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Effect of soil application of sulphur and zinc and foliar application of KNO_3, Borax, NAA and GA on chlorophyll, carotenoid and quality parameters of soybean (*Glycine max* L. Merrill)

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Abstract
The periodical observations on chlorophyll a, chlorophyll b and total chlorophyll concentration recorded at various dates under varied fertility levels. It was noted that the application of nitrogen over no nitrogen (control) enhanced the chlorophyll a content at all the growth stages. However chlorophyll b concentration was relatively lower than chlorophyll a concentration. Each additional soil and foliar application of nutrient from F_1 to F_6 improved the chlorophyll content of soybean. The higher chlorophyll synthesis in treatment F_6 followed by F_5 due to additional foliar sprays of potash and boron. The quality parameters of soybean *viz*., protein and oil content significantly influenced by soil and foliar application of nutrients and growth regulators. Treatment F_6 (RDF + Zn + foliar K, B and GR) recorded numerical higher protein and oil content which was found at par with all other treatments.

Keywords: sulphur, zinc, foliar, chlorophyll, carotenoid's, quality parameters, soybean

Introduction
Soybean (*Glycine max* L.) is important oil and protein crop belongs to family Fabaceae, it contains high quality protein (40-42%), oil (18-20%) and other nutrients like calcium and iron. (Devi *et al*, 2012) [2]. Soybean is preferable for human nutrition due to its high protein content. It is a good source of is flavones and therefore, it helps in preventing heart disease, cancer and HIV's. Soybean oil is leading vegetable oil in the world and is used in many industrial applications including biodiesel. Because of its high nutritional value and myriad form of uses, it is recognized as "Golden Bean" in India. The annual soybean production in India was 12.21 million tons (2011-12) with its area under cultivation was 10.1 million hectares. Zinc was assumed to be greater significance due to wide occurrence of its deficiency. The magnitude of sulphur removal is much higher due to intensive cropping. (Jaga, 2013) [4]. In Maharashtra, soybean occupied an area of 5 lakh ha. with production of 6 lakh tones. Sulphur is an essential macronutrient in plant growth and development. It is increasingly being recognized as the fourth major plant nutrient after nitrogen, phosphorus and potassium. Among the fertilizer elements, sulphur requirement of oilseed crops s quite high as compared to other crops. (Das and Das, 1994) [1].
The soils of Marathwada region, which are largely deficient in nutrients like N, P, K, S, Zn and B are mainly responsible for the greenness of the crops. Among all these nutrients S, Zn and B deficient in pulses growing regions of Marathwada. Therefore for the efficient exploitation of high yielding potential and oil content of soybean and monitoring growth attributes by spectral reflectance, with this background the present investigation was undertaken.

Material and Methods
The research experiment was carried out for two consecutive years 2016-17 and 2017-18 on experimental farm of Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. The material used and methods adopted for planning and conduct of field experiments for soil and plants chemical analysis, stastical analysis was done. Geographically, Parbhani district is situated in the Godavari drainage basin in the central part of India between $76^0 46'$, east longitude and $19^0 16'$ North latitude, having elevation of 423.46 m above the mean sea level in Marathwada division of Maharashtra State. The region has a semi-arid climate. It is under assured monsoon rainfall agro climatic zone with an average annual precipitation between 800 to 850 mm. The major portion of precipitation (75 per cent) being received through south-west monsoon from June to

September. The mean maximum temperature varies from 26.9 ^{0}C in winter to 42.4 ^{0}C in summer and means minimum temperature varies from 5.8 ^{0}C to 25.6 ^{0}C. The climate is suitable for *Kharif* crops like Soybean, Cotton, Sorghum, Green gram, Black gram and Red gram. The soils of the region are medium to deep black (Inceptisol/ Vertisol).

The meteorological data on rainfall, maximum and minimum temperature and relative humidity recorded during the experimental period at meteorological observatory. In order to determine the soil properties of experimental soil before sowing, the representative surface (20 cm depth) soil sample was collected from randomly selected spots covering experimental area. A composite soil sample was prepared and analyzed for its various soil properties. After completion of preparatory tillage operations, the experiment was laidout in Randomized Block Design comprising seven (07) treatments replicated thrice. The land of the experimental site was prepared by one ploughing and two harrowing and layout was done after the onset of monsoon. The certified seed of soybean MAUS 162 were sown in *kharif* by dibbling one seed per hill at 45 x 5 cm^2 distances; gap filling was done 12 days after sowing to maintain plant population. Schedule of cultural operation was carried out as per recommendations. The soybean crop was harvested and plot wise seed and straw yield per plot was recorded.

