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Effect of seed priming and foliar spray of bio-regulators on biochemical properties of chickpea (*Cicer arietinum* L.) under conserved moisture condition of Bhal region of Gujarat

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Abstract
Three year field experiment conducted at Bhal region of Gujarat to examine the effect of seed priming and foliar spray of stress mitigating bio-regulators under conserved moisture condition on biochemical properties of chickpea. Drought is the most limiting factor for production in semi -arid ecosystem of Gujarat. Under changed climate scenario due to variability in rain fall pattern problem of drought aggravated. Experiment treatments were applied as seed soaking with thiourea (500 ppm), salicylic acid (50 ppm), thioglycolic acid (50 ppm) before sowing and foliar spray of water, thiourea (1000 ppm), salicylic acid (100 ppm) thioglycolic acid (100 ppm) at vegetative & flowering stage & one is absolute control in both of factors. Bio-regulator spray was done at vegetative & flowering stage and samples were collected after 10 days of each spray for analysis. Among the treatments imposed, result revealed that seed priming of thiourea @500 ppm recorded significantly higher values of chlorophyll A (mg/g/ fresh weight), chlorophyll B (mg/g/ fresh weight), total chlorophyll (mg/g fresh weight) and proline content (mg/100g) in second sampling. Foliar spray with thiourea @1000 ppm recorded significantly higher data of chlorophyll A (mg/g/ fresh weight), chlorophyll B (mg/g fresh weight), total chlorophyll (mg/g/ fresh weight) and proline content (mg/100g) were recorded in second sampling.

Keywords: Chickpea, seed priming, foliar spray, thiourea, TGA, chlorophyll, proline

Introduction
Chickpea is a major pulse crop of India accounting for more than 40% of the total pulses area and production. From a mere 3.86 million tonnes (mt) during 2000-01, chickpea production rose steadily to an all-time high of 11.23 mt during 2017–18. The central and southern states such as Madhya Pradesh, Maharashtra, Rajasthan, Andhra Pradesh, Karnataka, Gujarat, Chhattisgarh and Jharkhand have benefited most from chickpea revolution in the country, with remarkable increase in both area and production. This has been achieved through development of suitable technologies like high-yielding varieties along with matching production and protection technologies, quality seed producing units, use of bio-regulators etc. India contributes to a major share of the world's chickpea area (70%) and production (67%) and continues to be the largest chickpea-producing nation. Dixit *et al.,* (2019) [4]. Drought stress is a serious situation for agriculture in the context of climate change and the ever-increasing world population. Extreme drought conditions reduce crop yields through negative impacts on plant growth, physiology and reproduction. Often drought is accompanied by relatively high temperatures, which promote evaportranspiration and hence could accentuate the effects of drought and thereby further reduce crop yields. (Sabaghpour, 2003) [9]. Rahangdale *et al.,* (1994) [7] reported that water stress decreased seed yield 15.2%. Yield reduction differ range 30 to 60 percent in chickpea, which depend on geographical region and length of crop season. The role of Bio-regulators in enhancing the production of crop has long been recognized and now this low cost technology has emerged as a boon for enhancing the agricultural production at an unprecedented rate. It hasbeenobserved that synthesis and translocation of photosynthates into sink is very poor at later stages of the crop besides poor vegetative growth and flowering. Plant hormones play important role as the small quantities regulate the various physiological processes and balance the source and sink thereby increase the productivity. (Wankhade *et al.,* 2020) [14]. Number of various bio-regulators like Thiourea (TU), Salicylic acid (SA), and thioglycolic acid (TGA) are being used on different crops as seed priming and foliar

Spray at different stages of development. In view of this, the present study was undertaken to access the effect of bio-regulators on yield, yield attributes and quality in chickpea.

