

CHANGES IN CHEMICAL, BIOCHEMICAL PARAMETERS, YIELD AND YIELD ATTRIBUTING CHARACTERS IN CHICKPEA THROUGH EXOGENOUS APPLICATION OF ASCORBIC ACID AND ZINC SULPHATE

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ABSTRACT

This study was carried out at farm of Botany, College of Agriculture, Nagpur, during *rabi* 2018-2019 to investigate the response of chickpea to foliar application of ascorbic acid (100, 200, 300 and 400 ppm) and zinc sulphate (0.5 %) along with their combined effects on chemical, biochemical characters, yield and yield attributing characters. The experiment was set in a randomized block design with three replications and ten treatments. Spraying was done at 25 and 40 DAS. Spraying the plants with ascorbic acid at 200 ppm + zinc sulphate at 0.5 % (T₈) significantly enhanced the total chlorophyll content, nitrogen content, phosphorus content, potassium content, protein content, number of filled pods, number of unfilled pods and 100 seed weight. Seed yield plant⁻¹ and plot⁻¹ were also increased due to combine application of 200 ppm ascorbic acid + 0.5 % zinc sulphate (T₈).

(Key words: Chickpea, ascorbic acid, zinc sulphate, foliar application, chemical characters, biochemical characters, yield, and yield contributing characters)

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important legume as well as cool season crop. It belongs to family "Leguminosae" and sub-family "Fabaceae". It also known as gram or bengal gram, garbanzo or garbanzo bean and Egyptian pea. Chickpea is the largest produced food legume in South Asia and in India Madhya Pradesh is a major chickpea producing state contributing 30 to 35% to the national production. The reproductive stage of chickpea is more sensitive to temperature extremities than vegetative stage. Two types of chickpea are recognized viz., white seeded "kabuli" and brown colored "Desi" types.

Ascorbic acid is a naturally occurring organic compound with antioxidant properties. In relation to cell growth, ascorbic acid is a cofactor for prolyl hydroxylase that post translationally hydroxylates proline residues in cell wall hydroxyproline-rich glycoproteins required for cell division and expansion. Additionally, high ascorbic acid oxidase activity in the cell wall is correlated with areas of rapid cell expansion.

Zinc sulphate is an inorganic chemical compound with the formula ZnSO₄. Zinc sulphate is a compound containing zinc, such as zinc oxide and zinc chloride. Zinc affects several biochemical processes in the plant, such as cytochrome and nucleotide synthesis, auxin metabolism, chlorophyll production, enzyme activation and membrane

integrity. The foliar application of zinc @ 0.1 per cent had a positive impact on reproductive structures development, number of pods plant⁻¹, number of seeds pod⁻¹, seed size, weight and seed yield in zinc deficient black gram plants (Nalini *et al.*, 2013).

MATERIALS AND METHODS

The present study was conducted during the *rabi* season of 2018- 2019 at farm of Botany, College of Agriculture, Nagpur. Treatments comprising of T₁- control, T₂- Foliar application of zinc sulphate @ 0.5 per cent, T₃- ascorbic acid @ 100 ppm, T₄- ascorbic acid @ 200 ppm, T₅- ascorbic acid @ 300 ppm, T₆- ascorbic acid @ 400 ppm, T₇- zinc sulphate @ 0.5 per cent + ascorbic acid @ 100 ppm, T₈- zinc sulphate @ 0.5 per cent + ascorbic acid @ 200 ppm, T₉- zinc sulphate @ 0.5 per cent + ascorbic acid @ 300 ppm and T₁₀-zinc sulphate @ 0.5 per cent + ascorbic acid @ 400 ppm were evaluated in a randomized block design with three replications. Each plot measured 2.20 m × 3.00 m gross and 2.00 m × 2.40 m net. At 25 and 40 DAS two foliar sprays of ascorbic acid and zinc sulphate were given. In the present study analysis of total chlorophyll and NPK content in chickpea leaves were taken on 25, 45, 65 and 85 DAS. Total chlorophyll content of oven dried leaves was estimated by colorimetric method as suggested by Bruinsma (1982). Nitrogen content in leaves was determined by micro

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kjeldhal's method as given by Somichi *et al.* (1972). Phosphorus content in leaves was determined by vanadomolybdate yellow colour method as given by Jackson (1967). Potassium content in leaves was determined by flame photometer by di-acid extract method given by Jackson (1967). Also, data on protein content in seed, number of filled pods, number of unfilled pods, 100 seed weight, seed yield plant⁻¹ and plot⁻¹ were recorded after harvest. Statistical analysis was done as per procedure given by pause and Sukhatme (1954).