In the statistical analysis these data obtained from field experiment and the ground truth of the crop soybean available soil nutrients, plant nutrient concentrations should be computed and correlation regression technique to be followed (Panse and Sukhatme, 1985) [6]. The biomass and economic yield data should also be derived and exposed for design analysis and critical difference is to be calculated for the treatment comparison.

Result and Discussion

Chlorophyll a

The periodical observations on chlorophyll a concentration recorded at various dates under varied fertility levels during year 2016-17 and 2017-18 are presented in table 1. On an average chlorophyll a concentration of soybean was found to be increased with growth of soybean up to 75 days after sowing during year 2016-17 and 2017-18. Thereafter there was decrease in chlorophyll a concentration. The increase was from 2.53 to 6.45 and 2.47 to 6.90 mgg^{-1} fresh weight. It was further noted that the application of nitrogen over no nitrogen (Treatment F_1) enhanced the chlorophyll a content at all the growth stages. The role of nitrogen in chlorophyll synthesis is of vital importance. Further, treatments F_6, F_5 and F_7 shown significant influence in chlorophyll a synthesis over control (F_1). The highest chlorophyll a is recoded in treatment receiving (RDF + Zn + Foliar K, B + GR) in both the years of experimentation. Further, it was also noticed that, application of RD+ Zn + foliar K & B synthesized more chlorophyll than other treatment combinations. Spraying of potash and boron with growth regulators significantly increased the chlorophyll concentration in leaves. The pooled mean showed lowest chlorophyll a content 2.75 mg g^{-1} fresh weight in control treatment. Whereas highest chlorophyll a content 5.68 mgg^{-1} fresh weight was observed in treatment receiving RD+ Zn + K, B with growth regulators (F_6).

Table 1: Effect of soil application of sulphur and zinc and foliar application of KNO_3, Borax, NAA and GA on chlorophyll a (mg/g fresh wt) content of soybean

Treatments	Chlorophyll 'a'										
	2016-17				Mean	2017-18				Mean	Pooled Mean
	30 DAS	60 DAS	75 DAS	90 DAS		30 DAS	60 DAS	75 DAS	90 DAS		
F1: Ctrl	1.42	3.60	4.00	3.14	3.04	0.97	2.82	3.70	2.42	2.47	2.75
F2: NPK	2.54	6.00	6.38	4.42	4.83	2.60	6.00	6.82	5.00	5.10	4.96
F3: RDWS	2.68	6.31	6.52	4.61	5.03	2.64	6.30	7.12	5.22	5.32	5.17
F4: RDZn	2.72	6.54	6.81	4.87	5.23	2.70	6.65	7.54	5.58	5.61	5.42
F5: RDZnKB	2.80	6.72	7.22	5.12	5.46	2.81	6.73	7.70	5.62	5.71	5.58
F6: RDZnKBGR	2.84	6.80	7.28	5.42	5.58	2.87	6.76	7.78	5.71	5.78	5.68
F7: RDZnGR	2.76	6.68	7.00	4.92	5.34	2.75	6.70	7.66	5.52	5.65	5.49
Mean	2.53	6.09	6.45	4.64	4.93	2.47	5.99	6.90	5.01	5.09	5.01
SE+	0.10	0.50	0.28	0.22	--	0.078	0.19	0.37	0.23	--	--
CD at 5%	3.11	1.56	0.86	0.68	--	0.24	0.61	1.16	0.71	--	--

Chlorophyll-b

On an average chlorophyll b content of soybean was found to be increased with growth of soybean crop up to 75 DAS in 2016-17 and 2017-18 (Table 2). There after there was decrease in chlorophyll b content up to the harvesting stage of soybean during 2016-17 and 2017-18. The increase was from 1.87 to 4.14 and 2.0 to 4.88 mg g^{-1} fresh weight of soybean. At various growth stages and under various fertility levels, chlorophyll b showed a similar pattern as that of chlorophyll a. However chlorophyll b concentration was relatively lower than chlorophyll a concentration. Chlorophyll b concentration was highest in treatment receiving RDF + Zn + K, B and GR at 75 DAS.