Matreials and Methods

The experiment was conducted *rabi* session 2013-14 to 2015-2016 for three years at Agricultural Research Station, Anand Agricultural University, Dhandhuka, Gujarat. The soil of the experiment field is medium black clay loamy with pH 8.7 and medium rang in available in nitrogen (215 kg/ha), low in available phosphorus (5.46kg/ha) and high in available potassium (475 kg/ha) with 0.46 % organic carbon. The average rain fall of station is 625 mm. The experiment was laid out in randomization block design (Factorial) with 20 treatment combinations of seed priming (S_0 - No seed soaking, S_1- seed soaking with thiourea @ 500 ppm, S_2 - seed soaking with salicylic acid @ 50 ppm, and S_3 - seed thioglycolic acid @50 ppm) for one hour before sowing and foliar spray (C_0 - No spray, C_1 - water spray, C_2 - thiourea spray @1000 ppm, C_3 - salicylic acid spray @100 ppm and C_4 - thioglycolic acid spray @100 ppm) with three replications. The chickpea variety GG-2 was sown at 30 cm spacing in conserved moisture which is received during month of June to August and no supplemental irrigation given to crop during the seasons and two foliar sprays of bio-regulators were applied at vegetative and flowering stage of crop. Leaf samples were collected 10 days after each spray for analysis like chlorophyll A, B, total chlorophyll and proline content.

Chlorophyll (mg/g of fresh weight) estimation

The representative fresh leaf samples were taken to determine chlorophyll content of leaves. These were washed with distilled water and dried with blotting paper. Out of this, 50 mg sample was taken in Mortar and ground well by Pestle with 5 ml 80 per cent acetone and centrifuged at 2000 rpm and made the final volume to 10 ml. Absorbance of clear supernatant was measured by Spectronic-20 at 652 nm. The chlorophyll content was expressed in mg g^{-1} of fresh weight of leaves (Arnon, 1949) [1].

$$\text{Total chlorophyll} = \frac{A\,(652) \times 29 \times \text{Total volume (ml)}}{\alpha \times 1000 \times \text{weight of sample (g)}}$$

Where

α is the path length = 1 cm

Proline estimation

Proline content in plants is determinate by the colorimetric assay method at acidic pH, nin-hydrin can from a red product with proline & omithine used for the estimation of concentration of these amino acids in pure solution (Sunkar R, 2010) [13]

$$\text{Total chlorophyll} = G.F \times O.D \times \frac{\text{Total volume}}{\text{Taken volume}} \times \frac{\text{sample weight}}{1} \times 10^{-6}$$

Where

G.F = Graf factor (94.67)

O.D = Optical density

The data from various characters under study were analyzed by the statistics department of Anand Agricultural University-Anand.

Result and Discussion

The seed priming and foliar spray of bio-regulators significantly influence on the chlorophyll A, B, total chlorophyll and proline content of rainfed chickpea under conserved moisture condition and data was submitted on pooled analysis of three year basis (Table 1).

Table 1: Effect of seed priming and foliar spray of stress mitigating chemical on biochemical properties after first & second sampling of rainfed chickpea

Treatment	Ist spray				IInd spray			
	Chlorophyll A (mg/g/fresh weight)	Chlorophyll B (mg/g/fresh weight)	Total Chlorophyll (mg/g/fresh weight)	Proline content (mg/100g)	Chlorophyll A (mg/g/fresh weight)	Chlorophyll B (mg/g/fresh weight)	Total Chlorophyll (mg/g/fresh weight)	Proline content (mg/100g)
(A) Seed Priming								
S_0: Control	1.71	0.64	2.36	11.38	1.35	0.50	1.86	21.09
S_1: Thiourea (500ppm)	1.83	0.70	2.53	13.03	1.70	0.63	2.33	22.90
S_2: SA (50ppm)	1.76	0.66	2.42	11.90	1.53	0.55	2.09	22.53
S_3: TGA (50ppm)	1.74	0.65	2.39	13.22	1.52	0.56	2.08	22.81
S.Em	0.02	0.01	0.03	0.34	0.04	0.02	0.05	0.55
CD@5%	0.07	NS	0.10	0.97	0.12	0.05	0.15	1.58
(B)Chemical Spray								
C_0: Control	1.76	0.67	2.43	11.36	1.37	0.50	1.87	20.68
C_1: Water spray	1.71	0.62	2.33	12.00	1.48	0.57	2.05	22.56
C_2:Thiourea (1000ppm)	1.84	0.68	2.52	13.84	1.67	0.63	2.31	24.92
C_3: SA(100ppm)	1.77	0.68	2.45	12.73	1.53	0.55	2.09	22.41
C_4: TGA (100ppm)	1.74	0.66	2.40	11.98	1.56	0.56	2.12	21.10
S.Em	0.02	0.02	0.04	0.38	0.04	0.023	0.06	0.62
CD@5%	0.08	NS	NS	1.09	0.137	0.065	0.17	1.78
Interaction S x C	Sig	NS	NS	Sig	NS	NS	NS	Sig.
CV%	5.62	10.86	5.93	10.59	10.73	13.88	10.26	9.56