RESULTS AND DISCUSSION

Total chlorophyll content

Chlorophyll is an antioxidant compounds which are present and stored in the chloroplast of green leaf plants and mainly it is present in the green area of leaves, stems, flowers and roots. However, chlorophyll production mainly depends on penetration of sun light and it is the main source of energy for plant.

At 45 DAS chlorophyll content in leaves ranged from 1.36 - 2.09 mg g⁻¹. Significantly highest chlorophyll content was found in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid) and T₁₀ (0.5 % zinc + 400 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study. Whereas treatments T₇ (0.5 % zinc + 100 ppm ascorbic acid), T₄ (200 ppm ascorbic acid) and T₂ (0.5 % zinc) were also found significantly superior over treatment T₁ (control) and rest of the treatments in chlorophyll content. At 65 DAS chlorophyll content in leaves ranged from 1.15 - 2.22 mg g⁻¹. Significantly highest chlorophyll content was found in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid), T₁₀ (0.5 % zinc + 400 ppm ascorbic acid), T₇ (0.5 % zinc + 100 ppm ascorbic acid) and T₄ (200 ppm ascorbic acid). Treatments T₂ (0.5 % zinc) and T₅ (300 ppm ascorbic acid) were found significantly superior over treatment T₁ (control) and rest of the treatments. At 85 DAS chlorophyll content in leaves ranged from 1.47 - 2.16 mg g⁻¹. Significantly highest chlorophyll content was noticed in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid) and T₁₀ (0.5 % zinc + 400 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study. Whereas treatments T₇ (0.5 % zinc + 100 ppm ascorbic acid), T₄ (200 ppm ascorbic acid) and T₂ (0.5 % zinc) also enhanced chlorophyll content in leaves significantly over treatment T₁ (control) and rest of the treatments.

It is obvious from the data that chlorophyll content in leaves was maximum at 45-65 DAS but thereafter, gradual decrease in chlorophyll content was noticed at 85 DAS. Nitrogen is present in ample quantity at vegetative stage and it is a constituent element of chlorophyll. Increase in chlorophyll content during 45 to 65 DAS might be because of the increase in uptake of N, P, K and other nutrients.

Amin *et al.* (2008) recorded highest values of chl a and chl. b content in leaves of wheat plants treated with salicylic acid at 100 or 200 mg l⁻¹ + ascorbic acid at 200 or 400 mg l⁻¹ compared to other treatments and control plants at two stages of growth. Purushottam *et al.* (2018) observed 3-8 % increment in leaf chlorophyll of pigeonpea with foliar application of 0.5% ZnSO₄.

Leaf nitrogen content (%)

Nitrogen is most imperative element for proper growth and development of plants which significantly increases and enhances the yield and its quality by playing a vital role in biochemical and physiological functions of plant.

At 45 DAS significantly maximum nitrogen content was examined in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid) and T₁₀ (0.5 % zinc + 400 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study. Whereas treatments T₇ (0.5 % zinc + 100 ppm ascorbic acid) and T₄ (200 ppm ascorbic acid) also found significantly superior over treatment T₁ (control) and rest of the treatments. At 65 DAS and 85 DAS significantly maximum nitrogen content was found in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatment T₉ (0.5 % zinc + 300 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study. Similarly, treatments T₁₀ (0.5 % zinc + 400 ppm ascorbic acid), T₇ (0.5 % zinc + 100 ppm ascorbic acid), T₄ (200 ppm ascorbic acid), T₂ (0.5 % zinc), T₅ (300 ppm ascorbic acid) and T₆ (400 ppm ascorbic acid) were also found significantly superior over treatment T₁ (control) and rest of the treatments.

From this data it is observed that younger leaves and developing organs such as seeds, act as strong sink demand and hence may draw nitrogen heavily from the older leaves. So, decrease in nitrogen content might occur in the later stages of growth in a plant (Gardner *et al.*, 1988).