Table 2: Effect of soil application of sulphur and zinc and foliar application of KNO_3, Borax, NAA and GA on chlorophyll b (mg/g fresh wt) content of soybean

Treatments	Chlorophyll 'b'										
	2016-17				Mean	2017-18				Mean	Pooled Mean
	30 DAS	60 DAS	75 DAS	90 DAS		30 DAS	60 DAS	75 DAS	90 DAS		
F1: Ctrl	0.98	2.00	2.22	1.30	1.62	0.68	1.96	2.60	1.56	1.70	1.66
F2: NPK	1.62	4.12	4.20	2.10	3.01	1.82	3.47	4.82	3.12	3.30	3.15
F3: RDWS	1.78	4.18	4.34	2.21	3.12	2.12	3.50	5.12	3.41	3.53	3.32
F4: RDZn	2.01	4.21	4.46	2.32	3.25	2.24	3.54	5.28	3.62	3.67	3.46
F5: RDZnKB	2.24	4.45	4.50	2.48	3.41	2.40	3.58	5.42	3.71	3.77	3.59
F6: RDZnKBGR	2.30	4.60	4.76	2.76	3.60	2.47	3.82	5.56	3.77	3.90	3.75

F7: RDZnGR	2.21	4.42	4.52	2.27	3.38	2.31	3.61	5.40	3.68	3.75	3.56
Mean	1.87	3.99	4.14	2.20	3.05	2.00	3.35	4.88	3.26	3.37	3.21
SE+	0.06	0.26	0.28	0.24	--	0.10	0.17	0.18	0.33	--	--
CD at 5%	0.21	0.83	0.86	0.75	--	0.33	0.52	0.56	1.01	--	--

Total chlorophyll

The data on total chlorophyll content in soybean leaves are presented in (Table 3). The data indicated that the total chlorophyll concentration in soybean leaves ranged from. 4.41 to 10.60 mg g^{-1} (fresh weight) with an average 8.23 mg g^{-1} fresh weight in 2016-17. Similarly, in the year 2017-18, it was ranged from 4.48 to 11.78 mg g^{-1} (fresh weight) with an average of 8.46 mg g^{-1} fresh weight of soybean. Further, N, P, K, S, Zn and B fertilization contributed significantly to total chlorophyll content, which can be seen from the treatments administered. Each additional soil and foliar application of nutrient from F_1 to F_6 improved the chlorophyll content of soybean. The maximum total chlorophyll content of soybean was observed at 75 DAS in both the years of experimentation. The behavior of various treatments in respect of total chlorophyll synthesis adopted a similar pattern as that of synthesis of chlorophyll a and b. The higher chlorophyll synthesis in treatment F_6 followed by F_5 due to additional foliar sprays of potash and boron.

Chlorophyll concentrations of leaves influences the leaf biochemical properties and biochemical interactions are the result of molecular/ atomic composition of the leaf. In turn they are responsible for color changes resulting from differences in pigment concentration. In this whole chain nitrogen, sulphur, zinc and foliar applications of potash and boron play important role.

Table 3: Effect of soil application of sulphur and zinc and foliar application of KNO_3, Borax, NAA and GA on total chlorophyll (mg/g fresh wt) content of soybean

Treatments	Total chlorophyll										
	2016-17				Mean	2017-18				Mean	Pooled Mean
	30 DAS	60 DAS	75 DAS	90 DAS		30 DAS	60 DAS	75 DAS	90 DAS		
F1: Ctrl	2.40	5.60	6.22	4.44	4.66	1.65	4.78	6.30	3.98	4.17	4.41
F2: NPK	4.15	10.12	10.58	6.52	7.84	4.42	9.47	11.63	8.12	8.41	7.97
F3: RDWS	4.46	10.49	10.86	6.82	8.15	4.76	9.80	12.24	8.63	8.85	8.31
F4: RDZn	4.73	11.75	11.27	7.19	8.48	4.94	10.10	12.82	9.20	9.26	8.76
F5: RDZnKB	5.04	11.17	11.72	7.60	8.88	5.21	10.30	13.12	9.33	9.49	9.03
F6: RDZnKBGR	5.14	11.40	12.04	8.18	9.19	5.34	10.50	13.34	9.48	9.66	9.42
F7: RDZnGR	4.97	11.10	11.52	7.19	8.69	5.06	10.30	13.07	9.20	9.40	9.04
Mean	4.41	10.09	10.60	6.84	8.23	4.48	9.32	11.78	8.27	8.46	8.34
SE+	0.36	0.36	0.37	0.26	--	0.26	0.29	0.30	0.23	--	--
CD at 5%	1.13	1.10	1.15	0.82	--	0.82	0.91	0.92	0.72	--	--

Carotenoid

The carotenoids content ranged from 0.67 to 1.67 mg g^{-1} fresh weights with a mean of 1.27 mgg^{-1} fresh weights in year 2016-17 and in year 2017-18 it increases from 0.81 to 1.93 mgg^{-1} fresh weights with a mean of 1.51 mgg^{-1} fresh weights (Table 4). During the process of senescence, the chlorophyll concentration decreased and allowed the dominance of carotenoids. They showed an accumulation in carotenoid towards physiological maturity. The carotenoid concentration was lower than chlorophyll a, b and total chlorophyll and it was demonstrated inverse relationship with concentration particularly at senescence.