Seed priming

I^st^ spray

An examination of data from Table 1.0 indicate that seed priming treatments significantly increase the chlorophyll A, total chlorophyll and proline content after first spray. Seed priming with thiourea @ 500 ppm (S_1) recorded significantly higher chlorophyll A content (1.83) over control (1.71) which is 7.01 percent higher over control. Treatment SA @ 50 ppm (S_2) and TGA @ 50 ppm (S_3) gave numerically higher chlorophyll A, 1.74 and 1.76 respectively, over control. A perusal of data revealed that seed priming of all bio-regulators though could not differ for chlorophyll B content after first spray. Further examination of data from Table 1.0 for total chlorophyll content (mg/g fresh weight) clearly indicated that seed priming respond positively. Seed priming with thiourea @ 500 ppm (S_1) recorded significantly higher total chlorophyll content (2.53) over control (2.36) which is 7.20 percent higher over control after first spray. A perusal of the data also exhibited that seed priming with stress mitigating bio-regulators significantly increased the proline content over no seed priming (control). foliar spray of thioglycolic acid (TGA) @ 50 ppm (S_3) (13.22) exhibited significantly superior data over control (11.38) and found remain at par with thiourea @ 500 ppm (13.03). Seed priming registered an increase of 16.16 percent higher proline content over control with thioglycolic acid (TGA) @ 50 ppm (S_3).

II^nd^ spray

Data pertaining to chlorophyll A, chlorophyll B, total chlorophyll and proline as influenced by seed priming of stress mitigating bio-regulators are presented in Table 1.0 after second spray. It was clear from the results that seed priming treatments significantly influenced the chlorophyll A (mg/g fresh weight). Substantially the highest value was observed with seed soaking with thiourea @ 500 ppm (1.70) (S_1) as compared to control (1.35) (S_0). However, it was found at par with SA @ 50 ppm (1.53) (S_2) and TGA @ 50 ppm (1.52) (S_3). Application of seed priming with thiourea @ 500 ppm recorded an increment of 25.92 per cent over no seed priming on pooled basis. Application of SA @ 50 ppm (1.53) (S_2) and TGA @ 50 ppm (1.52) (S_3) recorded 13.33 & 12.59 percent higher over control respectively. The data further revealed that seed priming with thiourea @ 500 ppm significantly influence chlorophyll B (mg/g fresh weight) and total chlorophyll (mg/g fresh weight) content. Application of seed priming with thiourea @ 500 ppm recorded significantly higher chlorophyll B (0.63) and total chlorophyll (2.33) over control (S_0) and an increment of 26.0 & 25.26 percent higher over no seed priming respectively, on pooled basis. Nathawat *et al.*, (2016) [6] found that application of thiourea (500, 750 and 1000 mg/L^{-1}) and thioglycolic acid (200, 300 and 400 mg/L^{-1}) would alleviate deleterious effects of water stress on clusterbean (*Cyamopsis tetragonoloba* L.) and found that application of SH compounds (Thiourea and Thioglycolic acid) significantly ($P < 0.05$) improved the contents of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid during both the years of experimentation. A perusal of data further revealed that seed priming of thiourea @ 500 ppm (S_1) significantly increased the proline content(22.90) after second spray over control (21.09) (C_0) treatment, which registered an increase of 8.58 percent at second spray in proline content over control on pooled basis. Treatment S_2 (SA 50 ppm) and S_3 (TGA 50 ppm) found remain at par with S_1 treatment (thiourea 500 ppm). Solanki (2002) [12] reported that thiourea being a sulphydryl compound significantly improved the root growth. This might be due to metabolic role of –SH group in root physiology and biochemistry. Dipping wheat seeds in 6.6 mM solution of thiourea and exposing the plants to heat stress enhanced the chlorophyll content (2.37%) whilst reducing the membrane injury (3.91%) compared to non-dipped seeds (Asthir *et al.*, 2013) [2]. Thiourea increased the chlorophyll content of leaves up to 3.6% over control in maize (Sahu *et al*., 1993) [10].