Gad *et al.* (2012) indicated that nitrogen % in pea was markedly increased by ascorbic acid foliar application at the concentration of 200 ppm.

Leaf phosphorus content

Phosphorus (P) is part of the nuclei acid structure of plants which is responsible for the regulation of protein synthesis. Without phosphorus, photosynthesis do not occur. Phosphorus plays a key role in complex energy transformations that are necessary to all life, as a main ingredient in form of ATP (adenosine triphosphate).

At 45 DAS significantly maximum phosphorus content was noted in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid), T₁₀ (0.5 % zinc + 400 ppm ascorbic acid), T₇ (0.5 % zinc + 100 ppm ascorbic acid), T₄ (200 ppm ascorbic acid), T₂ (0.5 % zinc) and T₅ (300 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study. At 65 DAS significantly maximum

phosphorus content was recorded in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid), T₁₀ (0.5 % zinc + 400 ppm ascorbic acid), T₇ (0.5 % zinc + 100 ppm ascorbic acid), T₄ (200 ppm ascorbic acid), T₂ (0.5 % zinc) and T₅ (300 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study. Whereas treatment T₆ (400 ppm ascorbic acid) was also found significantly superior over treatment T₁ (control) and rest of the treatments. At 85 DAS significantly maximum phosphorus content was observed in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid), T₁₀ (0.5 % zinc + 400 ppm ascorbic acid), T₇ (0.5 % zinc + 100 ppm ascorbic acid), T₄ (200 ppm ascorbic acid) and T₂ (0.5 % zinc) when compared with treatment T₁ (control) and remaining treatments under study.

The reason behind the increase in phosphorus content gradually upto 65 DAS might be due to translocation of leaf phosphorus and its utilization for the development of food storage organs. Amira (2014) recorded that maximum phosphorus content in soybean leaves were observed due to exogenous application of ascorbic acid at 200 ppm.

Leaf potassium content

Potassium (K) essentially plays a major role in plant physiological processes. Therefore, it is required for proper growth and reproduction in plants.

At 45 DAS significantly maximum potassium content was recorded in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid) and T₁₀ (0.5 % zinc + 400 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study. Similarly, treatments T₇ (0.5 % zinc + 100 ppm ascorbic acid), T₄ (200 ppm ascorbic acid) and T₂ (0.5 % zinc) were also found significantly superior over treatment T₁ (control) and rest of the treatments in potassium content in leaves. At 65 DAS significantly maximum potassium content was examined in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid) and T₁₀ (0.5 % zinc + 400 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study. Treatments T₇ (0.5 % zinc + 100 ppm ascorbic acid), T₄ (200 ppm ascorbic acid), T₂ (0.5 % zinc) and T₅ (300 ppm ascorbic acid) also increased potassium content in leaves over treatment T₁ (control) and rest of the treatments. At 85 DAS significantly maximum potassium content was found in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid) and T₁₀ (0.5 % zinc + 400 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study. Treatments T₇ (0.5 % zinc + 100 ppm ascorbic acid), T₄ (200 ppm ascorbic acid), T₂ (0.5 % zinc), T₅ (300 ppm ascorbic acid) and T₆ (400 ppm ascorbic acid) were found significantly superior over treatment T₁ (control) and rest of the treatments.

Decrease in potassium content in later stages of crop growth from 65 DAS to 85 DAS may be due to the

diversion of potassium towards developing parts i.e. pods of chickpea crop at maturity stage.

Hussein and Khursheed (2014) recorded that potassium content in wheat leaves were significantly affected by foliar spray of ascorbic acid at 100 and 200 ppm.

Protein content in seed

Grain legumes are known to be an important source of protein. The variability in seed protein content among different varieties of legumes are due to the genetic difference and the interaction with different physiological conditions of the plants. Protein content of the seed is one of the considerable factors for seed quality determination also.

From the analysis result indicate that treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) caused significant increase in protein content in seeds followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid), T₁₀ (0.5 % zinc + 400 ppm ascorbic acid) and T₇ (0.5 % zinc + 100 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study.

Nitrogen is the constituent of protein. Hence, increase in nitrogen content ultimately resulted in the increase in protein content in seeds of chickpea in the present investigation.