Table 4: Effect of soil application of sulphur and zinc and foliar application of KNO_3, Borax, NAA and GA on carotenoid (mg/g fresh wt) content of soybean

Treatments	Carotenoid										
	2016-17				Mean	2017-18				Mean	Pooled Mean
	30 DAS	60 DAS	75 DAS	90 DAS		30 DAS	60 DAS	75 DAS	90 DAS		
F1: Ctrl	0.34	0.69	0.74	0.47	0.56	0.60	1.32	0.78	0.84	0.88	0.72
F2: NPK	0.59	1.20	1.52	0.94	1.06	0.72	1.42	1.86	1.00	1.25	1.15
F3: RDWS	0.62	1.23	1.78	1.04	1.16	0.76	1.45	2.00	1.18	1.34	1.25
F4: RDZn	0.68	1.25	1.82	1.15	1.22	0.82	1.47	2.18	1.26	1.43	1.32
F5: RDZnKB	0.78	1.32	1.94	1.22	1.31	0.94	1.52	2.24	1.34	1.51	1.41
F6: RDZnKBGR	0.83	1.35	2.00	1.26	1.36	0.97	1.56	2.28	1.36	1.54	1.45
F7: RDZnGR	0.74	1.28	1.90	1.18	1.27	0.91	1.50	2.20	1.30	1.47	1.37
Mean	0.65	1.18	1.67	1.03	1.13	0.81	1.46	1.93	1.18	1.34	1.23
SE+	0.02	0.06	0.13	0.04	--	0.03	0.09	0.11	0.08	--	--
CD at 5%	0.06	0.19	0.42	0.12	--	0.09	0.28	0.35	0.25	--	--

Quality parameters of soybean

The data presented in Table 5 revealed that, the quality parameters of soybean *viz*., protein and oil content significantly influenced by soil and foliar application of nutrients and growth regulators. In pooled protein content in soybean seed ranged from 33.72 to 34.72 with mean value of 34.24 per cent. In pooled analysis, the treatment F_6 (RDF + Zn + foliar K, B and GR) recorded numerical higher protein (34.72%) which was found at par with all other treatments. As regards to oil content of soybean, oil content of soybean seeds ranged from 17.49 to 18.71 in pooled data of two years. The average oil content was 18.31 per cent. Maximum oil content was observed in soybean seed with treatment F_6 followed by F_5 and F_7. However, it could not reach to the level of significance.

Good seed is the basis for successful crop production. The quality seeds are ensured with uniform germination, rapid root and shoot development and resulted in increase in the yield per unit area. However realization of seed yield always depends on production of superior quality seeds as further dividends in cultivation of crops depends on utility of quality seeds without which targeted profits cannot be achieved. This beneficial effect of foliar application of micronutrient were exhibited only when it was applied in conjunction which could be due to synergistic role of micronutrient in increasing the nutrient availability and sustaining it over a period of time as compared to their individual application. Similar results were reported by Reddy (1983) [8] in alfalfa and Ramezani and Shekafandeh (2011) [7] in soybean. Further, Rubes (1984) [9], Hugar and Kurdikeri (2000) [3] who have reported an enhanced in seed quality parameters in pea and soybean respectively and they were of the opinion that the increased in seed quality parameters noticed in $ZnSO_4$ @ 0.3% + Boron @ 0.2 + KNO_3@ 0.5%, was due to its better translocation and metabolism as a carrier of phosphate nutrients particularly into the seed as well activator of enzymes like transphosphory lase, dehydrogenase and carboxylation.

Table 5: Effect of soil application of sulphur and zinc and foliar application of KNO_3, Borax, NAA and GA on protein and oil content (%) of soybean seeds

Treatments	Protein content			Oil content		
	2016-17	2017-18	Pooled	2016-17	2017-18	Pooled
F1: Ctrl	33.37	34.06	33.72	17.24	17.40	17.49
F2: NPK	35.18	35.75	34.01	18.12	18.24	18.18
F3: RDWS	35.31	35.87	34.20	18.24	18.28	18.26
F4: RDZn	35.37	35.93	34.21	18.30	18.34	18.32
F5: RDZnKB	36.12	36.68	34.24	19.07	18.87	18.57
F6: RDZnKBGR	36.31	36.81	34.72	19.18	19.08	18.71
F7: RDZnGR	35.50	36.06	34.60	18.31	18.37	18.70
Mean	35.30	35.88	34.24	18.19	18.26	18.31
SE+	0.44	0.35	0.70	0.32	0.28	0.48
CD at 5%	NS	NS	NS	NS	NS	NS

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