Chemical spray

I^st^ spray

A reference to data (Table 1.0) indicated that different foliar sprays of bio-regulators had their significant impact to chlorophyll A and proline content after first spray where as numerically higher data of chlorophyll B and total chlorophyll found with C_2 treatment over control and water spray. Substantially the significantly highest value of chlorophyll A was recorded with foliar sprays of thiourea @ 1000 ppm (1.84) (C_2) as compared to water spray (1.71) and control (1.76) (C_0). However, it was found at par with SA @ 100 ppm (1.77) (C_3) and TGA @ 50 ppm (1.74) (C_4). A perusal of data also revealed that foliar application of thiourea @ 1000 ppm (13.84) (C_2) significantly increased the proline content (mg/100g) after first spray over no spray (11.36) (C_0) and water spray (12.0) (C_1) treatment, which registered an increase of 21.83 and 15.33 percent at first spray in proline content, respectively, over no spray and water spray on pooled mean basis. Rana *et al.*, (2018) [8] reported that significantly higher proline content (2.512 μg/g fresh weight), total chlorophyll content at 75 DAS (1.916 mg /g fresh weight), RWC at 75 DAS (81.1 %) and WUE (16.1 kg/ha/mm) was observed with the foliar application of thiourea (1000 ppm) with water stress under normal and late sown conditions.

II^nd^ spray

Foliar spray with stress mitigating bio-regulators had significant influence on chlorophyll A, chlorophyll B, total chlorophyll (mg/g/fresh weight) and proline (mg/100g) on pooled basis, wherein; foliar spray of thiourea @ 1000 ppm significantly influence chlorophyll A (1.67), chlorophyll B (0.63), total chlorophyll (2.31) and proline content (24.92) over control and water spray and registered increase is 23.35, 26.0, 23.52 and 20.50 percent over control respectively, on pooled mean basis. Chlorophyll A, chlorophyll B, and total chlorophyll content found at par with C_4 treatment (TGA 100 ppm) whereas, proline content found at par with C_3 treatment (SA 100 ppm). Sharma and Singh (2005) found that foliar spray of 1000 ppm thiourea solution on maize significantly increased chlorophyll content over water sprayed plants. Two times (at 25 and 45 days after sowing) foliar spray of 0.1% thiourea increased net rate of photosynthesis, chlorophyll content, starch and nitrate reductase activity in cluster bean (Garg *et al.*, 2006) [5]. Under different environmental conditions like arid and semi-arid environments, foliar application of thiourea improved chlorophyll content in drought stressed clusterbean ultimately leading to significant improvement in plant growth by increased biomass and yield (Burman *et al.*, 2004) [4]. During drought stress, foliar application of thiourea improved net photosynthesis and chlorophyll content in cluster bean (Garg *et al.*, 2006) [5].