Gad *et al.* (2012) found that foliar spray of ascorbic acid at 200 ppm gave significant increase in protein % in pea seed. Sale *et al.* (2018) revealed that FeSO₄ spray (0.5%) + ZnSO₄ spray (0.5%) + seed fortification of molybdenum significantly increased protein content (42.99%) in soybean seed.

Yield and yield attributing parameters

Yield is complex character determined by several traits like internal plant processes and environmental factors.

Number of pods plant⁻¹

The output of total metabolic activities taking place in plant body is called pod. Pod yield mainly depends upon source sink relation. The economic part will obtain the assimilates synthesized by photosynthesis.

A close examination of the data indicated that significantly maximum number of pods plant⁻¹ were counted in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid), T₁₀ (0.5 % zinc + 400 ppm ascorbic acid), T₇ (0.5 % zinc + 100 ppm ascorbic acid) and T₄ (200 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study. Treatments T₂ (0.5 % zinc), T₅ (300 ppm ascorbic acid) and T₆ (400 ppm ascorbic acid) were also found significantly superior over treatment T₁ (control) and rest of the treatments.

Anitha *et al.* (2005) found that combined spraying of 0.5% FeSO₄ and 0.5% ZnSO₄ at 45 DAS recorded significantly higher pods plant⁻¹ in cowpea. Nandan *et al.* (2018) recorded foliar spray of 0.5% Zn and 0.5% Fe along with RDF produced maximum number of pods plant⁻¹ in chickpea.

Table 1. Effect of ascorbic acid and zinc sulphate on chemical and biochemical parameters in chickpea

Treatments	Total chlorophyll content (mg g ⁻¹)						Nitrogen content (%)						Phosphorus content (%)						Potassium content (%)						Protein content (%)
	25		45		65		85		25		45		65		85		25		45		65		85		
	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS	DAS		
T ₁ (control)	1.13	1.36	1.51	1.47	1.28	1.89	2.17	2.11	0.220	0.573	0.618	0.611	0.48	0.87	1.07	0.85	19.47								
T ₂ (0.5 % zinc)	1.17	1.67	1.97	1.73	1.23	2.14	2.83	2.54	0.213	0.678	0.753	0.703	0.53	1.11	1.36	1.33	21.38								
T ₃ (100 ppm ascorbic acid)	1.12	1.43	1.74	1.50	1.33	1.97	2.45	2.24	0.230	0.592	0.697	0.636	0.55	0.91	1.12	0.96	20.39								
T ₄ (200 ppm ascorbic acid)	1.23	1.72	2.04	1.80	1.32	2.33	2.87	2.80	0.221	0.690	0.779	0.753	0.51	1.16	1.44	1.42	21.97								
T ₅ (300 ppm ascorbic acid)	1.25	1.55	1.88	1.66	1.38	2.11	2.71	2.52	0.232	0.665	0.736	0.688	0.54	0.97	1.30	1.21	20.94								
T ₆ (400 ppm ascorbic acid)	1.22	1.47	1.74	1.64	1.31	2.07	2.66	2.49	0.223	0.613	0.712	0.672	0.50	0.95	1.24	1.15	20.53								
T ₇ (0.5 % zinc + 100 ppm ascorbic acid)	1.19	1.84	2.06	1.93	1.22	2.38	3.20	2.87	0.217	0.701	0.803	0.767	0.49	1.20	1.47	1.42	22.94								
T ₈ (0.5 % zinc + 200 ppm ascorbic acid)	1.21	2.09	2.22	2.16	1.10	2.81	3.73	3.50	0.209	0.729	0.827	0.786	0.54	1.39	1.69	1.59	24.00								
T ₉ (0.5 % zinc + 300 ppm ascorbic acid)	1.24	2.03	2.14	2.15	1.37	2.77	3.65	3.47	0.228	0.718	0.816	0.772	0.53	1.32	1.62	1.53	23.44								
T ₁₀ (0.5 % zinc + 400 ppm ascorbic acid)	1.15	1.99	2.10	2.06	1.40	2.65	3.22	3.10	0.219	0.706	0.812	0.769	0.52	1.27	1.58	1.46	23.03								
SE (m) ±	0.051	0.072	0.082	0.076	0.117	0.096	0.122	0.114	0.009	0.028	0.032	0.030	0.032	0.047	0.058	0.054	0.773								
CD at 5 %	-	0.205	0.234	0.216	-	0.276	0.349	0.327	-	0.080	0.092	0.087	-	0.133	0.166	0.154	2.298								

