similar tendency was evident when (S2) was combined with (P1) level. On contrast, when (S3) level interacted with (P2) level there was an antagonistic effect. Moderate or higher levels of S when combined with P could achieve fababean yield advantage ranging between 17 and 66% for both sulphur and phosphorus. Such increase in fababean yields is attributed to the positive effect of combining Sulphur and phosphorus compared to the sole application of both nutrients. In other words, synergism occurred through the interaction between sulphur and phosphorus at moderate or high levels. The obtained results are in harmony with those obtained by Aulakh *et al.* [41]; Nagar *et al.* [40] and Singh and Chauhan [51].

Table 4
The yield expected due to the interaction (y_{ab}) on the basis of the individual responses (y_a and y_b).

S-level / P-level	5 (S1)	10 (S2)	15 (S3)	Ya/Y0	Yb/Y0	yab*/ y0
P1	1.06	1.06	1.01	1.11	1.05	1.17
P2	1.14	1.11	0.74	1.09	1.52	1.66

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

4. Conclusions

It can be concluded from this study that including Sulphur to fababean plants which receive phosphorus may improve yield and increase the efficiency of S-amino acids formation in fababean seeds consequently improving yield and quality of fababean seeds.

5. Conflicts of interest

The authors declare that they have no conflict of interest.

6. Formatting of funding sources

Not applicable.

7. Acknowledgments

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