Interaction effect

Interaction effect (Table No 2. & 3.) of seed priming and foliar spray of stress mitigating bio-regulators in first spray for chlorophyll A and proline content were significant.

Highest and significantly value of chlorophyll A (1.97) and proline (15.71) found with S_1C_2 treatment on pooled basis. In case of second spray, significant interaction (Table No 4.) effect found in proline content (26.63) with S_1C_2 treatment on pooled basis.

Table 2: Interactive effect of seed priming and foliar spray of stress mitigating chemicals on chlorophyll A (mg/fresh weight) after first spray of rainfed chickpea in pooled analysis

Chemical spray	Seed priming				
	S_0	**S_1**	**S_2**	**S_3**	**Mean**
C_0	1.53	1.82	1.86	1.83	1.76
C_1	1.69	1.80	1.71	1.64	1.71
C_2	1.87	1.97	1.74	1.78	1.84
C_3	1.75	1.78	1.83	1.75	1.78
C_4	1.76	1.79	1.71	1.71	1.74
Mean	1.72	1.83	1.77	1.74	
Sem±	0.06				
C D @ 5%	0.17				
CV%	5.62				

Table 3: Interactive effects of seed priming and foliar spray of stress mitigating chemicals on proline content (mg/100gm) after first spray of rainfed chickpea in pooled analysis

Chemical spray	Seed priming				
	S_0	**S_1**	**S_2**	**S_3**	**Mean**
C_0	9.12	11.82	10.42	14.07	11.36
C_1	10.76	12.68	12.02	12.56	12.00
C_2	13.36	15.71	11.83	14.48	13.84
C_3	12.13	12.44	13.57	12.76	12.73
C_4	11.54	12.48	11.66	12.25	11.98
Mean	11.38	13.03	11.90	13.22	
Sem±	0.76				
C D @ 5%	2.19				
CV%	10.59				

Table 4: Interactive effects of seed priming and foliar spray of stress mitigating chemicals on proline content (mg/100gm) in pooled analysis (second spray) of rainfed chickpea

Chemical spray	Seed priming				
	S_0	**S_1**	**S_2**	**S_3**	**Mean**
C_0	16.15	21.70	21.14	23.73	20.68
C_1	21.47	24.61	23.08	21.09	22.56
C_2	25.41	26.63	22.87	24.77	24.92
C_3	20.93	21.70	23.59	23.41	22.41
C_4	21.48	19.88	21.98	21.05	21.10
Mean	21.09	22.90	22.53	22.81	
Sem±	1.23				
C D @ 5%	3.56				
CV%	9.56				

Economics

Economics deliberating to gross realization, net realization and benefit cost ratio (BCR) as influenced by seed soaking and foliar spray of different stress mitigating bio-regulators is presented in Table 5.

Table 5: Effects of seed priming and foliar spray of stress mitigating chemicals on economics of rainfed chickpea on the basis of pooled analysis.

Treatment	Seed Yield kg/ha	Straw yield kg/ha	Seed income (`)	Straw income (`)	Gross realization (`)	Total cost (`)	Net Realization (`)	B:C ratio
(A) Seed Priming								
S_0: Control	1305	682	65242	3410	68652	21000	47660	2.27
S_1: Thiourea (500ppm)	1452	740	72604	3700	76304	21235	55065	2.59
S_2: SA (50ppm)	1387	766	69328	3830	73158	21242	51938	2.45
S_3: TGA (50ppm)	1371	795	68530	3975	72505	21188	51337	2.42
(B) Chemical Spray								
C_0: Control	1275	726	63747	3630	67377	21000	46380	2.21
C_1: Water spray	1317	765	65854	3825	69679	21600	48075	2.23
C_2:Thiourea (1000ppm)	1532	694	76617	3470	80087	21822	58248	2.67
C_3: SA (100ppm)	1392	746	69622	3730	73352	21844	51486	2.36
C_4: TGA (100ppm)	1376	798	68789	3990	72779	21748	51042	2.35

Seed priming

Data presented in Table 5.0 clearly shown that highest gross realization (`76304 ha$^{-1}$) net realization (`55065 ha^{-1}) and B:C ratio (2.59) were obtained with seed priming through 500 ppm thiourea (S_1) followed by seed priming of SA @ 50 ppm (S_2) with gross realization (`73158 ha$^{-1}$), net realization (`51938 ha^{-1}) and B:C ratio (2.45).