Treatments	Number of filled pods	Number of unfilled pods plant ⁻¹	100 seed weight (g)	Seed yield plant ⁻¹ (g)	Seed yield plot ⁻¹ (kg)
T ₁ (control)	27.53	12.13	17.57	4.68	0.936
T ₂ (0.5 % zinc)	43.50	07.41	18.00	5.43	1.086
T ₃ (100 ppm ascorbic acid)	28.93	11.73	17.63	4.87	0.973
T ₄ (200 ppm ascorbic acid)	48.86	07.09	18.13	5.49	1.097
T ₅ (300 ppm ascorbic acid)	38.13	08.00	17.87	5.30	1.060
T ₆ (400 ppm ascorbic acid)	34.00	09.19	17.77	5.13	1.027
T ₇ (0.5 % zinc + 100 ppm ascorbic acid)	51.66	06.69	18.30	5.56	1.112
T ₈ (0.5 % zinc + 200 ppm ascorbic acid)	53.53	05.46	19.27	6.01	1.202
T ₉ (0.5 % zinc + 300 ppm ascorbic acid)	53.26	06.06	18.90	5.80	1.161
T ₁₀ (0.5 % zinc + 400 ppm ascorbic acid)	52.42	06.42	18.87	5.75	1.150
SE(m) ±	1.805	0.392	0.326	0.288	0.045
CD at 5%	5.152	1.119	0.969	0.653	0.131

Number of unfilled pods plant⁻¹

Scrutiny of the data revealed significant effect of foliar application of ascorbic acid and zinc sulphate on number of unfilled pods plant⁻¹. Significantly lowest number of unfilled pods plant⁻¹ was recorded in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid) and T₁₀ (0.5 % zinc + 400 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study. Next to these treatments T₇ (0.5 % zinc + 100 ppm ascorbic acid), T₄ (200 ppm ascorbic acid), T₂ (0.5 % zinc), T₅ (300 ppm ascorbic acid) and T₆ (400 ppm ascorbic acid) also showed lowest number of unfilled pods when compared with treatment T₁ (control) and remaining treatments under study.

100 seed weight

Among different treatments significantly maximum 100 seed weight was recorded in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid) and T₁₀ (0.5 % zinc + 400 ppm ascorbic acid) when compared with treatment T₁ (control) and remaining treatments under study.

Seed weight might be higher due to ability of plants to higher dry matter partitioning towards seed and greater sink capacity.

Thomson *et al.* (2017) recorded maximum 100 seed weight by the application of ascorbic acid @ 200 ppm on garden pea. Nandan *et al.* (2018) recorded higher 1000-seed weight due to foliar spray of 0.5% Zn and 0.5% Fe along with RDF in chickpea.

Seed yield plant⁻¹ and plot⁻¹

Seed yield is the economic yield which is final result of physiological activities of plant. Economic yield is the part of biomass that is converted into economic product. (Nichiporovic, 1960).

Significantly higher seed yield plant⁻¹ (g) and plot⁻¹ (kg) were produced in treatment T₈ (0.5 % zinc + 200 ppm ascorbic acid) followed by treatments T₉ (0.5 % zinc + 300 ppm ascorbic acid), T₁₀ (0.5 % zinc + 400 ppm ascorbic acid), T₇ (0.5 % zinc + 100 ppm ascorbic acid), T₄ (200 ppm ascorbic acid) and T₂ (0.5 % zinc) when compared with treatment T₁ (control) and remaining treatments under study.

Zarghamnejad *et al.* (2014) showed that application of higher doses of ascorbic acid (200 and 300 mg l⁻¹) led to significant increase in seed yield of chickpea at the rates of 30 and 27 per cent in comparison with control treatment. Anitha *et al.* (2005) revealed that foliar application of micronutrients like iron and zinc has significant influenced on the yield of cowpea. Combined spraying of 0.5% FeSO₄ and 0.5% ZnSO₄ at 45 DAS proved most effective and increased the seed yield by 43.09 per cent when compared with control.

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