Chemical spray

Data further reveled that highest gross realization (`80087ha$^{-1}$) net realization (`58248 ha^{-1}) and B:C ratio(2.67) were obtained with foliar spray of1000 ppm thiourea (C_2) followed by foliar spray of SA @ 100 ppm (C_3) with gross realization (`73352 ha$^{-1}$), net realization (`51486ha^{-1}) and B:C ratio (2.36)

References

1. Arnon DI. Copper enzymes in isolated chloroplast. I. Polyphenol oxidase in *Beta* vulgaris. Plant Physiology 1949;24:1-15.
2. Asthir B, Thapar R, Farooq M, Bains NS. Exogenous application of thiourea improves the performance of late sown wheat by inducing terminal heat resistance. Int. J Agric. Biol 2013;15:1337-1342.
3. Burman U, Garg BK, Kathju S. Interactive effects of thiourea and phosphorus on clusterbean under water stress. Biol. Plant 2004;48:61-65.
4. Dixit GP, Srivastava AK, Singh NP. Marching towards self-sufficiency in chickpea. Current science 2019;116:2-25. JANUARY, 2019.
5. Garg BK, Burman U, Kathju S. Influence of thiourea on photosynthesis, nitrogen metabolism and yield of cluster bean (*Cyamopsis tetragonoloba* (L.) Taub.) under rainfed conditions of Indian arid zone. Plant Growth Regul 2006;48:237-245.
6. Nathawat NS, Rathore VS, Meel B, Bhardwaj S, Yadava ND. Enhancing Yield of Clusterbean (*Cyamopsis Tetragonoloba* L. Taub) With Foliar Application of Sulphydryl Compounds Under Hot Arid Conditions. Experimental Agriculture 2016;52(3):418-433.
7. Rahangdale SL, Dhopte AM, Wanja KB. Evaluation of chickpea genotypes for yield stability under moisture deficit. Annals of plant physiology 1994;8:179-184.
8. Rana M, Solanki NS, Chouhan GS, Mali H, Kumar. Alleviating the Adverse Effects of Water Stress in Wheat by Foliar Sprays of Bio-Regulators. Int. J Curr. Microbiol. App. Sci 2018;7:3345-3354.
9. Sabaghpour SH, Sadeghi E, Malhotra RS. Present status and future prospects of chickpea cultivation in Iran. International chickpea Conference, Raipur, India, 2003.
10. Sahu MP, Solanki NS, Dashora NL. Effect of thiourea, thiamine and ascorbic acid on growth and yield of maize (Zea mays L.). J Agron. Crop Sci 1993;171:65-69.
11. Sharma OP, Singh GD. Effect of sulphur in conjunction with growth substances on productivity of clusterbean (*Cyamopsis tetragonoloba*) and their residual effect on barley (*Hordeum vulgare*). Ind. J Agron 2005;15:16-18.
12. Solanki NS. Effect of thiourea and demethylsulphoxide on phosphorus use efficiency, dry matter partitioning and productivity of clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.]. Ph. D. Thesis, Maharana Pratap University of Agriculture and Technology, Udaipur, 2002.
13. Sunkar R. *Plant Stress Tolerance*, Methods in Molecular Biology. Springer Science + Business Media, 2010. DOI 10.1007/978-1-60761-702-20.
14. Wankhade RS, Kubde KJ, Deshmukh MR, Goud VV. Effect of bioregulators on yield and yield attributes of Chickpea (*Cicer arietinum* L.). International Journal of Chemical Studies 2020;8(4):140-144.